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EFFECT OF PHENAZINE METHOSULPHATE ON COLOUR DEVELOPMENT IN NITRATE REDUCTASE ASSAY IN RICE SEEDLINGS (*ORYZA SATIVA* L)

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THE most sensitive method of assaying the assimilatory nitrate reductase (Enzyme code 1.6.6.1, NADH: nitrate oxido-reductase) (NR) in the plant extracts is by colorimetric determination of nitrite[1], the product of the assay. The cofactor nicotinamide adenine dinucleotide reduced (NADH), when present in excess in the assay mixture, interferes with the diazotization of the nitrite[2,3] and leads to under-estimation of NR activity.

Several methods for the removal of the residual NADH prior to colour development have been documented[3–6]. Zinc acetate-ethanol treatment increased the recovery of the nitrite[2,7,8] but not effective[3] in removing the NADH from the assay mixture. However, phenazine methosulphate (PMS) rapidly oxidized the excess of NADH[9] and hence, successfully employed[10] in the post-assay treatment of NR. Whether PMS used in conjunction with zinc acetate or zinc acetate-ethanol post-assay treatment, improved nitrite recovery in the NR assay, has been investigated in 5-day-old shoots of Taichung Native 1 (T(N)1) and Mashuri (Ponni) rice varieties grown in culture solutions[11] containing 84 μg/ml nitrate-N.

Nitrate reductase activity from crude extracts of shoots[11] was determined by adding 0.1 ml of 1 M zinc acetate followed by 1.9 ml of 70% (V/V) cold ethanol (zinc acetate-ethanol post-assay treatment) or PMS (15 nmoles/ml of reaction mixture[10]) (zinc acetate—PMS post-assay treatment) to the assay mixture[11]. Reaction tubes with cold ethanol were allowed to stand for 20 min at 0°C, while tubes with PMS were kept for 20 min at 30°C. Precipitate, if any, was removed by centrifugation. Reference tubes containing boiled leaf extracts served as controls. In suitable aliquots of the assay mixture, the quantity of nitrite formed was determined colorimetrically by adding sulphanilamide-N-1-naphthyl-ethylene diamine reagent and measuring the absorbance at 540 nm. Nitrate reductase activity was expressed in nmoles of nitrite formed per 15 min per mg protein[12].

Addition of zinc acetate enhanced NR activity by improving the nitrite colour development, but it could remove only a portion of the residual NADH that interfered with the colour development[3] (table 1). Pre-colour treatment with zinc acetate-ethanol resulted in slightly higher value for the recovery of the nitrite. The recovery was twice when PMS was used in conjunction with zinc acetate (table 1).

Table 1 *Elimination of NADH and other factor(s) that interfere with NO_2^- colour development in the in vitro NR assay in shoots of T(N) 1 and Ponni rice seedlings*

Nitrate reductase assay (*In vitro*)	NR activity	
	nmoles NO_2^- formed/15 min/mg protein	
Pre-colour treatment	T(N)1	Ponni
Standard assay (reference)	105.0	53.3
Zinc acetate	120.0†	69.5*
Zinc acetate + Ethanol	123.0†	73.5*
Zinc acetate + PMS	126.5*	78.6*

†Insignificant at 5% level; *Significant at 5% level.

Addition of cold ethanol following zinc acetate eliminated the excess of NADH from the assay mixture[2,7,8] to certain extent only. But, PMS added to assay mixture following zinc acetate and allowed to react for 20 min at 30°C was more effective in totally oxidizing the excess of NADH and the results are statistically significant (table 1). Further, the presence of zinc acetate in no way interfered with the ability of PMS to completely oxidize the NADH and the effects of PMS and zinc acetate on the colour development were independent[10].

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PTEROPHYLLUM MEDLICOTTIANUM FROM THE GANGAPUR FORMATION OF ANDHRA PRADESH

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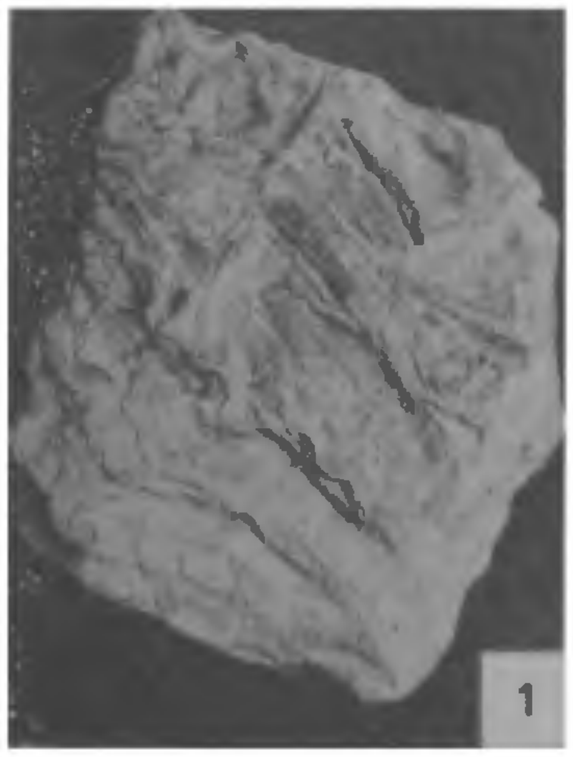
THE paper records for the first time the occurrence of *Pterophyllum medlicottianum* in the Lower Cretaceous (Neocomian–Aptian) Gangapur Formation of the Pranhita – Godavari basin, Andhra Pradesh. The fossil specimen has been collected from the Gangapur sediments near Anksapur (19°21′ N: 79°25′ E) on the left bank of Peddavagu in the Asifabad Taluk of Adilabad District.

The genus *Pterophyllum* referable to Cycadopsida, is a pinnately compound leaf with pinnae having almost parallel margins attached laterally on the rachis by their full width and showing parallel venation with local forking of veins.

Genus: *Pterophyllum* Brongniart 1828

Pterophyllum medlicottianum Oldham and Morris 1863 (*figures 1 and 2*)

Description: Leaf Pinnately compound, 11 cm long, rachis 4 mm wide, longitudinally striated, pinnae opposite, linear straight or slightly falcate, 8.5 cm long, 1.8 cm width, attached laterally on rachis by their full width, margin entire, veins many, 4–7, parallel, locally forked.



Figures 1 and 2. *Pterophyllum medlicottianum*. **1.** (× ¼). **2.** (× ⅓).