

A) Chromosomes of *Rattus rattus* (female) $2n = 38$. B) Karyogram of *Rattus rattus* (male) $2n = 38$.

Italian^{4,5} shows that the karyotype under study constitutes an intermediate condition and there is an inverse relationship of the number of biarmed chromosomes to the number of acrocentrics. Further the chromosomes of Mysore population differs from the standard type (Dr. T. C. Hsu, personal commun., July 1971) with $2n = 42$ in a) absence of 4 pairs of acrocentric chromosomes, b) absence of 1 pair of submetacentric chromosomes and c) presence of 2 pairs of metacentric and 1 pair of large subtelocentric chromosomes. While structural polymorphism exists in different populations, it is the authors' considered opinion that Robertsonian centric fusion and centric dissociation might have played their role in reducing the number from 42 to 38 in Mysore populations.

Résumé. Contribution à l'étude du polymorphisme chromosomique chez le rat noir *Rattus rattus* L.

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Evidence for a Transmissible Substance Affecting Pigment Synthesis in *Pisum*¹

Among the numerous chlorophyll mutants of *Pisum*, *alt* is especially distinctive²⁻⁵. Homozygous recessive plants (*alt/alt*) exhibit normal pigment development until they reach the 5 or 6 node stage; then above a rather sharply defined zone of transition the tissue is bleached white (Figure 1), further growth is checked, and the mutant plants die without producing seeds.

In order to investigate the nature of the mutant we first performed grafting experiments using *alt/alt* and normal (*Alt/Alt*) plants as partners. The graft partners were placed in contact after removing a thin tangential slice of nodal tissue from the normal (*Alt/-*) donor plant as well as from the recipient *alt/alt* plant. The site of the graft for the recipient plant was in the region of normal green tissue, usually the 3rd or 4th vegetative node.

The basal or axillary buds in the first 3-4 nodes of the main stem of the mutants are activated by decapitation or by loss of apical dominance following the death of the apical meristem, but such branches - which are chlorotic even though they arise from the region of the plant which is green - soon die. Successful graft transmission of an active substance resulted in the formation of chlorophyll in the basal or axillary branches of the mutant.

These branches ultimately produced flowers, fruits, and seeds. Seeds produced in this manner on *alt/alt* tissue, when planted, all gave rise to plants with the mutant phenotype.

The mutant expression was attenuated by low light intensity (< 500 ft-c) in a growth chamber. The leaves above node 6 or 7 were only partially chlorotic and sufficient pigment was present to sustain weak growth, the plants eventually reaching the fruiting stage of development but dying before mature, viable seeds were formed.

We next attempted to extract, isolate, and chemically identify the graft-transmissible substance present in normal plants but deficient in the mutant. For this a bioassay was devised (Figure 2). Mutant plants were decap-

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itated 1 cm above the 3rd node and a glass tube (6 mm OD by 10 cm) was placed over the cut end. The tube was sealed to the stem with paraffin film, attached to a support and partially filled with 0.3 ml of 1.5% agar. Then 0.5 ml of sample was placed in the tube. The treated and control (distilled water) plants were examined for activity 10 days later. A single application of the most active extracts led

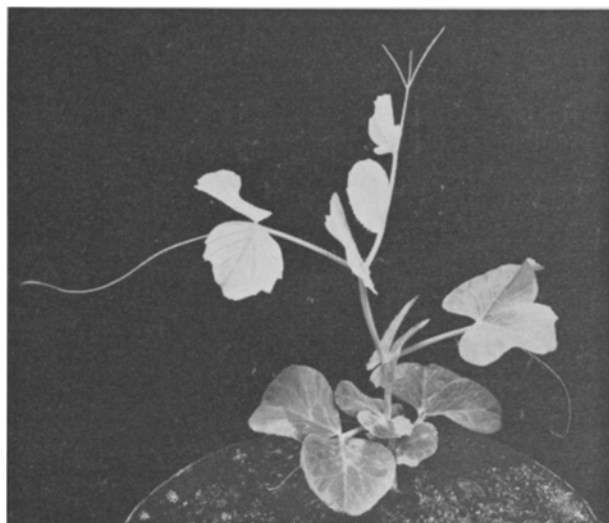


Fig. 1. Mutant *alt/alt* plant showing chlorosis of tissue appearing at and above the 5th node.

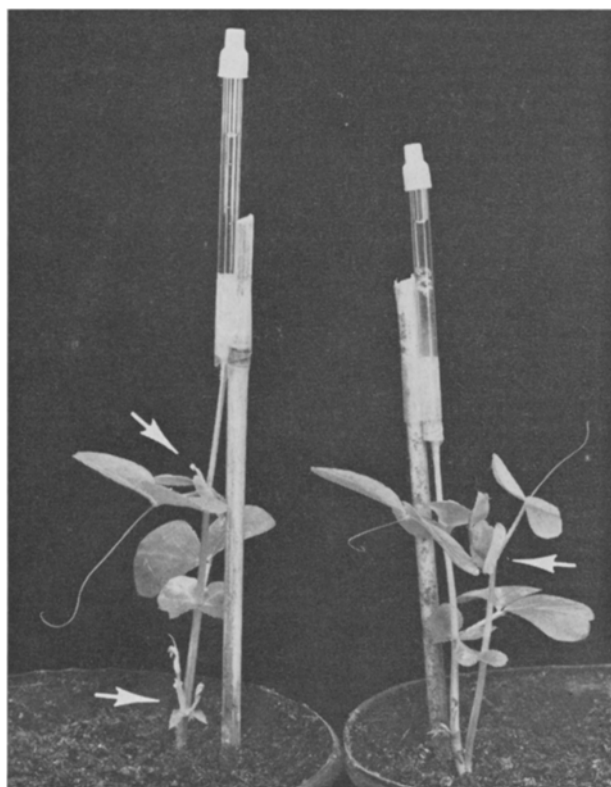


Fig. 2. Bioassay setup. Plant on right, supplied with crude extract, shows basal shoot with near-normal development, whereas control plant (left), supplied with H_2O only, shows 3 activated but undeveloped shoots. Photo taken 9 days after application.

to the production of shoots bearing flowers and small fruits but without mature, viable seeds.

A crude extract of pea seeds was prepared as follows: 1,300 g of dried seeds were soaked in 2 l of water for 16 to 18 h. An equal volume of acetone (2 l) was added and the peas were homogenized in a blender and enough 1:1 v/v acetone water solution was added to keep the homogenate liquid. This material was filtered through shark skin paper and the filtrate was extracted twice with an equal volume of petroleum ether. The aqueous phase was concentrated under vacuum at 45°C to 500 ml. This crude extract was active in the bioassay even after 6 months storage at -13°C. The results indicate that hydrated seeds of normal plants contain one or more chemicals which compensate, at least in part, for the deficiency caused by the gene.

Further studies of the crude extract revealed the following: 1. Passage of the crude extract through a column of strong cation exchange resin (Dowex 50, hydrogen form) removed all the activity of the extract. 2. However, passage of the extract through a column of an anion exchange resin Dowex 3 (OH^- form) reduced slightly but did not completely remove the activity. 3. The ash of this crude extract was inactive even when combined with the extract which was passed through the cation exchange resin. 4. The activity was removed when the extract was passed through the cation exchange resin and then through the anion exchange resin or vice versa. These results indicate that the principle in the crude extract is a cation and it is neither a metal ion nor a complex between a metal ion and an anion.

No activity was recovered when the resin was eluted with 0.5 N HCl. However, activity was partially recovered when the resin was eluted with a stronger acid (6 N HCl). The extracts were adjusted to pH 7 with NaOH or with Dowex 1 (OH^- form).

These results suggest that the active principle is a strong base. The cytokinins are characteristically strong bases⁶. However, zeatin, kinetin, and N^6 benzyladenine were inactive in the bioassay, as was a number of naturally occurring substances including 18 amino acids, 10 carbohydrates, and 15 vitamins and minerals. Clearly, the mutant lacks a translocatable substance present in normal peas which either controls or is a metabolic intermediate in the elaboration of the photosynthetic pigments. Further work is in progress to isolate and chemically identify this active principle.

Zusammenfassung. *Alt*-rezessive Erbsenpflanzen zeigen als Keimlinge normale Pigmentbildung, werden aber oberhalb des 5. oder 6. Knotens chlorotisch und sterben vor der Samenreife ab. Aufpfropfen des Wildtyps und Zugabe eines Extraktes regenerieren die Pigmentbildung in der Mutante, was auf die Anwesenheit einer übertragbaren, stark basischen Substanz schliessen lässt.

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