

escent supernatant on 7 $\frac{1}{2}$ % polyacrylamide gels. Gels were stained using standard enzyme incubation mixtures⁹ and Coomassie Blue for total protein.

The isoenzyme patterns of 5 enzyme systems plus total extractable protein were studied from friable callus material, haploid plant leaves and diploid plant leaves. Only cytologically confirmed haploids were examined. In all cases protein and enzyme patterns of callus material were identical to that from haploid plant material.

The patterns of glutamate and lactate dehydrogenases and esterase were monomorphic in haploid plants and consisted of a single band at R_F ($\times 100$) values of 21, 04 and 55 respectively. These results are identical to the patterns for these enzymes from diploid leaf material.

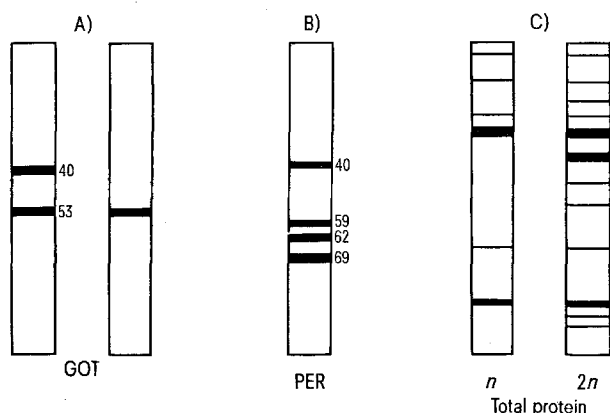
Glutamate oxaloacetate transaminase (GOT) patterns exhibited a polymorphism in haploid plants identical to that present in natural, diploid plant populations¹⁰ (Figure A). The occurrence of 2 bands in the GOT pattern of a haploid plant suggests that this enzyme is programmed by 2 loci (rather than multiple alleles at a single locus). These loci are designated GOT-1 (R_F 40) and GOT-2 (R_F 53). This interpretation is supported by the observa-

tion that a single-banded pattern at R_F 40 has never been detected in naturally occurring, diploid populations despite extensive sampling. GOT-2 appears monomorphic, whereas GOT-1 must possess a null allele in order to permit the occurrence of the single-banded R_F 53 pattern.

Peroxidase (PER) patterns of haploid plant leaves consist of 4 bands (Figure B). This pattern is identical with the root tissue isoperoxidase pattern from diploid plants¹⁰. Diploid plant leaf tissue characteristically lacks the PER band at R_F 40. These results would appear to reflect the loss of some tissue-specific regulatory mechanism in the haploid plant. These results also suggest the presence of a minimum of 4 isoperoxidase loci in this taxon.

The total protein banding pattern of haploid plants lacks one major and several minor bands which occur in the pattern of diploid plants (Figure C). Since the genetic interpretation of the complex, total protein band pattern is unknown, it can only be presumed that the loss of these several bands reflects the loss of potential for heterozygosity at several loci in the haploid organism.

The absence of any unique isoenzyme patterns in haploid plants may be explained in 2 ways: 1. any potential isoenzymically unique, recessive mutant may be lethal and therefore lost at the early plantlet states of culturing or 2. there may be little genetic variation (a low percentage of heterozygosity) in natural populations of this taxon. The former possibility cannot be rigorously tested at present, but preliminary results in our laboratory (unpublished) suggest that the latter possibility is accurate. Polymorphism has been identified in only 1 locus (GOT-1) out of the 9 loci examined. This percentage (11%) of polymorphic loci is considerably lower than that found for other organisms (ranging from 26 to 55%)^{11,12} and suggests that *Datura meteloides* may be somewhat depauperate genetically a situation which may be related to its 'weedy' biological character.



Isoenzyme patterns observed in haploid *Datura meteloides*. A) glutamate oxaloacetate transaminase and B) peroxidase. C) Total protein patterns observed in haploid and diploid plants. R_F values $\times 100$.

⁹ C. R. SHAW and R. PRASAD, *Biochem. Genet.* 4, 297 (1970).

¹⁰ R. L. SCOGIN, *Aliso* 8, 275 (1975).

¹¹ F. R. AYALA, J. R. POWELL, M. I. TRACEY, C. A. MOURAO and S. PEREZ-SALAS, *Genetics* 70, 113 (1972).

¹² D. A. LEVIN, G. P. HOWLAND and E. STEINER, *Proc. natn. Acad. Sci., USA* 69, 1475 (1972).

Mechanism of in vitro Gall Induction in *Zizyphus jujuba* Lamk.

P. TANDON, G. S. VYAS and H. C. ARYA

Plant Tissue Culture Laboratory, Department of Botany, University of Jodhpur, Jodhpur 342 001 (India), 16 July 1975.

Summary. *Zizyphus jujuba* Lamk. stem galls incited by *Eriophyes cernuus* Massee were induced aseptically on stem segments cultured on auxin and kinetin-free modified MURASHIGE and SKOOG's nutrient medium by 1. gall callus graft, 2. gall tissue extract and 3. incorporation of NAA into the medium.

Non-self-limiting tumor tissue in plants with etiological factors like bacteria, viruses and genetic constitution are on record as having tumefacient properties^{1,2}. Insect and mite-induced galls are reported to be self-limiting. Their transplantability and tumefacient properties are unknown. In our studies we have shown that gall induced by *Eriophyes cernuus* Massee (a mite belonging to family Eriophyidae) on *Zizyphus jujuba* Lamk. is of non-self-limiting type and the tissues possess tumefacient properties. This is probably the first report of such a phenomenon, and has significance for better understanding of abnormal growth in plants.

Zizyphus seedlings were raised aseptically from surface sterilized seeds and grown on auxin, kinetin-free MURASHIGE and SKOOG's³ (MS) medium in Erlenmeyer flasks at $26 \pm 2^\circ\text{C}$ maintaining 55% relative humidity and 1,000 lux light. Experimental material consisted of stem segments obtained after removing roots from 20-25-day-old seedling. Control comprised: stem segments planted

¹ A. C. BRAUN, in *Plant Physiology* (Ed. F. C. STEWARD; Academic Press, New York 1969), p. 379.

² A. C. BRAUN and T. STONIER, in *Protoplasmatologia* (Eds. L. V. HEILBRUNN and F. WEBER; (Springer-Verlag, Wien 1958), p. 93.

³ T. MURASHIGE and F. SKOOG, *Physiologia plant.* 15, 473 (1962).

'Gall' induction

Experimental treatment	No. of replicates	No. of 'galls' induced	Period of initiation (days)	Average size (mm)	Shape	Colour	Position of 'gall' on the stem segment
Gall callus graft	96	16	7-10	2.5	Spherical	Shining white	At the site of graft and above the graft
Normal callus graft	96	—	—	—	—	—	—
Gall extract in the medium (%)							
1.25	96	—	—	—	—	—	—
2.50	96	—	—	—	—	—	—
6.25	96	64	20-25	2.7	Lobed	Dull white	On the segment just above the surface of the medium and at the top.
12.25	96	96	20-25	3.4	Lobed	Dull white	On the segment just above the surface of the medium and at the top.
NAA (ppm)							
1.0	96	—	—	—	—	—	—
2.0	96	—	—	—	—	—	—
5.0	96	96	18-20	6.5	Spherical	White	At the base, at the internode and at the top.
10.0	96	96	18-20	6.5	Spherical	White	At the base, at the internode and at the top.
20.0	96	96	18-20	6.5	Spherical	White	At the base, at the internode and at the top.
Control	96	—	—	—	—	—	—

vertically with morphological basal end thrust 5-8 mm in the auxin, kinetin-free MS medium at pH 6.

The treatment given to this set of controls consisted of a) 20-day-old 200 mg, sterile gall and normal tissues grown on MS medium transplanted separately on the first node of the stem segment touching simultaneously the surface of the medium, b) stem segments grown on the control medium containing 1. 1.25 to 12.5% cold sterilized gall extract (prepared by homogenizing 1 kg fresh galls from *Z. jujuba* in 100 ml of phosphate buffer at pH 6 and sterilized by passing through bacteriological filter) 2. 1.0-20.0 ppm naphthaleneacetic acid (NAA).

Results obtained are given in the Table. With gall callus graft, 'galls' in the form of undifferentiated cell mass appeared after 7 days on the stem segment. In 2 weeks 'galls' showed 2.5 mm diameter growth and appeared white, compact and ill-defined in shape (Figure 1). During this period, the gall tissue graft being in contact

with the medium also showed growth. Normal callus graft induced no 'galls'. The grafted tissue turned brown and failed to grow. Gall tissue extract (6.25 and 12.5%) incorporated in the medium induced 'gall' development on the stem segment in 20 days (Figure 2) 2.7 mm in diameter which enlarged in another fortnight to 3.4 mm. They were dull white, irregular and lobed. NAA (5.0, 10.0 and 20.0 ppm) induced 'galls' on stem segment after 20 days which were 6.5 mm in diameter, white and compact.

'Galls' induced by gall callus graft, gall tissue extract and NAA were isolated and cultured on auxin, kinetin-free MS medium. Since they do not require any exogenous supply of auxin for growth, they resemble gall tissues, whereas normal tissue failed to grow on auxin, kinetin-free MS medium. The cultures obtained from different treatments are maintained for further investigations to elucidate the problem of abnormal growth in plants

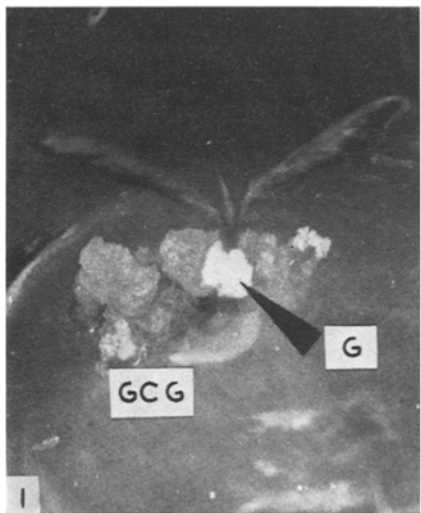


Fig. 1. Stem segment with gall callus graft (GCG) showing shining white 'gall' (G).



Fig. 2. Stem segment showing 'gall' (G) at the top under the effect of gall tissue extract in the medium.