Workshop Japan-Egypt “Pharmacognosy and Traditional Medicine”

July 20th to 23rd 2010, Tokyo

New strategy using monoclonal antibody on natural product investigation

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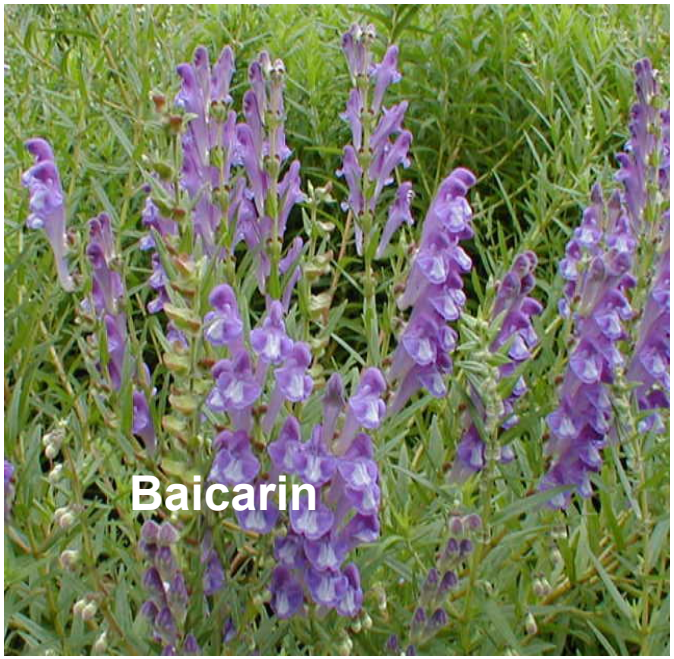
30 or more MAbs were prepared.



Prepared MAb

○indicates scFV cloning

Forskolin

Codeine, Thebaine

Solamargine

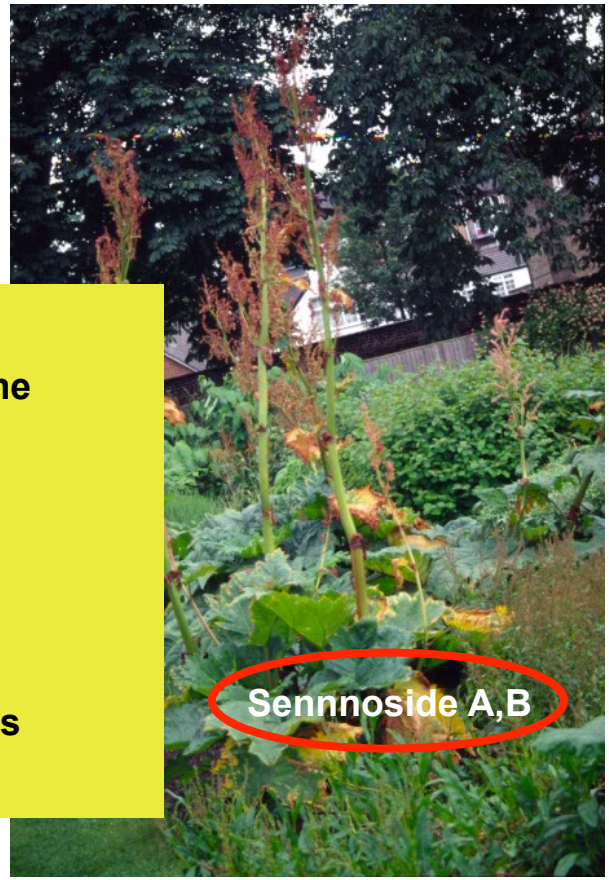
Cannabinoids

Saikosaponins

Berberine

Aristolochic acids

Ginkgoric acids



Preparation of MAb and its application

1) Competitive ELISA
2) Eastern blotting
3) Knockout extract (finding of active comps)
4) Cloning and expression of scFV and use for molecular breeding (breeding)

1) Competitive ELISA

GC Contents in Licorice Roots and Various Traditional Chinese Prescriptions Determined by ELISA

sample	content (mg/g dry drug)
licorice root	
G. glabra	34.9±3.4
G. inflata	32.5±2.8
G. uralensis	27.5±1.3
prescription	
芍药甘草汤	16.8±2.9
大黄甘草汤	4.7±0.4
小建中汤	0.8±0.1
小青龙汤	2.8±0.3
六君子汤	0.4±0
麻黄汤	2.3±0.1
加味麻黄汤	1.6±0.1
加味逍遥散	3.4±0.1
桂枝加芍药汤	5.2±0.1
柴胡桂枝汤	3.8±0.2
小柴胡汤	1.4±0.3
桂甘姜枣汤	2.2±0.4
桂枝汤	0.8±0.1
当归芍药散	nd
大柴胡汤	nd

nd = not detectable.

ELISA kit for glycyrrhizin analysis

Wako Chemical Inc.

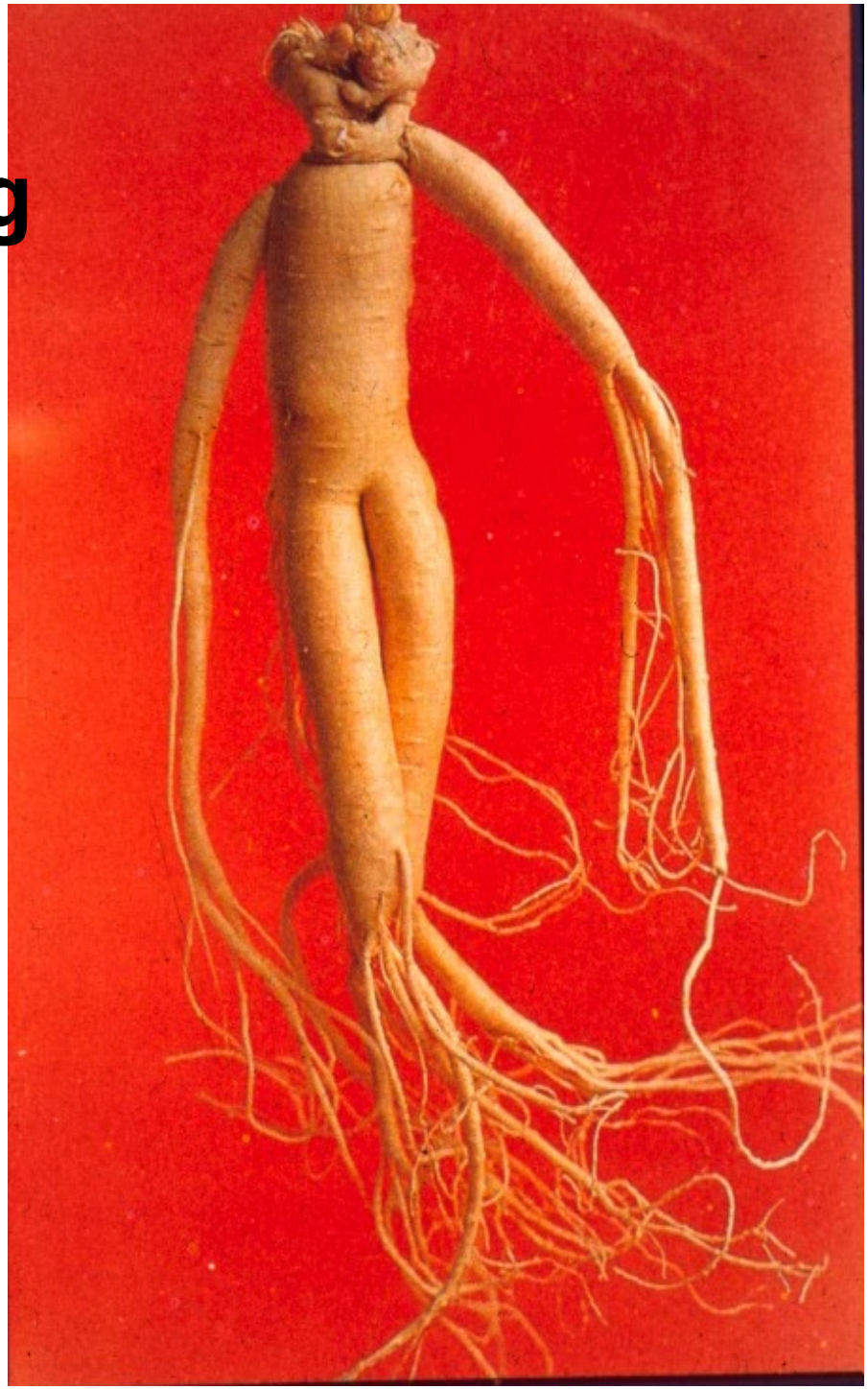
Breeding of *Glycyrrhiza urarensis* by selection

2) Eastern blotting

Panax ginseng

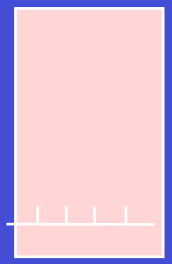
Ginsenosides

Rb1, Rg1, Re



Eastern blooting

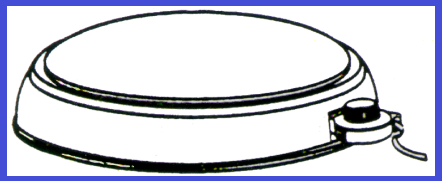
Easter blotting protocol



TLC-plate

Sprayed with blotting solution

Stainless-steel plate
TLC-plate
PVDF-membrane
Glass micro fiber filter



Hot plate

Transfered to PVDF-membrane

Dipping in $NaIO_4$ soln.

Incubated for 1 h
Washed with H_2O

Addion of Na_2CO_3-$NaHCO_3$ buffer soln. containing BSA

Shaked for 3 h
Washed with PBS

Blocking with SPBS for 3 h

Washed with PBS

Addition of anti-G-Rb1-MAb

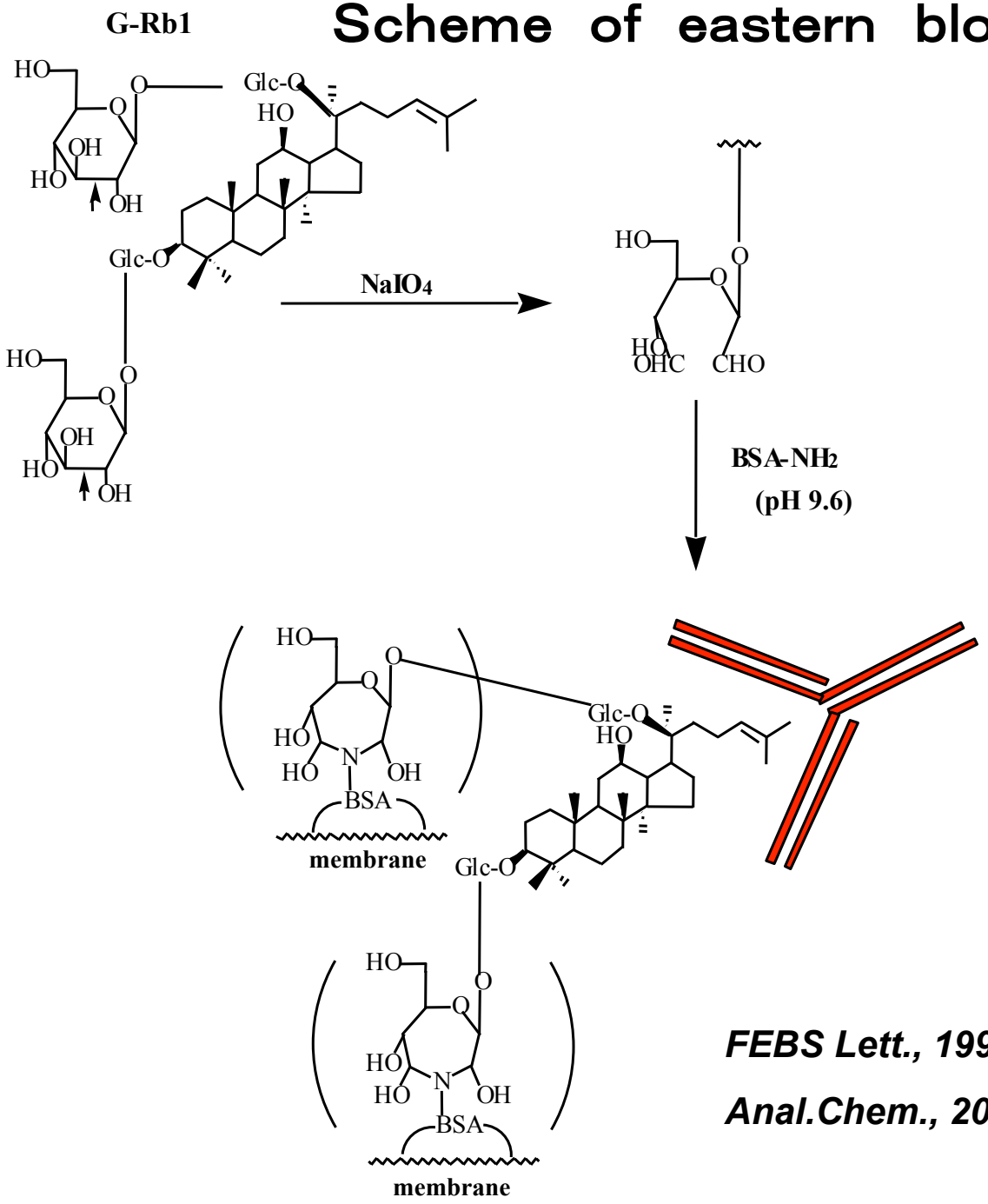
Shaked for 3 h
Washed with TPBS

Addition of POD-labeled goat anti-mouse IgG

Shaked for 1 h
Washed with TPBS

Addition of substrate solution (4-chloro-1-naphthol and H_2O_2)

Scheme of eastern blotting



FEBS Lett., 1997

Anal.Chem., 2001

Double staining of ginseng saponins by eastern blotting using monoclonal antibodies

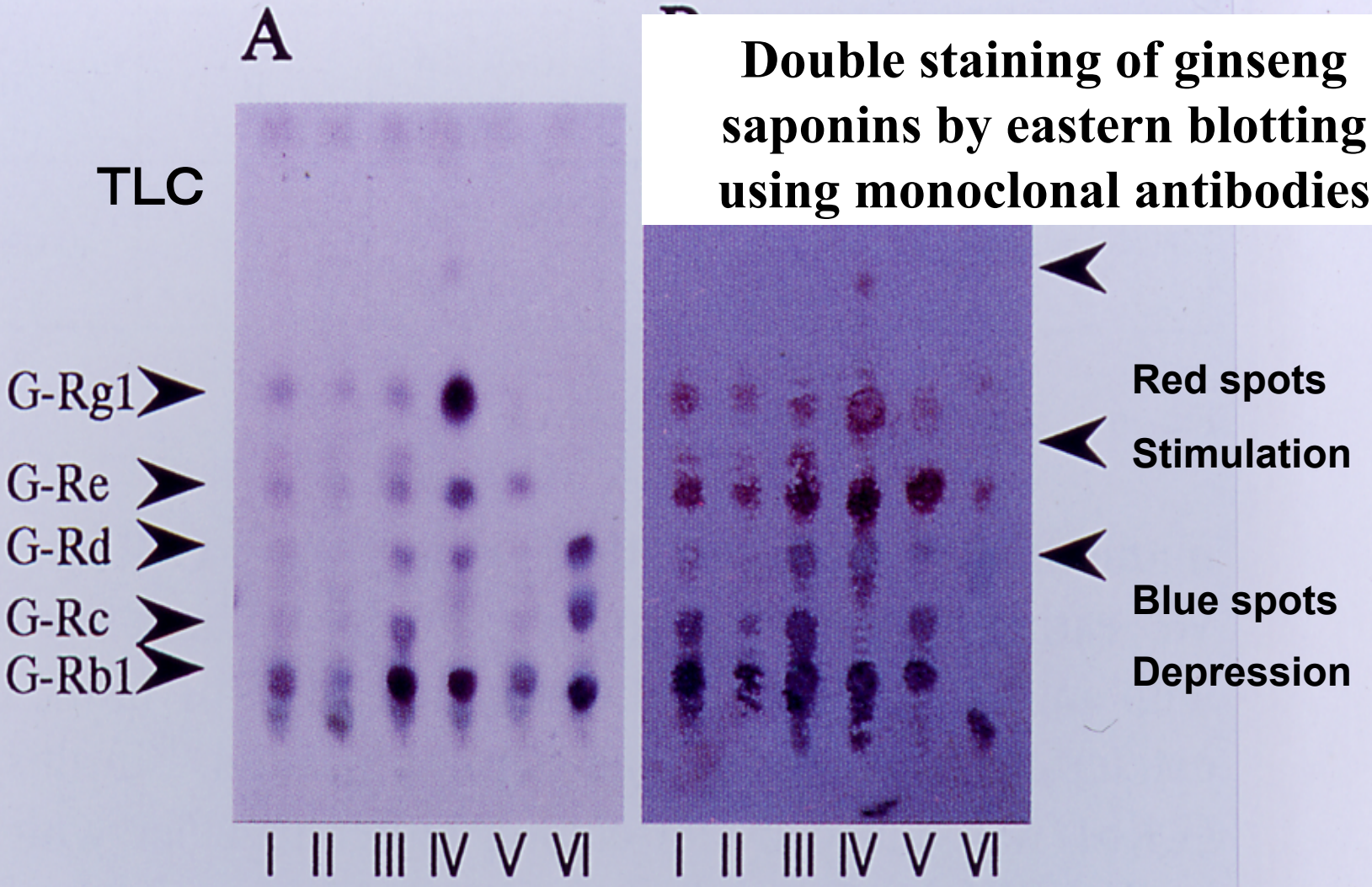
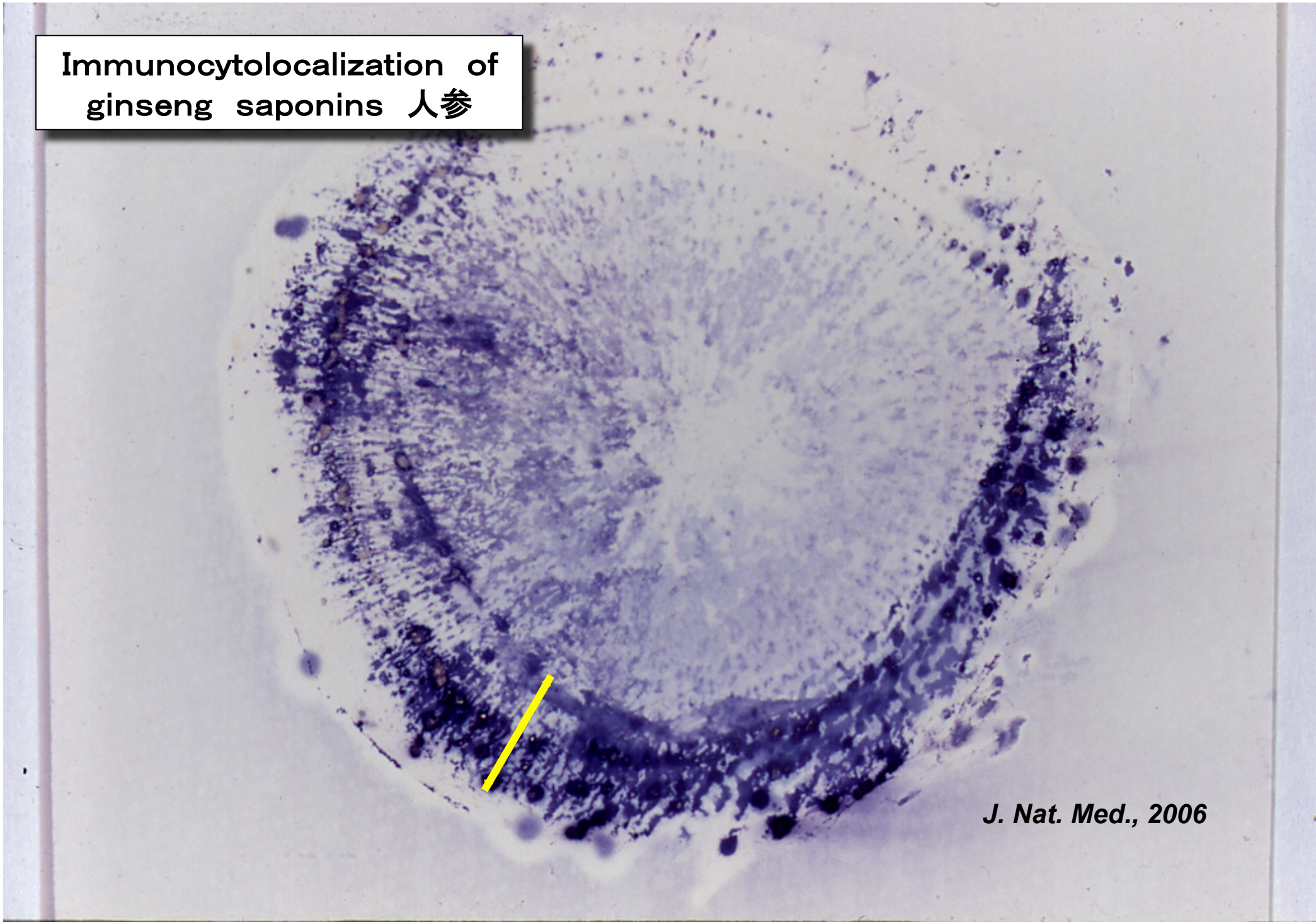


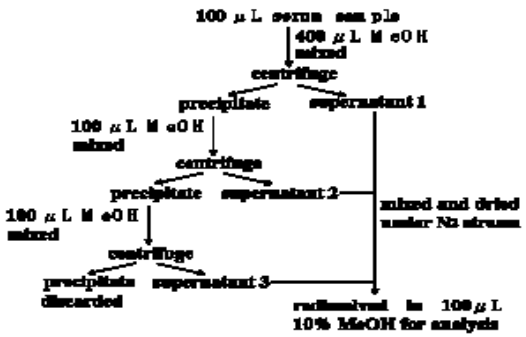
Fig. 4. Double Staining of Various *Panax* Samples

A, H_2SO_4 staining; B, Western blotting. Lanes I, II, III, IV, V, and VI indicate white ginseng, red ginseng, fibrous ginseng, *P. notoginseng*, *P. quinquefolium*, and *P. japonicus* (60 μg), respectively.

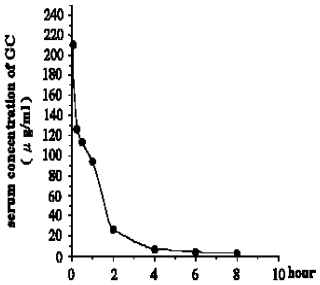
Analyst, 2000
Biol.Pharm.Bull., 2001


Immunocytolocalization of ginseng saponins 人参
J. Nat. Med., 2006

Serum Sample Preparation

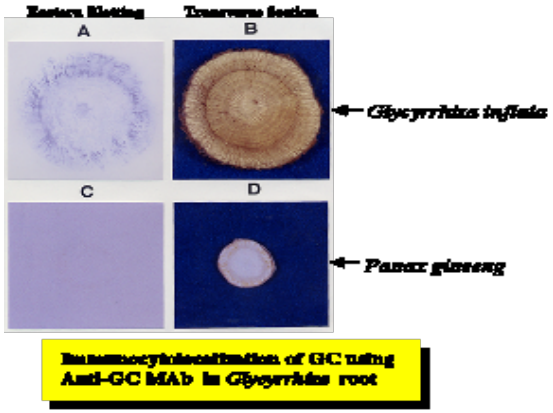


By ELISA



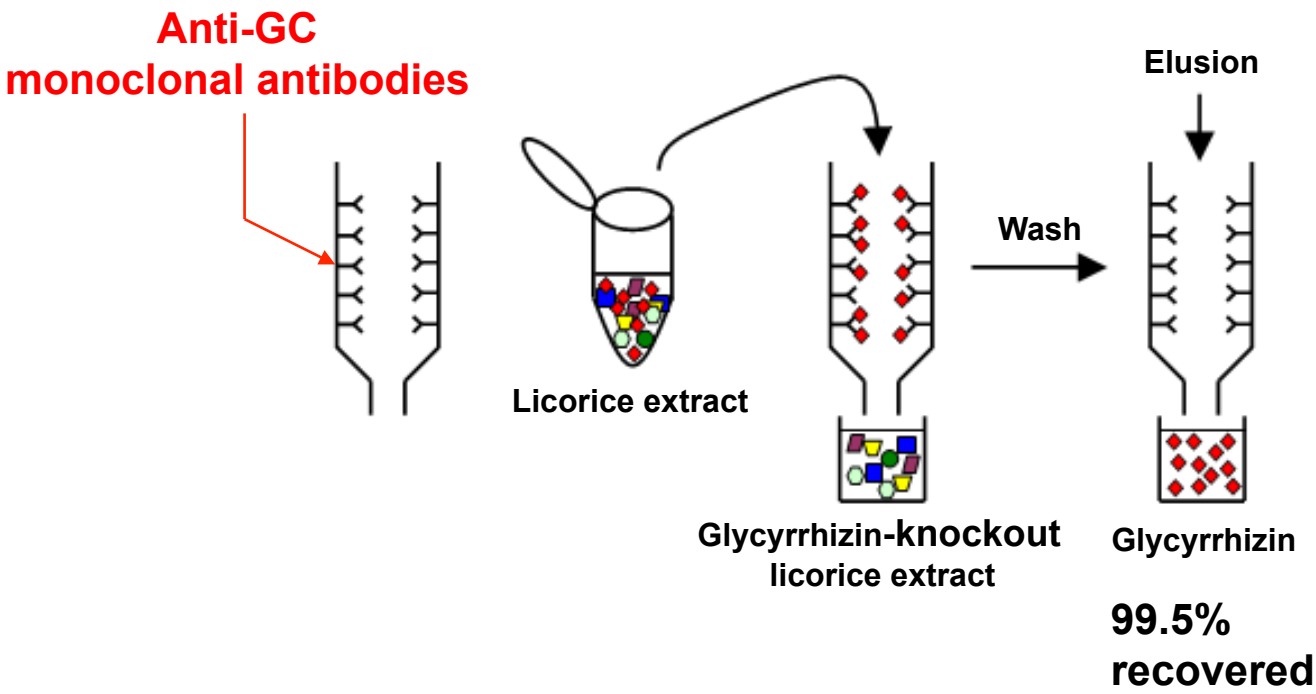
Time Course of GC in Rat after Intravenous Administration

Eastern blotting



Anal.Chem.,2002

3) Preparation of glycyrrhizin-knockout licorice extract



12 mg of licorice extract (GC content: 1275.8 μg) was applied on the anti-GC-MAb Affi-Gel Hz gel. After washing, fractions were deionized and the solvent was lyophilized. TLC profile indicated washing fraction contained all of the components in the licorice extract except GC, and ELISA showed 99.5 % GC (1269.3 μg) was eliminated.

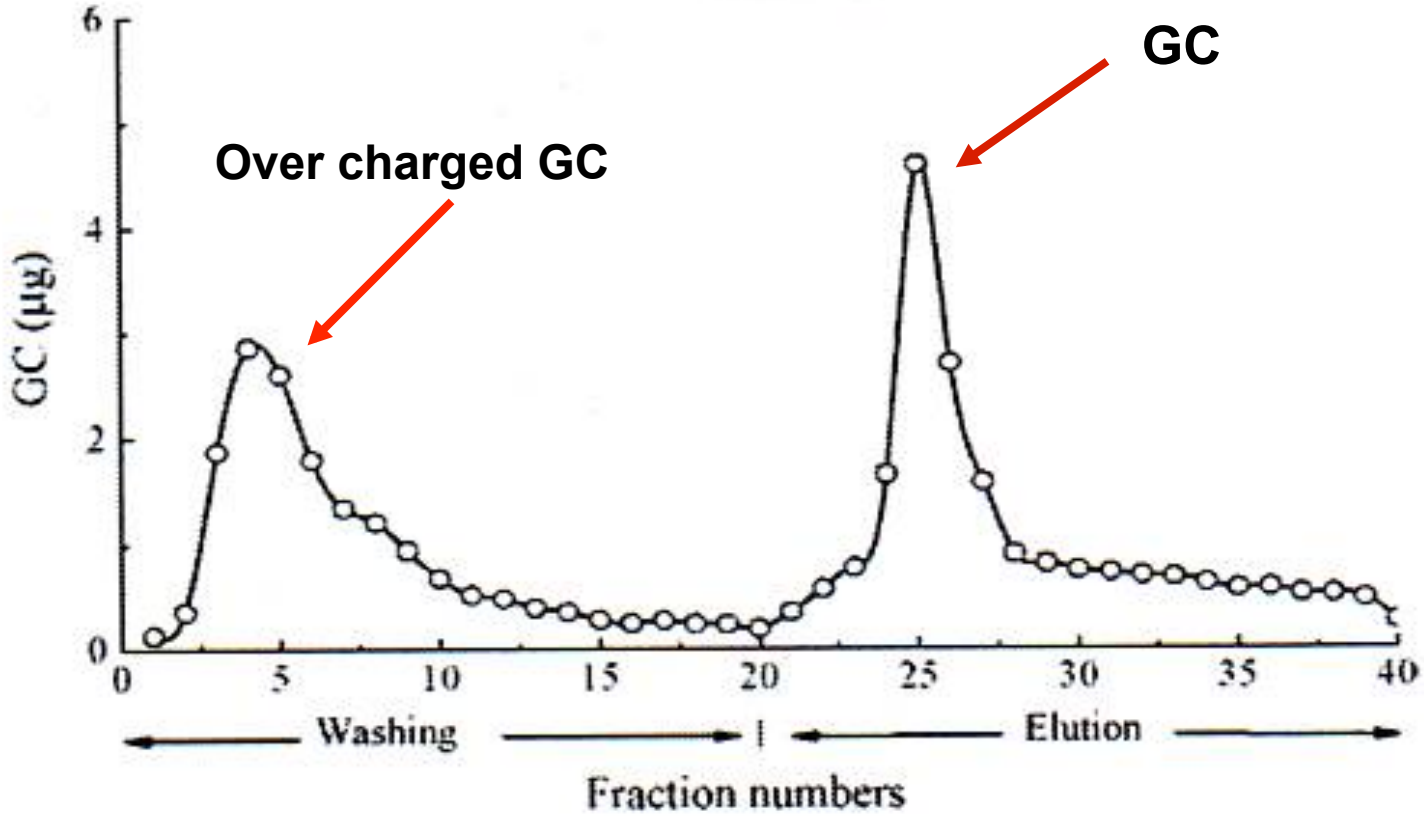
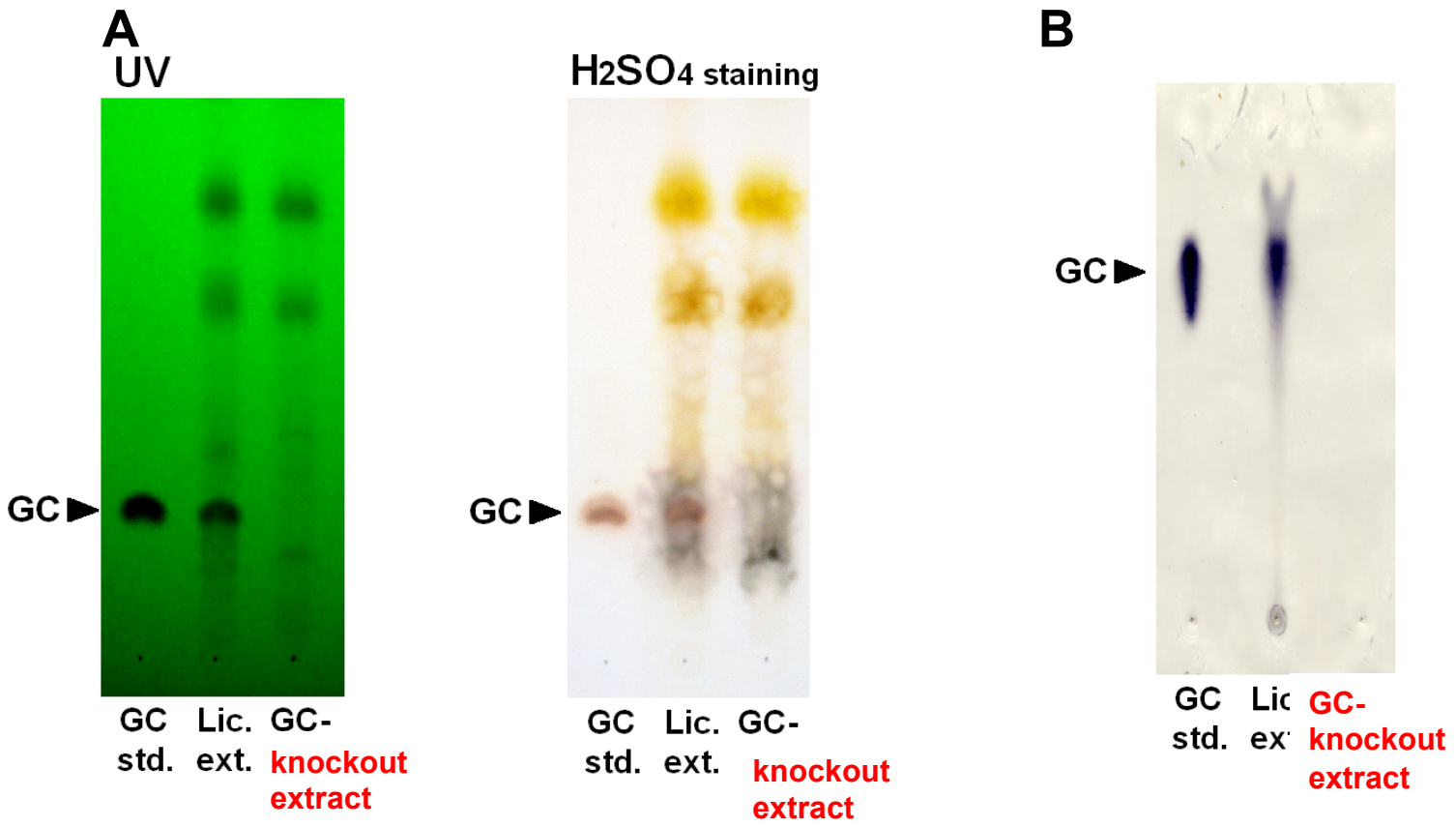


Fig. 3. Elution profile of GA from the crude extracts of *Shakuyaku kanzo to* (*Shaoyao gancao tang*) with an immunoaffinity column coupled with the anti-GA-MAb. For buffer systems and eluting conditions, see the text.

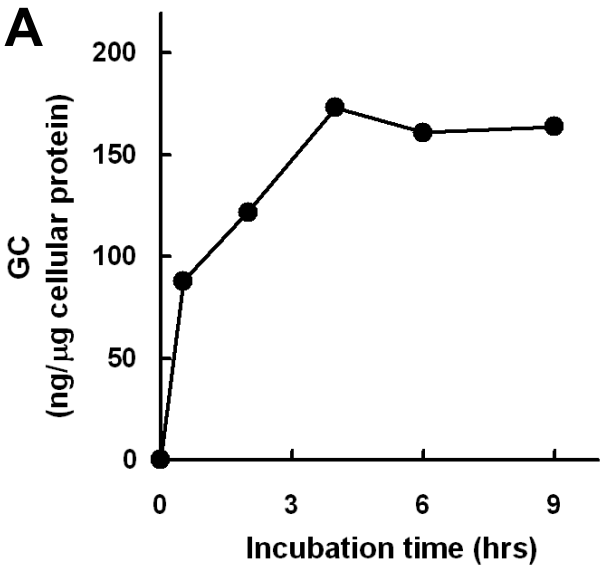
***J. Chromatog.B*, 2007**

TLC profiles (A) and eastern blotting (B) analysis of GC-knockout extract

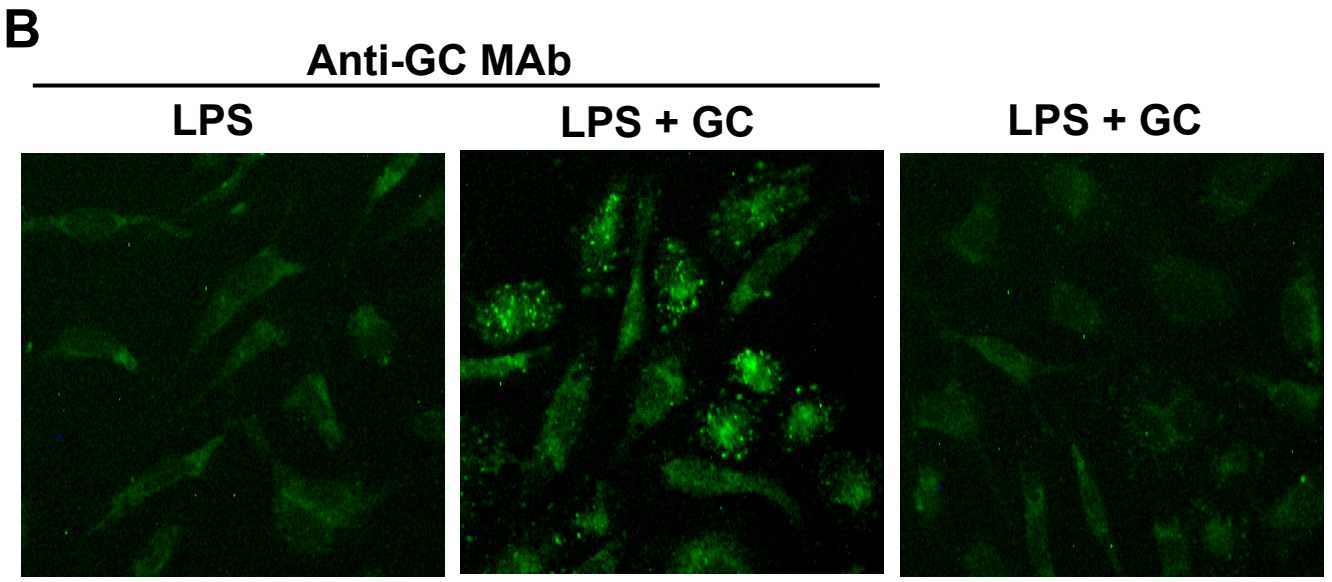


UV and H2SO4 staining of GC in licorice extract and GC-knockout extract (A).
Detection of GC by eastern bllott analysis using anti-GC MAb (B).

Accumulation and cellular localization of GC in LPS-treated RAW264 cells



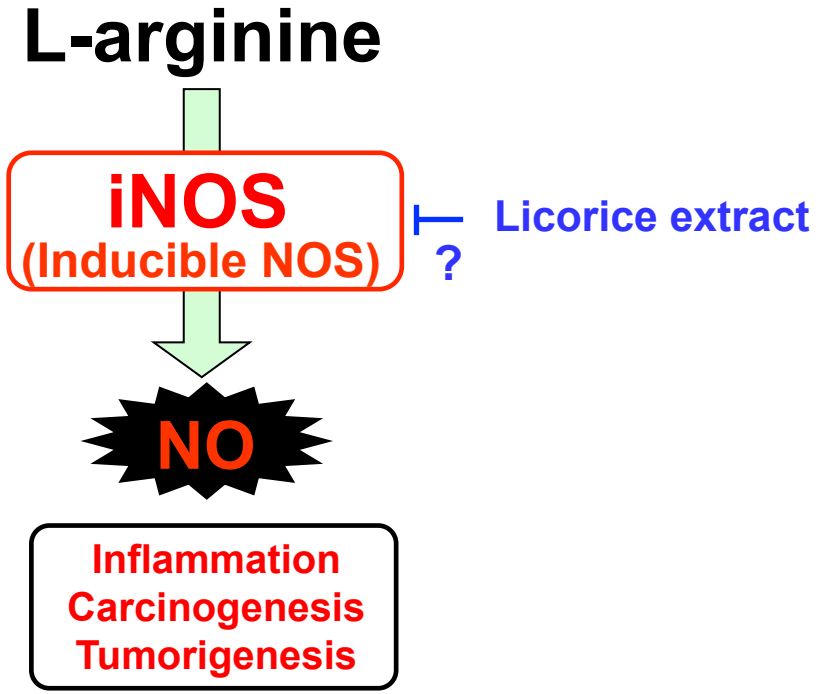
Time-dependent accumulation of GC (A). Immunocytochemical staining using anti-GC MAb in GC accumulated RAW264 cells (B).



Nitric oxide (NO) in physiology and disease

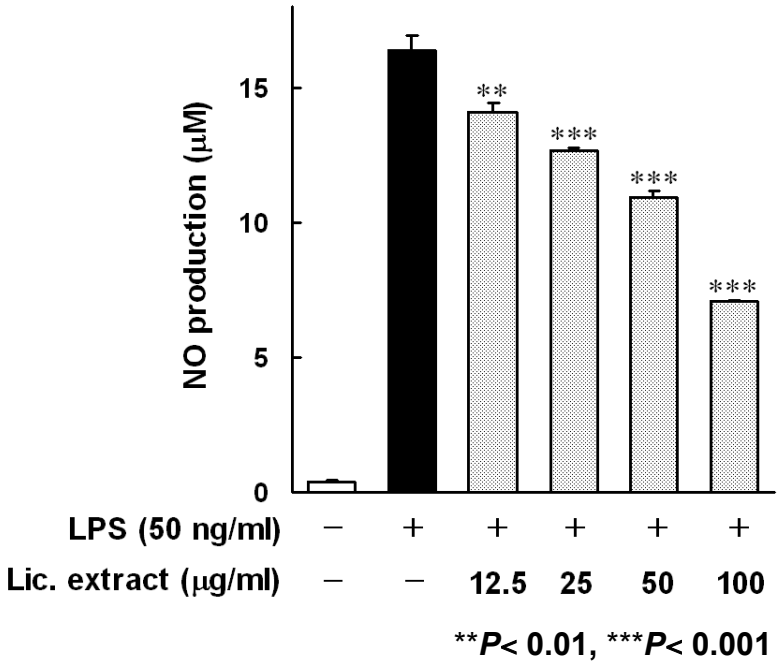
Nitric Oxide Synthases : NOS

NOS1	neuronal NOS (nNOS)	Neurotransmitter
NOS2	Inducible NOS (iNOS)	Inflammation carcinogenesis
NOS3	endothelial NOS (eNOS)	Blood pressure Vascular angiogenesis



Licorice extract suppressed LPS-induced NO production in a dose-dependent manner

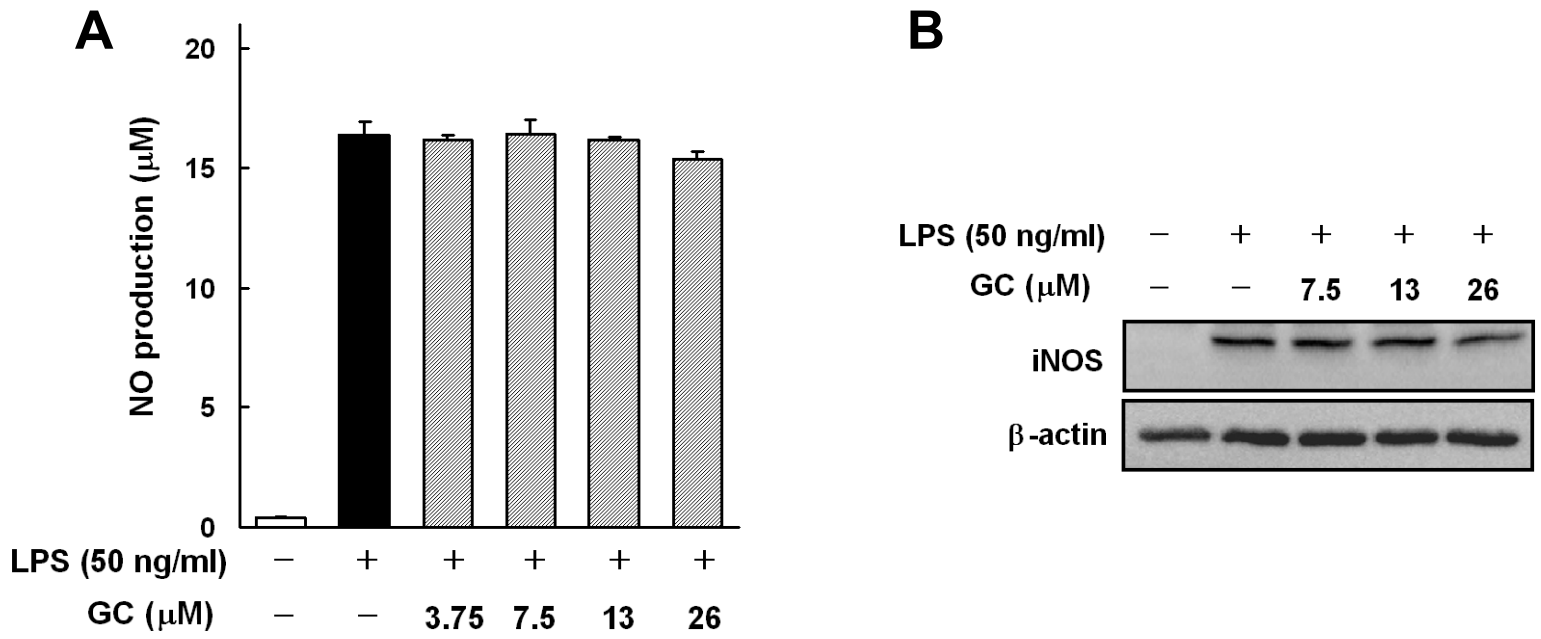
Preparation of licorice extract
Licorice root powder (*G. uralensis.* Japanese pharmacopeia grade) was purchased from Uchida Wakanyaku Corporation (Tokyo, Japan). The dried powder of licorice root (100 mg) was extracted with methanol (1.2 ml) under sonication five times, filtered and then evaporated with N_2 gas at 60 °C.



GC can not suppress LPS-induced NO production and iNOS expression

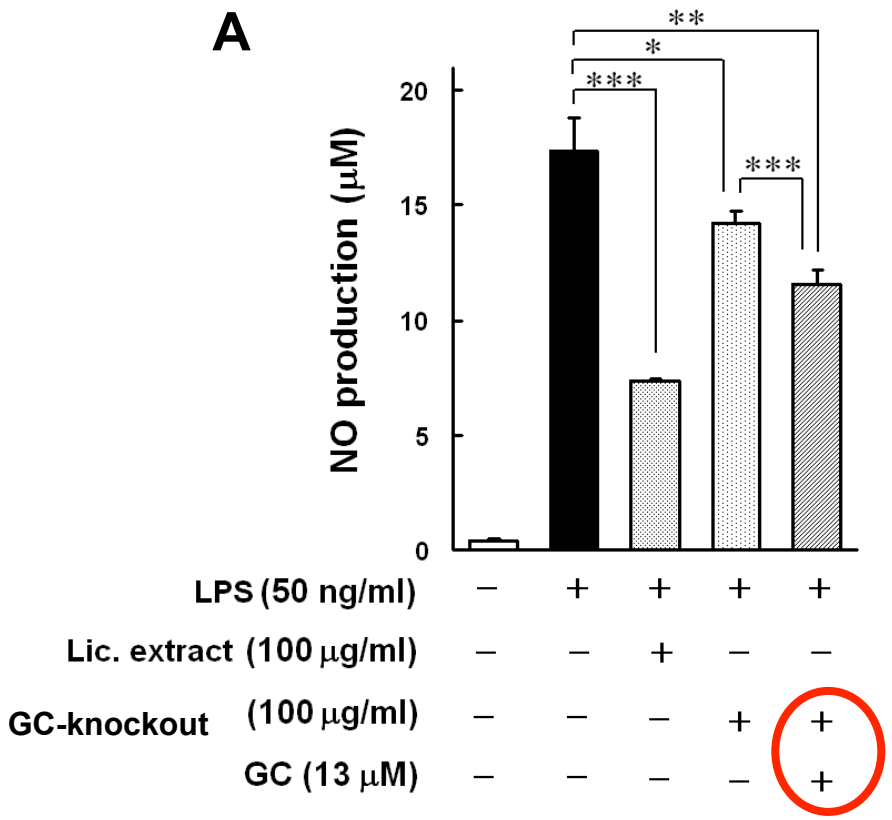
Glycyrrhizin concentration: **106.26 ± 6.18 μg/mg dry licorice extract**

ELISA by using anti-GC monoclonal antibody (MAb) demonstrated 100 μg/ml of licorice extract contains 10.6 μg/ml (≒ 13 μM) of GC.

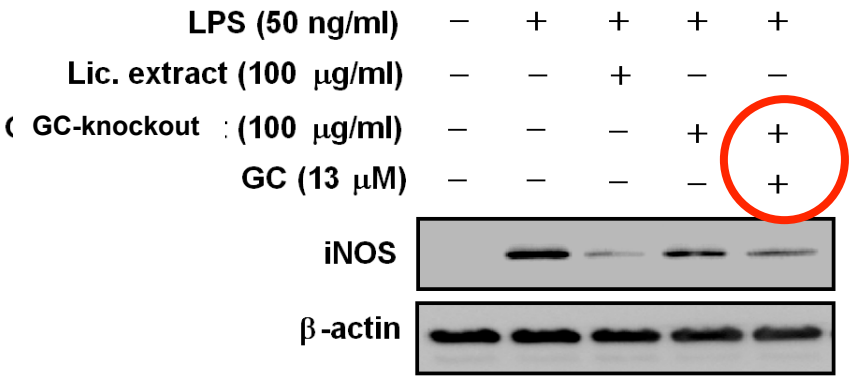


Effects of GC on LPS-induced NO production (A) and iNOS protein expression (B).

Effects of GC-knockout extract and the combination of GC-knockout extract and GC on LPS-induced NO production and iNOS protein expression



*P< 0.05, **P< 0.01, ***P< 0.001

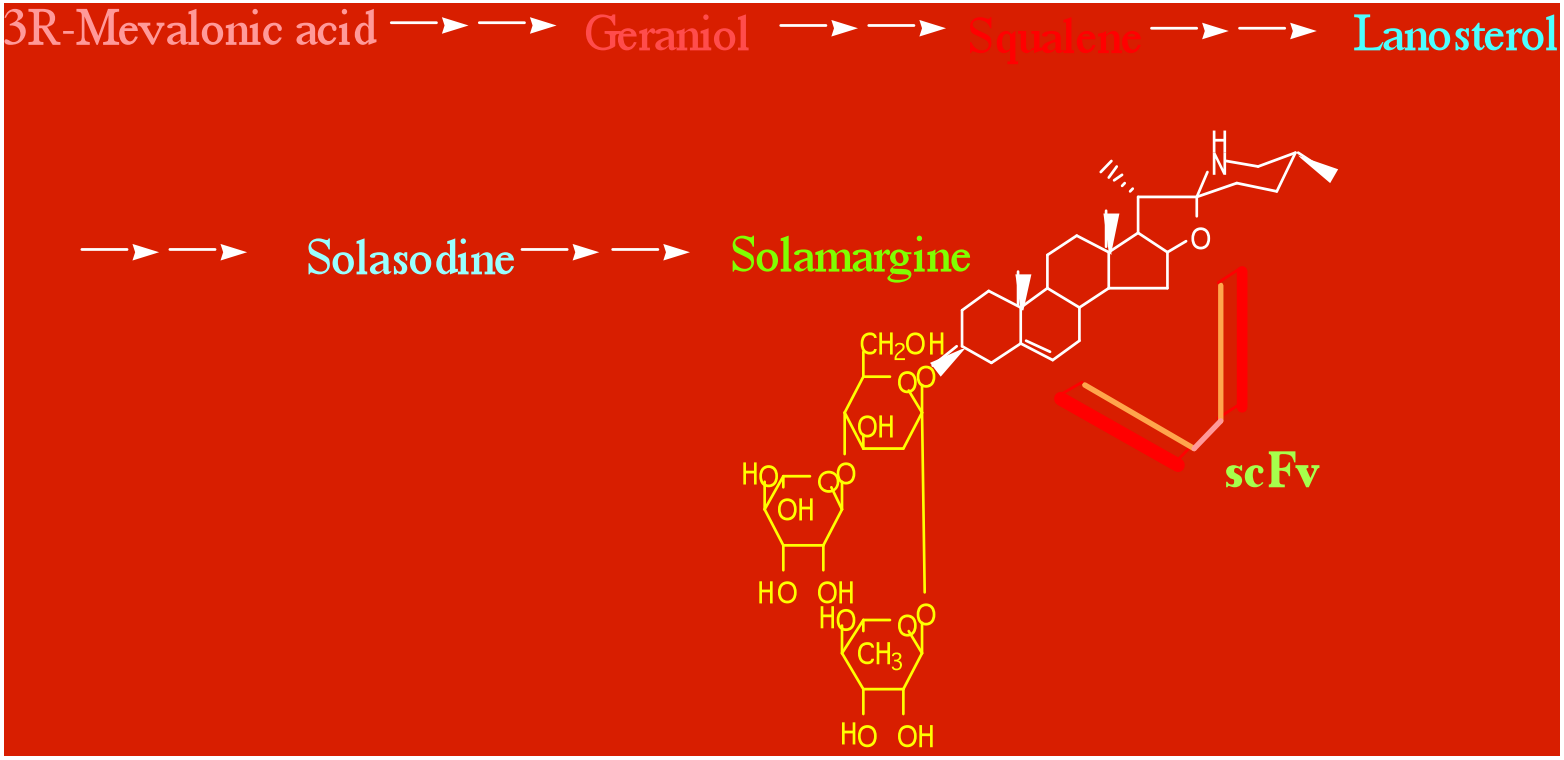


Effects of GC-knockout extract on LPS-induced NO production (A) and iNOS protein expression (B).

4) Application of single chain Fv gene for a missile type breeding (Direct targeting)

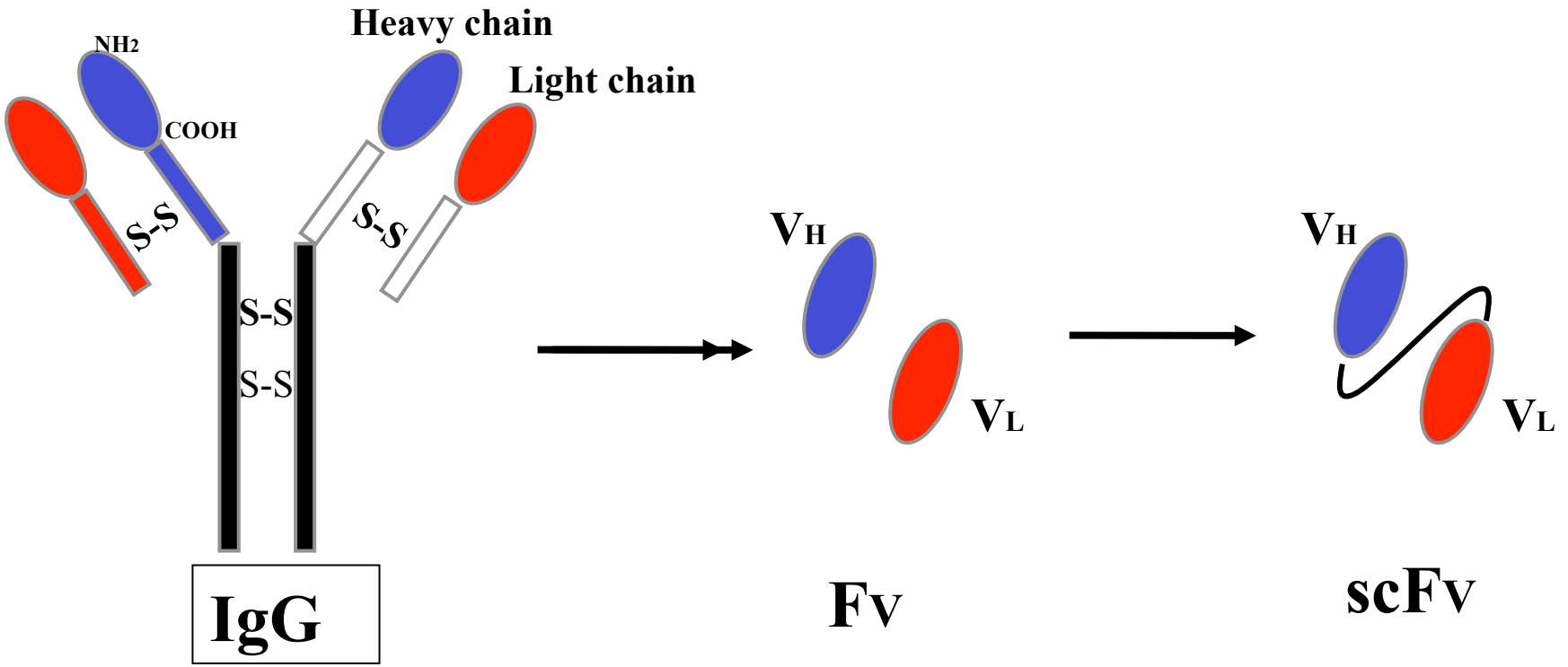

Solanum khasianum fruits
Containing solasodine glycosides which can be used for preparation of hormones

Targeting for final product using compact antibody (scFV)

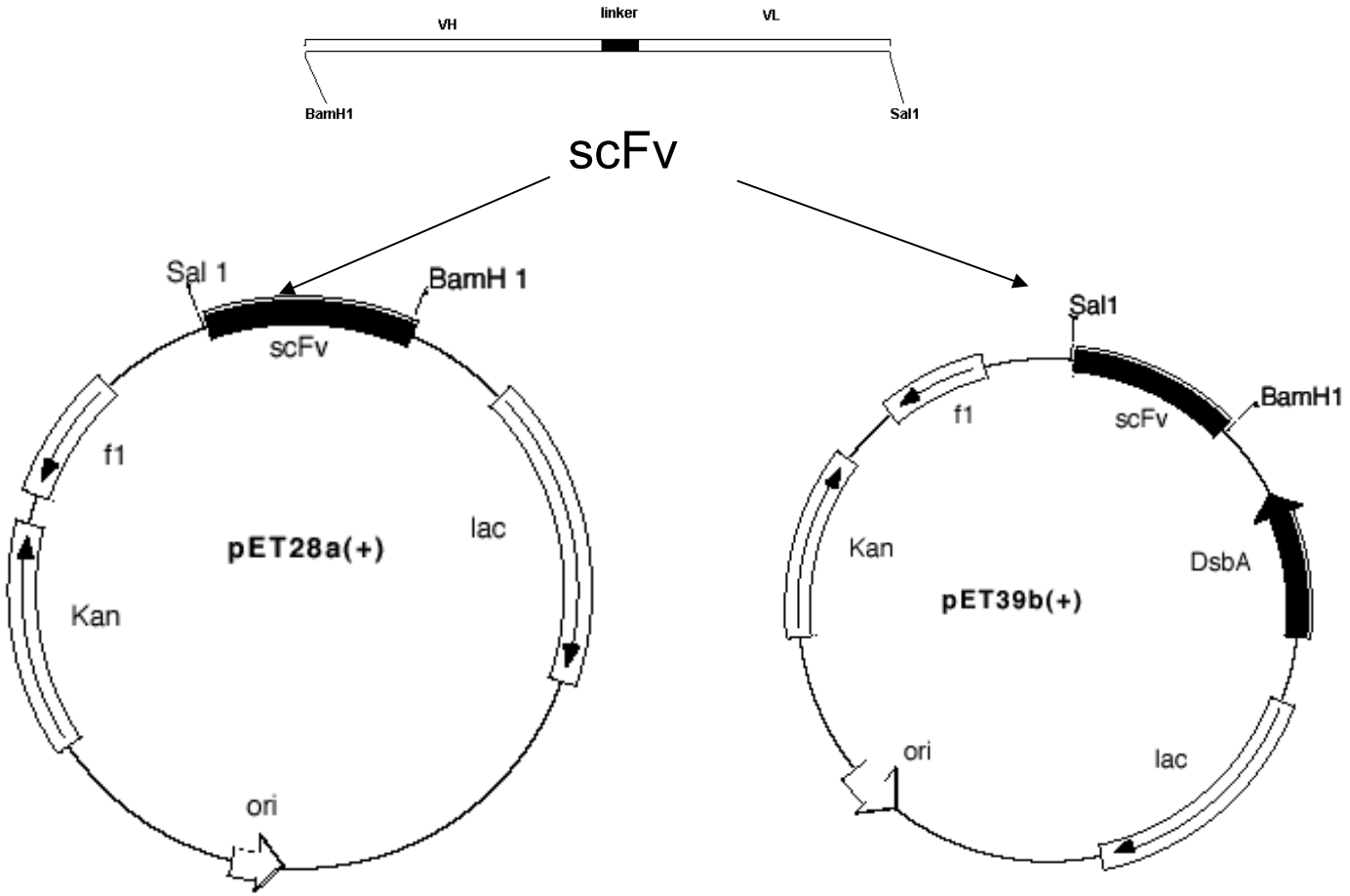


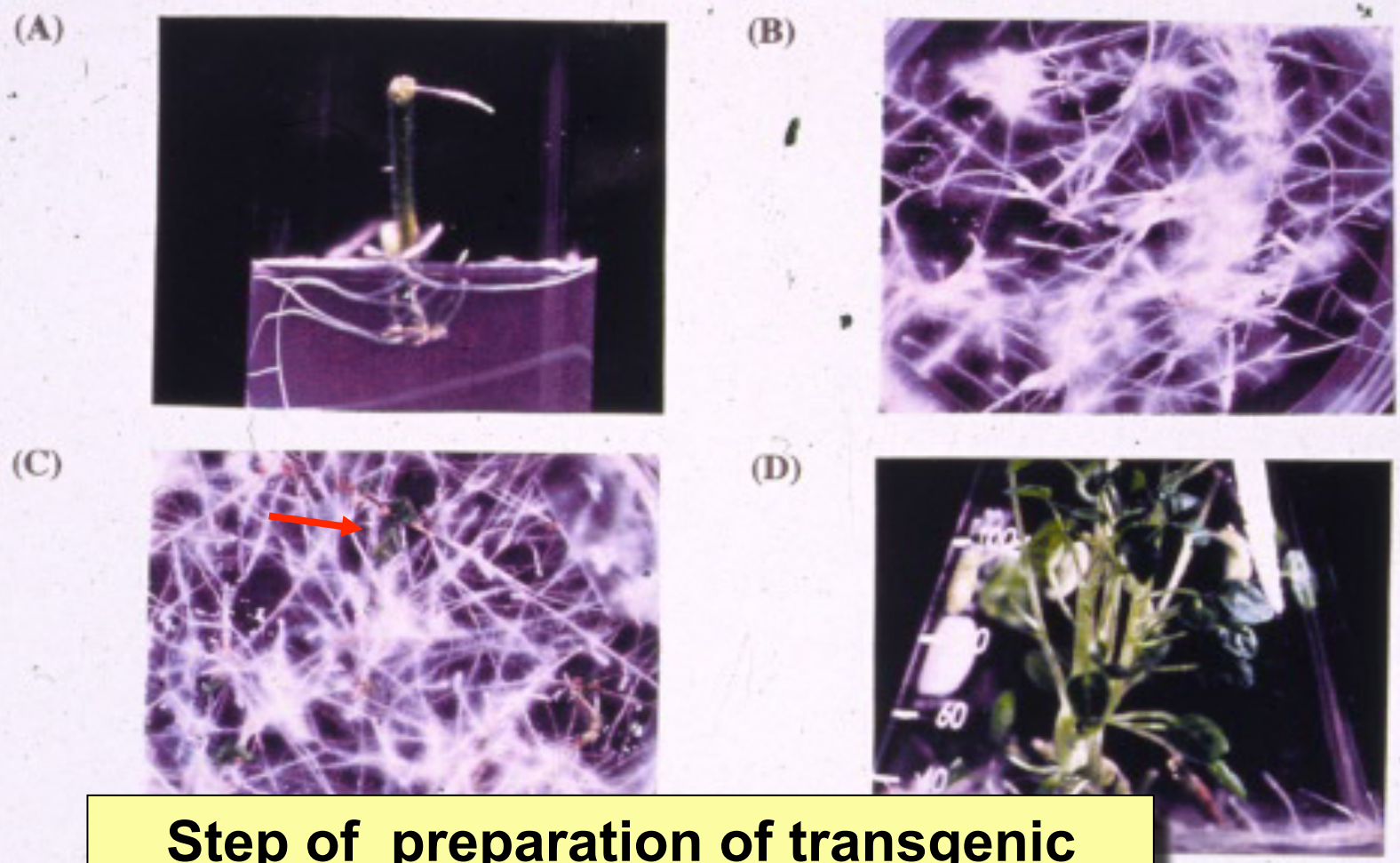
Propose of anti-solamargine scFv on biosynthesis of solasodine glycosides in transgenic *S. khasianum*.

Preparation of scFv



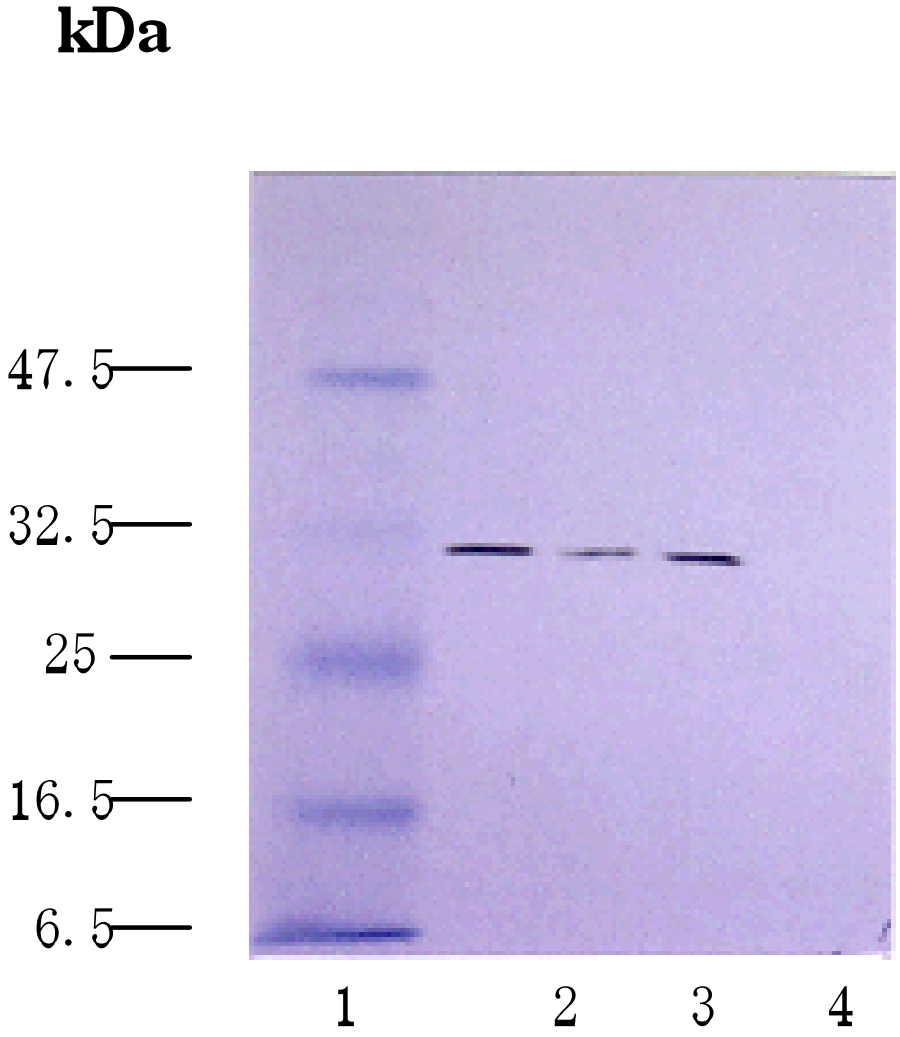
Construction of scFV gene





Step of preparation of transgenic plant induced scFV gene

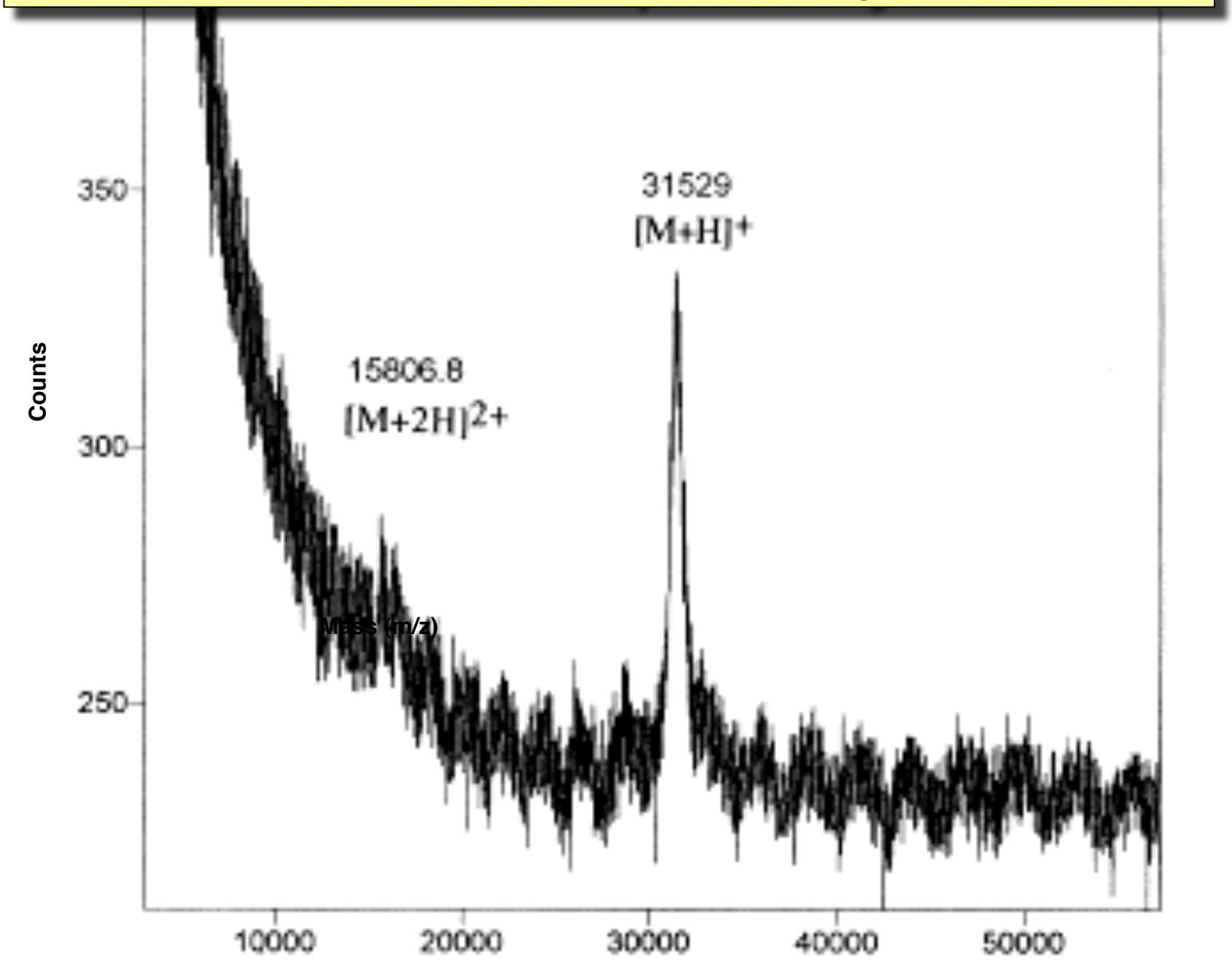
Fig. 8 Development of hairy root after infected with *A. rhizogenes* (A), transgenic hairy roots (B), shoot production from transgenic hairy roots (C) and regeneration of transgenic plant from hairy root of *S. khasianum* (D).



Determination of scFV protein in transgenic plant

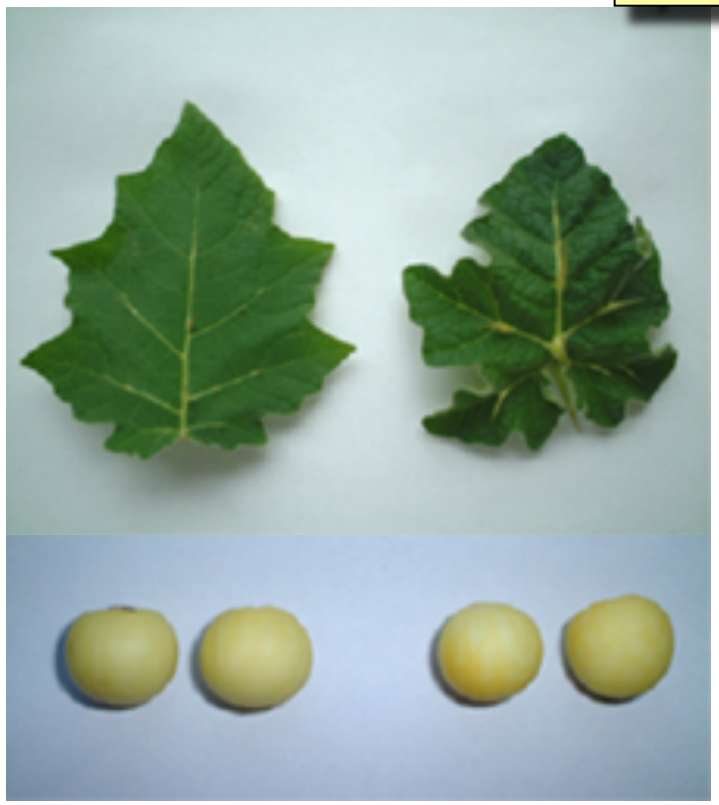
Determination of sc FV protein by MALDI-MS

B



Stimulation of biosynthesis of pharmacologically active compound

A B



Solasodine glycosides	μg/g dry wt.
Control	
Leaf	54.50±2.08
Fruit	53.43 ± 3.90x10^3
Transgenic plant	
Leaf	144.48 ± 4.64
Fruit	126.95 ± 6.03x10^3

Leaf and fruit of control (A) and transgenic plant (B)

Relation between solasodine glycoside content and scFV protein

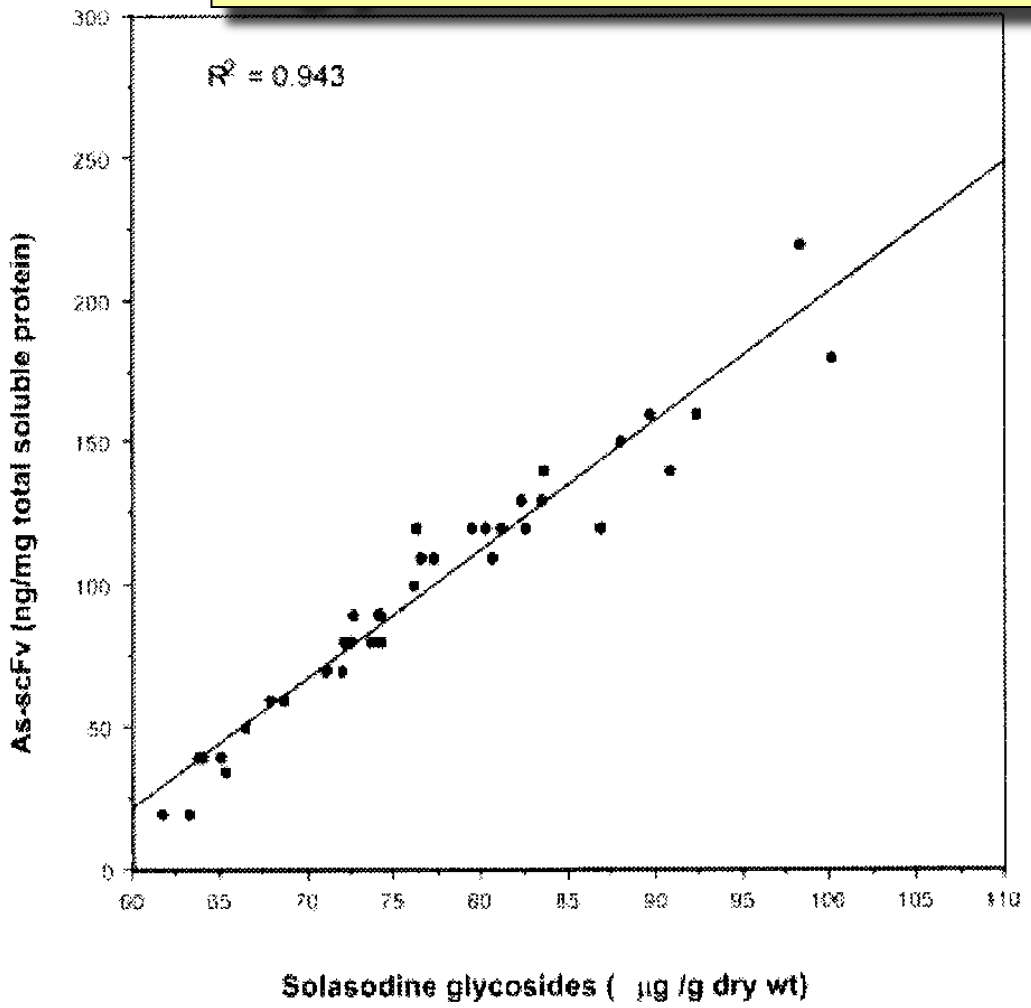
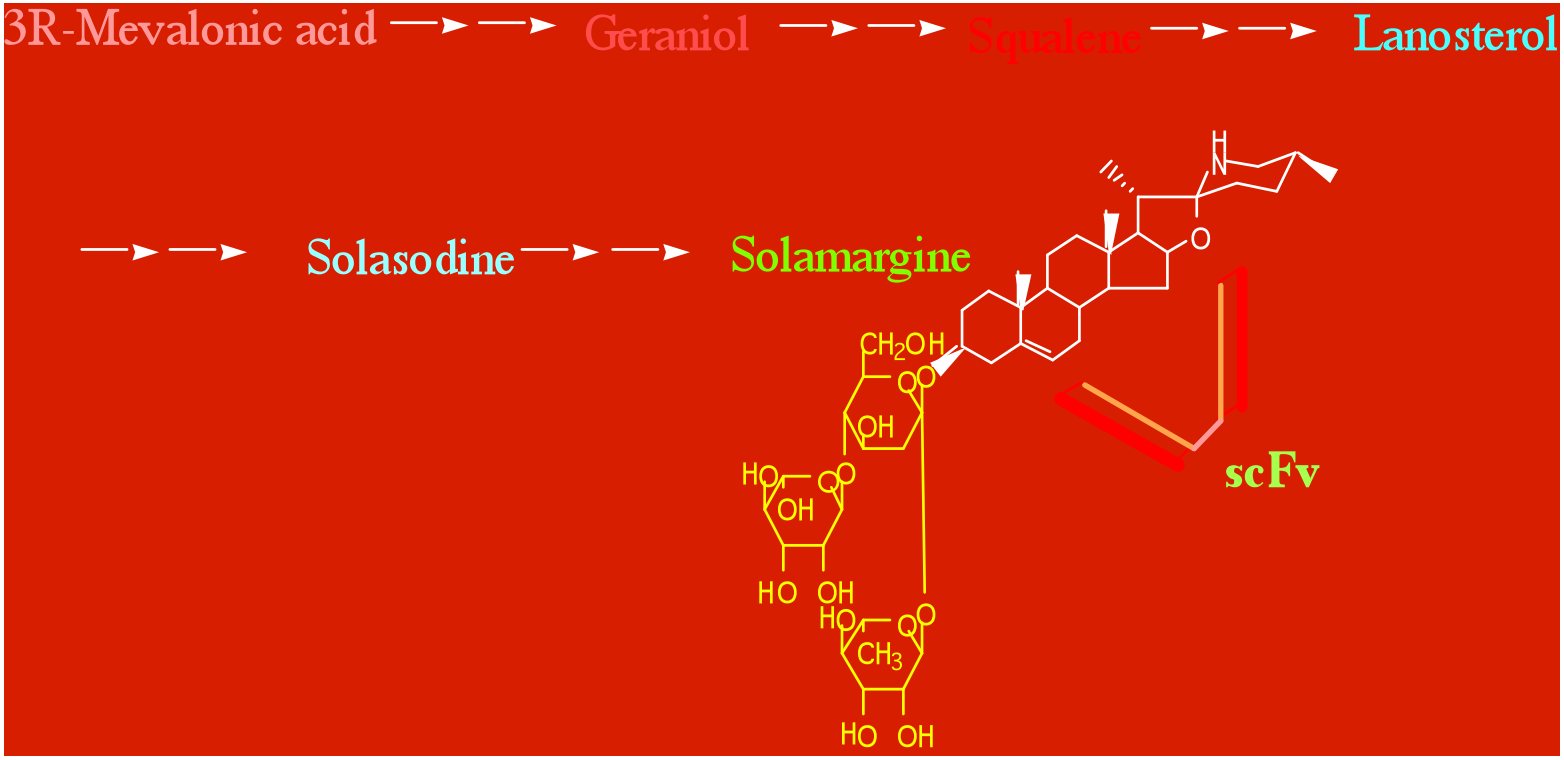


Fig. 6. Comparison of solasodine glycoside concentrations from hairy root clones and scFv expression levels

Plant Cell Rep., 2004



Propose of anti-solamargine scFv on biosynthetic of solasodine glycosides in transgenic *S. khasianum*.

Missile type breeding of *Glycyrrhiza urarensis* by induction of scFv gene

Thank you for kind attention !