

(the usual dose of MS), and double, four times, six times and eight times of the usual dose. The results were calculated after the third passage (one month each) of callus growth to avoid the influence of the previous nutritional condition. All the cultures grew in a room at 25°C and 400–600 lux light intensity for 10 hr per day. The tissues were extracted after pooling each set together (20 replicates) and drying at 60°C. For extraction, isolation and semi-quantitative estimation of nimbin and  $\beta$ -sitosterol by thin layer chromatography (TLC) and gas liquid chromatography (GLC) the process of Sanyal *et al*<sup>1</sup> was followed.

The growth value rose up to the two-fold dose of glycine (0.6 mg/l) added to the medium. But nimbin continued to increase and  $\beta$ -sitosterol continued to decrease up to the six-fold dose. With an eight-fold dose, nimbin decreased, but  $\beta$ -sitosterol increased considerably (figure 1).

Each of these two phenomena (growth value and synthesis of nimbin) required an optimum dosage of glycine which was higher for the latter. Nimbin accumulation had a reverse relation to  $\beta$ -sitosterol accumulation. It was similar to the redifferentiating tissues where nimbin is high and  $\beta$ -sitosterol low. The natural occurrence<sup>1</sup> of high endogenous glycine and nimbin, and low  $\beta$ -sitosterol in differentiated and cotyledonary tissues, and accumulation of nim-

bin and decrease of  $\beta$ -sitosterol with increase of exogenous glycine in callus, suggested glycine's role in the biosynthesis of these secondary metabolites in *A. indica*. Redifferentiation of calli appeared to bring about certain metabolic changes, helping nimbin biosynthesis of which increased production of glycine was one.

A key role of mevalonic acid and squalene as the precursor of all the known plant sterols and triterpenes<sup>4-6</sup> is well established. In that case, following the initial cyclization, many further transformations must occur. The stereochemic requirements for folding of the squalene molecule to conform with cyclization to different types of sterols (like  $\beta$ -sitosterol) and triterpenes (like nimbin) are also specific. So, in the changed tissue environment of callus, the stereochemical requirements for folding squalene to nimbin or to  $\beta$ -sitosterol must be different. Glycine probably triggered the biosynthesis of nimbin, blocking the other pathway to  $\beta$ -sitosterol.

28 April 1987; Revised 4 August 1987

1. Sanyal, M., Tikadar, S. and Datta, P. C., *Indian Drugs*, 1983, 81, 479.
2. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, 15, 273.
3. Street, H. F., In: *The biology of cell and tissue in culture*, 1966, Vol. 3, p. 631.
4. Good, L. J., In: *Terpenoids in plants*, (ed.) J. B. Pridham, 1967, p. 159.
5. Chakraborty, T. and Datta, P. C., *J. Sen. Mem. Volume*, 1969, p. 437.
6. Nicholus, H. J., In: *Biogenesis of natural compounds*, (ed.) P. Bermfeld, 1967.

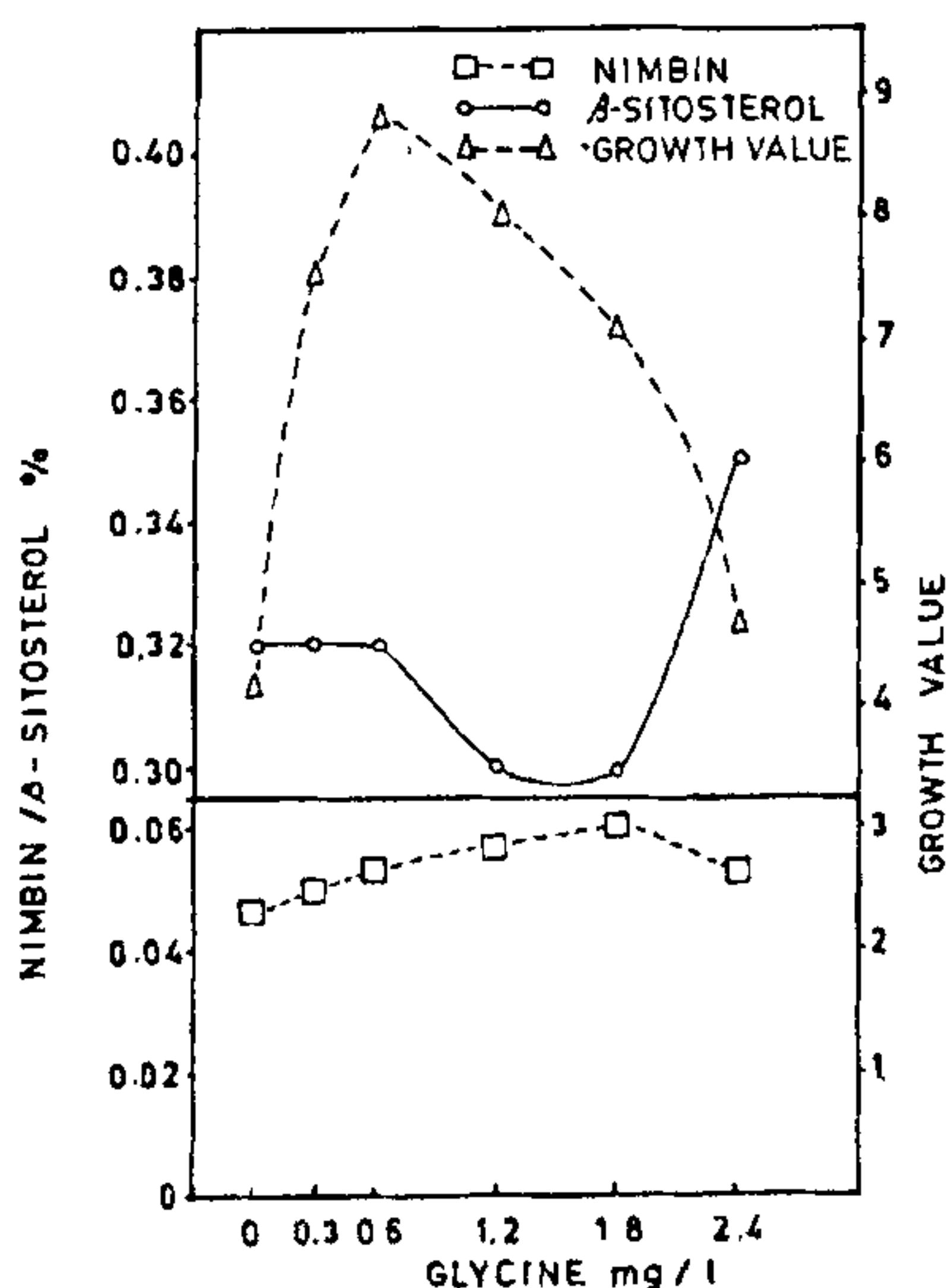


Figure 1. Glycine on nimbin,  $\beta$ -sitosterol and growth value.

## TWO LONG LOST THALLOSE LIVERWORTS RECOLLECTED FROM PINDARI RANGE (WESTERN HIMALAYA)

S. D. TEWARI, L. S. BISHT and GIRIBALA PANT

Department of Botany, D.S.B. Campus, Kumaun University, Nainital 263 002, India.

DURING a bryo-collection trip to Pindari Glacier (alt. 2900 – 4000 m) in Almora District of Kumaun Himalaya, two extremely rare and interesting thallose liverworts viz. *Sauchia spongiosa* Kash. and *Preissia quadrata* (Scop.) Nees were collected in September 1985. Both are restricted to the high altitude regions of Western Himalaya.

1. *Sauchia spongiosa* Kash. (Sauteriaceae), Monotypic endemic: The genus *Sauchia* was first procured and recorded by Kashyap<sup>1</sup> on the basis of collections made from high altitude localities of Middle and Main Himalaya ranging from 2700 to 4350 m (9000–14,500 feet). In 1928, more material was collected and the original description amplified. Since then, no one came across this rare liverwort. This is the first record of this taxon from the Kumaun region of Western Himalaya, which is referred to exist only in literature by Shimizu and Hattori<sup>2</sup>.

The present collection of both sterile and copiously sporiferous plants of *S. spongiosa* (figure 1a–c) was made from moist, grassy alpine soil enroute to Pindari Glacier at Phurkia (alt. 3400 m) and in between Dwali and Phurkia (alt. 2900–3400 m).

Our field observations reveal that this monotype is solely restricted to high altitude. Even at Pindari range, the plants grew in small isolated patches mixed with other hepatics like *Reboulia hemisphaerica*, *Jungermannia* sp., *Lophocolea bidentata* and *Plagiochila* sp. and mosses; *Brachythecium buchananii*, *Mnium lycopodioides* and *Bryum* sp. The plant can be easily recognized by its thick, delicate and sharply spongy texture.

The important taxonomic features are as follows: Plants thick, light-green, delicate and spongy; air

chambers wide and deep in one layer, empty, directed obliquely; pores bounded by cells with thin radial walls; scales hyaline, scattered; monoecious, antheridia on short ventral shoots in mid dorsal cluster, female receptacle stalked (figure 1b); stalk up to 0.7–3.5 cm long, receptacle 4 or 5 lobed; sporogonium with well-developed foot; spores 66–75  $\mu\text{m}$ , winged crenulate, lobate; elaters closely trispiral 200–225  $\mu\text{m}$  long.

2. *Preissia quadrata* (Scop.) Nees (Marchantiaceae): Rare in India, distributed in Western Himalaya, Pangi; Lahul; Baralacha Pass; Kaghan valley, Kinlung; Kashmir; (collected only once since 1929 from Sonmarg, Kashmir), Nepal, Europe, Japan, Siberia and North America. The genus was also recorded by Kashyap<sup>3</sup> and added to the list of Western Himalayan flora. Since then it has never been collected. This is the first record of this rare taxon from the Kumaun region of Western Himalaya after a lapse of 56 years.

The plants were found growing on moist alpine soil/rock together with other liverworts and mosses like *Jungermannia* sp., *Lophocolea cuspidata*, *Brachythecium buchananii*, *Bryum capillare* and *Hyophila involuta*.

The important taxonomic features are as follows: plants pale-green, margin tinged with red-brown to deep-violet turning purplish-brown in older parts of the thallus; thallus 3–3.5 cm long and 1 cm broad, dorsal surface with distinct areolae; epidermal cells 4–6 angled, marginal cells mostly quadrate, thin-walled, angle not thickened; pores small, barrel-shaped as in *Marchantia*, overlapping; ventral scales on either side of the midrib; midrib prominent below, containing brown fibrous cells; male receptacle stalked, stalk up to 1.8 cm long; female receptacle up to 5.5 cm long (figure 1c); receptacle usually 4–5 lobed; spores 65–75  $\mu\text{m}$ , reddish-brown, coarsely reticulate; elaters bi or trispiral, reddish-brown 300–375  $\mu\text{m}$  (these features tally with those given by Kashyap<sup>3</sup>).

It may be added here that at present even Pindari route is not free from biotic disturbances. With the exception of three winter months (November–February) it is under constant pressure of tourists, traffic, shepherds and their cattle. How long will these fragile, small and rare cryptograms be able to withstand the biotic stress is a matter of concern.

Financial assistance from DST, New Delhi is gratefully acknowledged. Thanks are due to Mr M. C. Pargaien for help during collection.

**Figure 1a–c.** a. A vegetative thallus of *Sauchia spongiosa* Kash. ( $\times 5$ ); b. Sporiferous plants of *S. spongiosa* Kash. showing archegoniophores ( $\times 2$ ); c. A thallus of *Preissia quadrata* (Scop.) Nees bearing male and female receptacles ( $\times 1.5$ ).

18 July 1987

1. Kashyap, S. R., *J. Bombay Nat. Hist. Soc.*, 1916, **24**, 347.
2. Shimizu, D. and Hattori, S., *J. Hattori Bot. Lab.*, 1954, No. 12, 53.
3. Kashyap, S. R., *Liverworts of the Western Himalayas and the Punjab Plain*, Part I, Lahore, 1929.

**RESPONSE OF PEARL MILLET (*PENNISETUM AMERICANUM*) TO INOCULATION WITH VESICULAR-ARBUSCULAR MYCORRHIZAE AND *AZOSPIRILLUM BRASILENSE* WITH DIFFERENT SOURCES OF PHOSPHORUS**

K. V. B. R. TILAK and C. S. SINGH

*Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110 012, India.*

It has frequently been shown that vesicular-arbuscular mycorrhizal (VAM) plants are much more efficient than non-mycorrhizal plants in utilizing insoluble phosphate fertilizers applied to soil<sup>1-3</sup>. An attempt has been made in the present study to assess the effects of combined inoculation of pearl millet with VAM fungi and *A. brasilense* with single super phosphate and rockphosphate.

A pot culture experiment was conducted using unsterilized farm soil of sandy-loam type from the fields of this Institute, which was highly deficient of

phosphorus content (5 ppm by Olsen's method)<sup>4</sup>. Ten kg of finely processed soil was taken in 30 cm dia pots. Phosphorus was added in the form of rockphosphate and single super phosphate at the rate of 50 kg P<sub>2</sub>O<sub>5</sub>/ha.

The top soil in pots up to a depth of 2-3 cm from 10 kg soil was mixed with 200 ml of soil containing finely chopped heavily infected root segments of the following endophytes, previously raised on cowpea separately as standard host: *Acaulospora* sp., *Gigaspora margarita* and *Glomus fasciculatum*.

The seeds of pearl millet var. BJ 104 were treated with a carrier-based (soil + farm yard manure in equal proportions) inoculant containing highly efficient strains (P-1 and P-2) of *A. brasilense*. The control treatment received neither VAM inoculum nor the seed inoculation with *A. brasilense*.

The treatments were replicated 6 times and the experiment was laid out in a randomized design.

The data on dry matter yield were recorded at 120 days of plant growth. The total phosphorus content in plants was estimated by the vanado-molybdate method after digestion with *tri-acid mixture*<sup>4</sup>. Percentage mycorrhizal infection in roots was determined by the slide technique on the 120th day of the plant growth after clearing the roots in 10% KOH and staining with trypon-blue lactophenon<sup>5</sup>. The percentage root colonization was calculated as follows: (number of VAM positive segments/total number of root segments scored) × 100.

Soil inoculation with VAM fungi in general,

**Table 1** Response of pearl millet to inoculation with *Azospirillum brasilense* and different VA-mycorrhizal fungi with varying sources of phosphorus fertilization (mean of 6 pots—each pot containing 4 plants)

Treatment	Control (0 kg P <sub>2</sub> O <sub>5</sub> /ha)			Single super phosphate (50 kg P <sub>2</sub> O <sub>5</sub> /ha)			Rock phosphate (50 kg P <sub>2</sub> O <sub>5</sub> /ha)		
	1	2	3	1	2	3	1	2	3
Uninoculated control	20	13.3	13.7	42	17.5	20.8	35	15.8	19.5
<i>A. brasilense</i>	35	14.4	20.1	45	20.5	28.2	48	18.0	26.2
<i>Acaulospora</i> sp.	55	15.5	22.5	68	17.6	20.5	65	15.8	24.2
<i>G. margarita</i>	65	16.5	26.0	68	18.5	25.7	62	16.5	22.2
<i>G. fasciculatum</i>	75	18.8	30.0	82	20.5	32.7	75	18.7	26.5
<i>A. brasilense</i> + <i>Acaulospora</i> sp.	62	17.4	25.5	75	19.2	23.5	70	18.5	22.5
<i>A. brasilense</i> + <i>G. margarita</i>	78	19.8	31.5	80	22.5	29.7	80	20.7	26.5
<i>A. brasilense</i> + <i>G. fasciculatum</i>	87	20.6	32.7	92	25.6	32.9	85	22.5	30.8

P = 0.05% - 1 : 10.57; 2 : 3.91; 3 : 7.41.

1. Root colonization by VAM fungi (%); 2. Dry matter yield (g/pot); 3. Total phosphorus uptake by plant (mg/plant).