Production of Steroid Saponin in Grafts: *Solarium aculeatissimum* and *Lycopersicon esculentum*

Toshihiko Ikenaga, Seiji Kikuta, Miyuki Kistuki, Midori Yamada, and Kenichiro Nakashima

Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852, Japan

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Abstract. *Solarium aculeatissimum* Jacq. contains the steroid saponins aculeatiside A and aculeatiside B at high levels in the root, whereas they are lacking in all organs of tomatoes (*Lycopersicon esculentum* Mill.). We examined the site of synthesis of these steroid saponins by using grafts between *S. aculeatissimum* and tomato plants. When *S. aculeatissimum* was grafted onto a stock of tomato (S.a./Tom.), a small amount of steroid saponin was found in the leaves and the stem of *S. aculeatissimum* but not in the roots of the tomato. However, when tomato was grafted onto stocks of *S. aculeatissimum* (Tom./S.a.), steroid saponin occurred only in the roots of *S. aculeatissimum*. These results suggest that the steroid saponins are mainly synthesized in the roots of *S. aculeatissimum*. The lack of transport of steroid saponins from the roots of *S. aculeatissimum* to the leaves and stem of the tomato remains unexplained.

Grafting of different species has been used to investigate the site of synthesis of compounds, to increase flowering and vigor, to make handling easy, and to build disease tolerance (Dukes and Jones, -1981; Lardizabal and Thompson, 1988). Grafts between tomato and tobacco and between tomato and *Datura* have been used to determine that alkaloids in these plants are synthesized in the roots (Dawson, 1942; Hasegawa, 1937; Shibata and Imaseki, 1953).

Solarium aculeatissimum contains the steroid saponins aculeatiside A and aculeatiside B at high levels in its roots (Ikenaga et al.,

1988a). Since nautigenin and isonutigenin, obtained by hydrolysis of aculeatiside A and aculeatiside B, can be transformed into pregnenolone (Imada et al., 1981; Saijo et al., 1983; Tschesche and Richert, 1964), it is conceivable that *S. aculeatissimum* may be a more useful source of pregnane derivatives than the *Dioscorea* species that contains a natural steroid saponin, diosgenin, in its tubers (Tal et al., 1984; Rokem et al., 1985).

In the present work, we used grafting between *S. aculeatissimum* and tomato to determine the site of synthesis of steroid saponins in *S. aculeatissimum*. If grafted plants [tomato grafted onto stocks of *S. aculeatissimum* (Tom./S.a.)] contained steroid saponins at the same levels as the roots of *S. aculeatissimum*, they could replace the shoot of *S. aculeatissimum*. This would be desirable because the stems and leaves of *S. aculeatissimum* have thorns and are difficult to handle.

Seeds of *S. aculeatissimum* were sown and plants produced in a greenhouse. Seeds of

'Daifuku' tomato, obtained locally, were sown and cultivated in a greenhouse until they were grafted.

Grafting. Plants of *S. aculeatissimum*, with stems 6 to 8 cm high and six to eight leaves, were reciprocally cleft grafted to tomato plants with 6- to 8-cm-long stems and three to four leaves.

The cut end of the scion, which was ≈ 5 cm long, was shaped like a wedge. The stem of the stock was cut off ≈ 5 cm above the soil surface. A cleft was made vertically along the width of the diameter in the stem of the stock. Then the scion was vertically inlaid into this cleft.

After grafting, the joint part of scions and stocks was firmly fixed with commercial grafting clips. Eighteen of the 20 Tom./S.a. plants developed. Sixteen of the 20 plants that had *S. aculeatissimum* grafted onto a stock of tomato (S.a./Tom.) developed. The plants, which were fertilized with a mineral fertilizer and treated monthly with a fungicide, were cultivated in the greenhouse for 172 days and then harvested.

Ten plants of each graft type were harvested at random, dried at 40C for 48 h, and the dry weights of leaves, stems, and roots determined. The dried tissues were powdered with a mill and dried again at 40C for 2 h before chemical analysis.

Chemical analysis. Crude extracts were obtained for quantitative analysis by adding 0.5 g powder from the roots of *S. aculeatissimum* to 100 ml ethanol. One gram of powder from leaves and stems of *S. aculeatissimum* and from leaves, stems, and roots of tomato were added to 200 ml ethanol. Aculeatiside A and B were identified in the extract by means of high performance liquid chromatography (HPLC) on C-8 phase with acetonitril as the solvent, as described previously (Ikenaga et al., 1988b). A chromatogram of grafted plant organs of *S. aculeatissimum* and tomato is shown in Fig. 1.

Accumulation of steroid saponins. Steroid saponins were not detected in any organs of the tomato or in the tomato scion in Tom./S.a. (Table 1). They were present in leaves, stems, and roots of *S. aculeatissimum* and in the scion of *S. aculeatissimum* in S.a./Tom. However, the steroid saponins in the leaves

Table 1. Production of steroid saponins in *Solarium aculeatissimum*, *Lycopersicon esculentum*, and grafts between them.

Scion/stock ²	Organ	Steroid saponin concn ¹ (% of dry wt) [*]			Yield ¹ (mg/plant) [*]	
		A	B	A + B	A	B
S.a./S.a.	Leaf	Trace	Trace	---	---	---
	Stem	0.34 $\pm$ 0.05	0.38 $\pm$ 0.02	0.72	12.4	13.9
	Root	1.37 $\pm$ 0.06	1.68 $\pm$ 0.07	3.05	33.3	40.8
Tom./Tom.	Leaf	n.d.	n.d.	---	---	---
	Stem	n.d.	n.d.	---	---	---
	Root	n.d.	n.d.	---	---	---
S.a./Tom.	Leaf	Trace	Trace	---	---	---
	Stem	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04	1.0	0.8
	Root	n.d.	n.d.	---	---	---
Tom./S.a.	Leaf	n.d.	n.d.	---	---	---
	Stem	n.d.	n.d.	---	---	---
	Root	0.35 $\pm$ 0.04	0.50 $\pm$ 0.03	0.85	3.7	5.3

¹S.a., *S. aculeatissimum*; Tom., tomatoes (*L. esculentum*).

²Aculeatiside A, B, or A + B.

^{*}Each value is the mean of 10 plants $\pm$ standard error. Trace is <0.01%, n.d. = not detected.