

characters of the male parent. The reflection of female dominance in the pollen of *Erythrina* is substantiated by both vegetative and floral characters, and it may be presumed that it is a case of maternal inheritance. In an elaborate study of the pollen morphology of *Erythrina*, Graham and Tomb² recorded the occurrence of the increased abortion and multiaperturate grains as a result of hybridation, although the present study does not provide sufficient evidence in that direction.

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REPORT OF TWO NATURAL ENEMIES OF *SPIRODELA POLYRHIZA* (L.) SCHLEID

THE menace of water weeds is reaching alarming proportions in specially tropical countries. Duckweeds are next important aquatic weeds after waterhyacinth. Amongst duckweeds, *Spirodela polyrhiza* (L.) Schleid is one of the most vigorously growing plant on the earth (National Academy of Sciences¹). The chemical method cannot be adopted for its control because effective chemicals cause many harmful affects in aquatic ecosystem. No natural enemies of *S. polyrhiza* have been reported uptill now.

During the present study, two potent natural enemies, viz., *Lymnaea luteola* f. *impura* (Troschel), a snail and *Bagous* sp., a coleoptral insect were found. They were found naturally in rare water-bodies of Gorakhpur. Both the enemies were found to show their maximum feeding capacity during January and February, which is also their maximum population period.

Lymnaea luteola f. *impura* (Troschel) is a snail of family Lymnaeidae, found in organically rich water-bodies. They were found to scrap the fleshy thallus of *S. polyrhiza*, causing permanent injury, which finally leads to disintegration of thallus very quickly. It was found that 50 starved individuals can cause death of 25 gm of *S. polyrhiza* in 7 days. *L. luteola* is also

not known to serve as an intermediate host for any trematode causing diseases in human beings.

Bagous sp. is small semi-aquatic insect of family Curculionidae. The insect generally makes a small hole on the thallus of *S. polyrhiza*. Sometimes, the process is so frequent that they cause complete death of the plant. It was found that 100 individuals can cause 30% loss of fresh-weight in 7 days. The insect has been found most active during morning hours. Both the natural enemies were found to co-exist.

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PRODUCTION OF AMINO ACIDS IN *EPHEDRA FOLIATA* SUSPENSION CULTURES

THE role of amino acids in the biosynthesis of secondary plant products has been speculated¹⁻⁴. There is little information regarding the quantitative estimation of amino acids from plant tissue cultures, although amino acids have been reported from *Datura stramonium*,⁴ *D. metel*, *D. tatula*, *Momordica charantia*, *Trigonella foenum-graecum*,⁵ *Ginkgo* species,⁶ *Sesamum indicum*, *Tephrosia purpurea* and *T. vogelii*⁷. The present investigation deals with the production and estimation of amino acids in *Ephedra foliata* Boiss., suspension cultures.

Sterilized stem pieces of *E. foliata* were inoculated in 100 ml flasks containing 30 ml of revised⁸ Murashige and Skoog's⁹ medium (RT) supplemented with 1 ppm of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 1% agar. The tissue was grown and maintained for a period of twelve months as static cultures after frequent subculturing of 6-8 weeks in fresh RT medium. The static tissue was then transferred to RT liquid medium supplemented with 0.1 ppm of 2, 4-D. Suspension cultures were grown on rotary shaker for a period of five months. Three and six weeks old tissues were harvested separately and growth indices calculated (GI = Final fresh weight of tissue—Initial fresh weight of tissue/Initial fresh weight).

For the extraction of free amino acids the fresh tissue was homogenized in a Waring Blendor in 90% ethanol (1 gm/5 ml). Each of the homogenized tissue was separately centrifuged for 30 min and the residue washed three times with 90% ethanol. The

supernatants were separately mixed with chloroform (1 : 3) and aqueous layer concentrated *in vacuo*.

For bound amino acids the residual tissue was hydrolysed with 7% sulphuric acid for 24 hr at room temperature. The mixture was filtered and dried under reduced pressure. Both free and bound amino acid fractions were dissolved in 50% ethanol before application.

The isolated fractions were separately applied on Whatman No. 1 filter paper strips along with 21 reference standard amino acids by using *n*-butanol-acetic acid-water; 60 : 20 : 20 in one dimension and phenol-acetic acid-water; 74 : 1 : 19.2 in the second dimension. Chromatograms were developed at 90° C for 5 min after spraying with 0.25% (wt./vol.) ninhydrin in acetone. The ninhydrin positive spots were eluted⁵⁻⁷ in 90% ethanol and read in Spectronic 20 (Bausch & Lomb) colorimeter at 400 nm. Different amino acids were identified with reference to authentic samples of amino acids.

The maximum (8.0) growth index was observed in six weeks old tissue rather than three weeks old tissue (4.0). Eleven free and ten bound amino acids were detected; of them one remained unidentified (Rf 0.7). The total amino acid concentration was more in six weeks old cultures (Table I). Glutamic

acid in free form and arginine in bound form were comparatively higher in concentration whereas leucine and serine had lower concentration. Arginine only in bound form and lysine and valine in free form were present.

Tulecke and Rutner¹⁰ have reported that the presence of amino acids in the tissue was due to biochemical reactions at the cell surface whereas Khanna and Jain⁷ and Khanna and Nag⁵ reported it to be due to biochemical reaction and preferential synthesis of particular amino acid under the described conditions of cultivation of tissues. The latter view also suggests that the concentration of amino acids varies with the type and age of tissue. The wide variation in the individual and total amino acid content can be attributed to the biosynthesis of other primary and secondary products during the growth of the tissue.

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TABLE I

Amino acid composition of Ephedra foliata Boiss., suspension cultures

Amino acid	Concentration (mg/g.d.w.) ^a			
	(Three weeks old)		(Six weeks old)	
	Free	Bound	Free	Bound
L-Alanine	0.62	0.35	0.90	0.83
L-Arginine	..	1.15	..	3.00
L-Aspartic acid	3.00	0.68	7.04	2.50
L-Cystine	1.94	1.74	1.20	2.94
L-Glutamic acid	1.13	0.85	7.57	2.04
Glycine	0.17	0.42	0.76	0.45
L-Leucine	1.38	0.73	0.42	0.33
L-Lycine	1.10	..	1.14	..
L-Phenylalanine	1.75	0.45	0.90	2.65
L-Serine	0.10	0.10	0.45	0.21
L-Valine	0.38	..	0.53	..
TOTAL	11.57	6.47	20.91	14.95

^a Milligram per gram dry weight (Data based on 5 chromatographs of each sample).

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ON THE OCCURRENCE OF THE NEMATODE *ASPICULURIS LAHORICA*

Aspiculuris laborica Akhtar¹ is recorded for the first time from India. The worms were collected from the colon and the rectum part of the alimentary canal of the mouse, *Mus musculus*, at Jodhpur (Rajasthan).