

Preparation of complex and competence media for growth and development of competence for *Rhizobium* and intraspecific and interspecific transformation in *Rhizobium* were done as per the method given by Raina and Modi¹. For growing streptomycin resistant mutant and screening transformants, streptomycin was added at the concentration of 1000 $\mu\text{g}^{-\text{ml}}$ to the complex media. DNA was extracted⁶ from *R. cowpea* Str. + grown on complex medium with streptomycin for 24 h. The isolated DNA dissolved in sterile saline citrate (0.15 M NaCl and 0.015 M sodium citrate pH 7.0) and preserved over a layer of chloroform at 0° to 4° C, was used for transformation experiments. The concentration of DNA was estimated by the diphenylamine reaction⁷. For intergeneric transformation, *A. chroococcum* grown on Waksman 77 broth for 45 h was used as the recipient culture. Cells were washed with sterile distilled water and resuspended in fresh medium at a concentration of 10^6 cells $^{-\text{ml}}$. The transformant mixture contained 0.5 ml of this cell suspension and 0.5 ml of donor DNA (50 $\mu\text{g}^{-\text{ml}}$). Recipient cells and DNA were incubated for 30 min at 30° C in a rotary shaker, after which period, DNase (100 μg in 10^{-3} M MgCl_2) was added and further incubated for another 30 min. After suitable dilution, plating was done on Waksman 77 agar medium and incubated for 48h and the colonies were counted. Appropriate platings were done for total count, transformants and controls.

TABLE

Transformation frequency of streptomycin sensitive recipients

Recipient culture	Frequency of Transformation
<i>Rhizobium cowpea</i> Str ⁻	0.04-0.08%
<i>Rhizobium japonicum</i> Str ⁻	0.002-0.006%
<i>Azotobacter chroococcum</i> Str ⁻	0.00001-0.0001%

Donor DNA from *Rhizobium cowpea* Str⁺.

The frequency of intraspecific transformation appears to be the same as that of the one, reported by Raina and Modi for the same marker in *R. japonicum*⁸. The frequency is low in interspecific transfer and it is still lower in the intergeneric transfer (Table). Despite repeated attempts, we could not obtain the high value of frequency reported by Sen *et al.*⁴ for intergeneric transfer of streptomycin resistance between *Rhizobium* and *Azotobacter*. Perhaps the short life of competence and the progressive inactivation of the transformable DNA may be the causes for lower

frequency in intergeneric transformation observed in the present study⁹.

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Tamil Nadu Agricultural
University,
Coimbatore 641 003,
March 9, 1978.

S. SADASIVAM.
S. PANDIAN.

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INDUCTION OF TENDRIL MUTATIONS IN LENTIL (*LENS CULINARIS* MEDIC.)

DRY seeds of the lentil variety L-235 were treated with 6 and 10 kR doses of Co⁶⁰ gamma rays and 0.005 and 0.01% N-nitroso-N-methyl urea (NMU). M₂ progenies were raised from M₁ seeds for screening various mutations. A progeny in 6 kR and 3 progenies in 0.01% NMU treatment segregated for tendril mutations in M₂ generation, which had similar phenotypic expression. The mutation was named as Tendril-1. Another mutation, which was slightly similar to the first one, was isolated when normal M₂ progenies of 0.01% NMU treatment were grown in M₃ generation. This mutation was designated as Tendril-2. The characteristics and the inheritance of the two types of mutations are presented.

Tendril-1: The stem and branches were smooth, wiry and prostrate. The lanceolate leaflets showed modifications for shape, size, number and tendrils. The modified characteristics are not conspicuous at

the initial stages of plant growth and hardly one or two leaves are affected, while gradual increase in the frequency was noticed with the advancement of plant growth. The leaflets get modified into a long tendril (Fig. 1). Tendril mutations were also reported in peas^{1,7}. Occasionally the leaves of the mutant were changed from unipinnate to bipinnate. A spontaneous single recessive gene has been reported to modify the unipinnate leaves into bipinnate structures in gram⁸. Sometimes, the leaflets fused into a funnel-like structure at the base due to inward folding of the basal part and were petiolated. In addition to this, some leaflets are also modified into bifoliate, trifoliate and tetrafoliate structures (Fig. 2), unlike in funnel leaf mutation, already reported in lentil⁵.



FIG. 1. Upper : Development of leaflets modified into tendrils. Lower : Control leaf (left) and of Tendril-1 mutant (right),

All the plants were late flowering and possessed small malformed actinomorphic flowers, normal anthers and pollen grains, but rudimentary gynoecium. There was no pod set and the plants remained green for longer period than control. The mutation had pleiotropic effect. The four mutated M_2 progenies altogether had 76 normal and 8 mutant plants. This indicates a good fit to monohybrid ratio ($\chi^2 = 1.266$; $P > 0.05$). Thus the mutation is controlled by a pair of recessive genes. The gene symbol *Ten* is proposed for this mutation.

Among the several lentil species, only *Lens montretii* (*Lens kotschyana*) and *Lens culinaris* are known to possess tendrils^{2,6}. The tendril in *Lens culinaris*

whenever present, is solitary and always in apical position. The tendrils in the wild species *Lens montretii* are frequently branched. The mutation displays changes beyond the limits of the species, more towards *Lens montretii*. Consequently the mutant is totally sterile. This is one of the examples of the interspecific or trans-specific mutations described previously in other crops^{3,4}.



FIG. 2. Modifications of leaflets in the mutation Tendril-1.



FIG. 3. Leaf and pods of control (left) and the Tendril-2 mutant (right).

Tendril-2 : In this mutation, the terminal two or three leaflets get modified into tendril-like structures (Fig. 3). Other distinguishing characteristics between these two tendril mutations along with the parental variety are presented in Table I. The mutation causes less drastic change within the limits of the species *Lens culinaris* and the fertility is reduced only partially. The mode of segregation suggested single recessive gene control of the mutation.

These two induced mutations are of great interest and are reported for the first time in *Lens*. They are important from the phylogenetic and evolutionary point of view. Their allelic interrelationship, however, remains to be determined.

TABLE I
Characteristics of the parental variety L-235, Tendril-1 and Tendril-2 mutations

Character	Parent variety (L-235)	Tendril-1	Tendril-2
Height (cm)	33.8±0.39	25.4±0.31	28.5±0.34
Stem	Normal	Thin and smooth	Normal
Leaf modifications (unipinnate to bipinnate)	Absent	Present	Absent
Tendril modifications	Absent	Present (any leaflet)	Present (two or three terminal leaflets)
Leaflet modifications	Absent	Present	Absent
Petiolated, funnel-shaped and multifoliate leaflets	Absent	Present	Absent
Pollen grains	Fertile	Fertile	Fertile
Seed set	Normal	Nil	Normal
Pod size	Normal
Seed weight (gm/1000)	21.6	..	20.2

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Division of Genetics,
Indian Agricultural Research
Institute,
New Delhi 110 012, March 15, 1978.

S. K. SHARMA.*
B. SHARMA.

* Present address: Central Potato Research Station, Kufri, Simla 171 006.

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LIGHT MICROSCOPIC DETECTION OF MYCOPLASMA-LIKE ORGANISM (MLO) IN SESAMUM PHYLLODY

PHYLLODY is a serious and widespread disease of sesamum in India^{1,2}. The disease has been reported from almost all parts of the country where sesamum is grown. The electron microscopic studies of the infected tissues of sesamum have revealed presence of Mycoplasma-like organism (MLO) associated with

phyllody³. Though electron microscopy is the surest method for the detection of these organisms, but, for rapid diagnosis, light microscopy proves better. The disadvantages and importance of light and electron microscopy have been discussed⁴. Light microscopic detection of plant mycoplasma in *Nicotiana tabacum* L. var. *Xanthi* and *Vinca rosea* L. (*Catharanthus roseus*) has been reported⁵.

In this paper we report observations on the light microscopic detection of mycoplasma associated with sesamum phyllody using Feulgen's staining procedure.

Healthy and diseased plant material was collected from experimental plot at University Campus. Stem portions (10 cm) (from the top of the old plants) were used. The stem segments were cut into 0.5 cm pieces and fixed in Helly's fixative, following the method given by Kartha *et al.*¹. Transverse sections (10 μ) were cut with the help of ordinary microtome and stained with basic fuchsin in dark for 1 hour. The sections were examined under phase contrast.

When the sections from diseased plants were compared with those from healthy plants, difference in the staining pattern was observed. The nuclei in both the types were uniformly stained light purple; in the case of diseased material, groups of stained bodies of different shapes and forms were observed in the phloem elements (Figs. 1, 2). Since the phloem elements in the diseased plants show an intense stain reaction it is assumed to have been attributed by the stained DNA of the mycoplasma bodies. The presence of these types of bodies in the infected tissues of sesamum has been confirmed by electron microscopy³.