The present fossil wood in having concentric rows of gum canals belongs to the 1st group. The present fossil wood shows close resemblance to *Pentacme*[1–3] and within the genus *P. suavis* and *P. mindamensis* come closest to the fossil wood[4].

As far as the authors are aware no petrified fossil wood of *Pentacme* of the family Dipterocarpaceae has been reported so far. This is the first record of this genus from Mio-Pliocene Formations of Siwaliks from Kalagarh. It is, named as *Pentacmeoxylon ornatum* gen. et sp. nov.

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LIMITATIONS IN NITROGEN FIXING ABILITY OF RHIZOBIA IN HETEROLOGOUS LEGUME HOSTS

The root callus-*Rhizobium* association studies from several laboratories have shown that the nitrogen fixing ability of rhizobia can be expressed in calli of non-leguminous plants[1,2]. The rhizobial specificity for nodulation in legumes therefore, appears to be only at the level of infection which incidentally, forms the basis for taxonomic grouping of the root nodule bacteria. Recently Johnston *et al.*[3] have reported that the bacterial factors determining the host specificity can be manipulated to develop multiple host infective rhizobial strains. In legume-*Rhizobium* symbiosis, the problem however, is not of host infectivity but of symbiotic effectivity—the ability to fix atmospheric nitrogen. It is well known that the native rhizobia infecting a single host species show wide variations in their nitrogen fixing capacities in the same host. Whether a strain developed for multiple host infectivity character will be effective in nitrogen fixing ability with all the hosts, should be a matter of prime consideration. Using effective strains from different cross inoculation groups of *Rhizobium* and calli of cow pea miscellany hosts for *in vitro* association, we have found that the effectiveness of the strains in heterologous systems is drastically reduced. The multiple host infective strains, therefore, may not be of much importance unless the genes for nitrogen fixation are also manipulated so that they function with equal efficiency.

Studies were conducted in root calli of two cow pea miscellany hosts-green gram (*Vigna radiata* var. *aureus*) and chick pea (*Cicer arietinum* L.). The green gram is a symbiotically promiscuous host that can be nodulated by different groups of rhizobia while the chick pea is symbiotically a very specific host nodulated with its own rhizobia[4]. The various *Rhizobium* strains used are *R. japonicum* SB16 (*glycine max*), *R. lupini* 3001 (*lupinus*), *R. meliloti* Su216 (*Medicago sativa*), *R. leguminosarum* P2 (*Pisum sativum*), *R. trifolii* T19 (*Trifolium alexandrinum*) *R.* spp. cow pea miscellany—M1, S24, M8 (*Vigna radiata* var. *aureus*), Ca121 and Ca181 (*Cicer arietinum*). These strains were isolated from the nodules of the hosts mentioned in the parenthesis and were maintained on yeast extract mannitol agar slopes[5]. Nodulation and nitrogen fixation efficiency of the various *Rhizobium* strains in homologous hosts was tested under aseptic conditions in sterilized Leonard jars using acid washed sand and Sloger's nitrogen free medium[6].

Table I shows that all strains nodulated their respective hosts and fixed nitrogen efficiently except M8 of *V. radiata* which was infective but ineffective. The nodule nitrogenase activity was comparable with nitrogen ratio in most of the host systems except Lucerne and *Trifolium*. In these hosts the nodule mass was comparatively less but the nitrogenase activity per unit weight of nodule was high suggesting that it could be the property of the symbiosis in these hosts.

The root callus of green gram cultivar (Cv) T51 was developed on Phillips medium[8] and that of Chick pea Cv C235 on Gamborg's B5 medium[9] containing 0·75% agar and pH adjusted to 6·5. Surface sterilized seeds of these two Cvs were germinated on 1% agar in petri plates at 28° C. After 72 h, 2 to 5 mm long root bits from epical region were transferred aseptically on respective callus medium slopes in tubes (15 cm × 2·5 cm dia) and incubated at 25° C. Sufficient callus growth was observed in the tubes after 20 to 25 d which was subcultured on fresh medium slopes. The rhizobia were inoculated in 15 d old solid calli (0·5 to 0·8 g callus/tube) by stabbing 3 to 7 d old cultures of rhizobia from yeast extract mannitol agar slopes with a straight needle. The rhizobia were allowed to establish in the growing calli for 5 d and then transferred to slopes of SCN agar ndium[8] in 30 ml vials. After 7 d growth on SCN medium, the cotton plugs of the vials were replaced with serum stoppers and the C_2H_2 reduction activity of the calli was determined gas chromatographically.

Table II shows nitrogenase activity of cow pea group rhizobia in root calli of green gram and chick pea. In homologous host callus, the M1 and S24 strains of green gram and Ca121 and Ca181 of chick pea showed very high activity. The ineffective

but infective strain M8 of green gram showed very low activity in callus of its homologus host indicating that the root callus nitrogenase activities could be compared with nitrogen fixing capacity of these strains. In heterologous associations, the strains although expressed nitrogenase, the values were low and comparable with ineffective strain M8.

TABLE I

Efficiency of different Rhizobium *strains on their homologous hosts*

Rhizobium strain	Test host	Efficiency (av. value 9 pl.) No. of nodules/ plant	Nitrogen ratio	Nodule N_2ase activity
R. japonicum				
SB16	soybean	20	3·42	3142
R. lupini 3001	lupin	15	2·31	3410
R. meliloti				
Su216	lucerne	18	3·72	32100
R. leguminosarum				
P2	pea	149	2·04	2881
R trifolii T19	berseem	23	3·89	28800
R. spp. M1	green gram	30	2·01	2960
S24	do.	32	1·81	3567
M8	do.	41	1·08	940
Ca121	chick pea	23	2·24	2485
Ca181	do.	19	2·11	2204

Surface sterilized seeds of the test hosts were inoculated with respective *Rhizobium* suspension from yeast extract mannitol agar slopes and sown in Leonard jars in triplicate Uninoculated seeds were sown as controls. The jars were kept in a net house and plants uprooted after 30 to 50 d of growth depending on the host plant Nodules were counted and nodule nitrogenase activity was determined gas chromatographically by C_2H_2 reduction technique[7] Nitrogen content in dried plants was determined by Kjeldahl method. Nitrogen ratio being calculated as nitrogen uptake/plant in treated divided by nitrogen uptake/plant in control. Nodule nitrogenase activity is expressed as nM C_2H_2 reduced h^{-1} g^{-1} fresh weight of callus.

The establishment of the rhizobia in calli was confirmed by examining microscopically and by dilution plating of all these calli after the nitrogenase assay. Microscopic studies showed that the rhizobia, irrespective of host affinity, established themselves in intracellular spaces with the same density. In most of the cases bacteria were also observed inside the callus cells. Dilution plating of the root calli revealed a total count between $3·5 \times 10^{10}$ to 8×10^{10} bacteria/g fresh wt. of calli. From this we conclude that the low nitrogenase activity in heterologous associations was not due to a failure of establishment of the bacteria.

TABLE II

Nitrogenase activity of cow pea miscellany rhizobia in root calli of green gram and chickpea

Rhizobium strain	Specific activity in callus: green gram	chick pea
M1	11·71±0·76	5·29±0·43
S24	13·77±0·83	6·04±0·41
M8	4·06±0·26	3·09±0·37
Ca121	2·52±0·18	19·61±0·24
Ca181	2·37±0·33	19·55±0·27
Endogenous	0·21±0 01	0 22±0·01

Specific activity is expressed as nM C_2H_2 reduced h^{-1} g^{-1} fresh weight of calli. The activity was determined by incubating the calli in an atmosphere of air : C_2H_2 (9 : 1) for 4 h at 28° C. The calli after assay were removed from agar surface to record fresh weight. These were then examined microscopically and plated for viable bacterial count to confirm *Rhizobium* establishment. The plus-minus (±) values indicate standard error of replication mean.

TABLE III

Nitrogenase activity of rhizobia from different cross inoculation species in association with root calli of green gram and chick pea

Rhizobium strain	Specific activity: green gram	chick pea
R. japonicum SB16	5·38±0·24	4·34±0·21
R. lupini 3001	4·01±0·06	4·12±0·14
R. meliloti Su216	2·70±0·08	6·05±0·13
R. leguminosarum P2	3·32±0·16	2·19±0·14
R trifolii T19	3·29±0·19	2·98±0·14
Endogenous	0·21±0·02	0·23±0·03

Specific activity is expressed as nM C_2H_2 reduced h^{-1} g^{-1} fresh weight of calli.

The nitrogenase activity of the effective *Rhizobium* strains from different cross inoculation species in calli of these two cow pea hosts is shown in Table III. The values in all the cases are comparable to those of

inefficient strain M8. The endogenous values for C_2H_2 reduction of calli were ten times less than the lowest activity observed with any bacterial association, indicating that the values are not influenced by the endogenous C_2H_2 reduction or C_2H_4 production activity of the calli. These studies suggest that it is not only the infectivity for which a strain of *Rhizobium* differs but the nitrogen fixing ability is also associated with the host it recognises for nodulation. A strain manipulated for cross infectivity therefore, will not be efficient in all the hosts infected.

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REGENERATION OF RHIZOME SEGMENTS OF *REGNELLIDIUM DIPHYLLUM* LINDM. IN ASEPTIC CULTURES

DURING the recent comparative *in vitro* studies on a few Phyletically unrelated ferns (both homo- and heterosporous), *Regnellidium diphyllum* has proved to be an excellent experimental material for morphogenetic studies[1].

Regnellidium diphyllum, the monotypic Brazilian genus belonging to the family Marsiliaceae is an aquatic heterosporous fern with globular sporocarps containing numerous gradate sori. Rhizome segments used as inocula were obtained from sexually reproduced sporophytes raised in sterile cultures on Knop's medium supplemented with 3% sucrose. Equal-sized rhizome segments, with and without nodal parts were cut and inoculated separately in Knop's medium supplemented with 3% sucrose. All the cultures were maintained under 12 h photoperiod at 25° 2° C.

Some variations regarding the activation and growth pattern of rhizome segments with and without nodal part were observed. Segments with nodal part were the first to sprout, while the rhizome segments without nodal part remained quiescent for about two months. Further growth of these segments was interesting. Segments with nodal part, after a few days of inoculation, turned brown. A week later, swelling up of rhizome segments was observed and the first sign of growth was evidenced by the production of multicellular, uniseriate haris. Subsequently, a normal sporophyte was formed from each of these segments.

Segments of the internodal parts behaved quite differently. They turned brown and remained in the same state for two months. Their prolonged cultures resulted in the production of small-sized cylindrical structures herein designated as 'protophylls' and chlorophylous, positively geotropic roots. These 'protophylls' were comprised of compactly arranged sporophytic cells with discoid chloroplasts without any vascular supply and stomata. A large number of multi-cellular, uniseriate hair was seen on the surface of these 'protophylls'.

In the present investigations, it is seen that the regeneration response of the rhizome segments, with and without nodal part intact is different on the same nutrient medium. Rhizome segments with nodal part regenerated whole plants; on the other hand, those without nodal part produced 'protophylls' only. This differential response may be explainable on the basis of the presence-absence of the quiescent bud. Segments with nodal part intact, on coming in contact with the nutrient medium, resumed mitotic activity. As a result, the endogenous auxin level is raised, which in turn, helps in differentiating the whole plant. The rhizome segments without nodal parts, regenerating into whole plant is limited by the low endogenous auxin level and consequently the 'protophylls' are formed.

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BAITED RAGI CULM CULTURE, AN EASY TECHNIQUE FOR MAINTENANCE OF VIRULENCE IN *HELMINTHOSPORIUM NODULOSUM* BERK. & CURT.

REPEATED subculturing and/or their survival in soil saprophytically, resulted in the loss of virulence and subsequent sporulation in *Helminthosporium nodulosum* Berk. and Curt. which causes pre- and post-emergence seedling blight[1] in ragi (*Eleusine coracana* Gaertn.).