

regime should induce a modulation of the activity pattern (shape of oscillation) by advancing the maximum and giving rise to a steeper increase of activity. Thus, bimodal activity patterns of the alternans-type⁷ may finally change into the bigeminus-type. Since this model deduction has scarcely been tested on an experimental basis, we carried out experiments with 5 species of bats.

Species used for activity measurements under different light-darkness conditions were *Myotis myotis* ($n = 9$; duration of each registration period $n' = 10$ days), *Rousettus aegyptiacus* ($n = 5$; $n' = 20$), *Phyllostomus discolor* ($n = 4$; $n' = 10$), *Molossus ater* and *Molossus molossus* ($n = 3$; $n' = 12-17$ days). In order to determine the gross locomotory activity of the bats, we used an electroacoustic registration equipment according to BAY⁸, climbing bars mounted upon micro-switches for *Rousettus* respectively, and an ELMG multi-channel digital printer. Every hour compiled activity data were calculated as percentage of the respective total amount of activity per day. Thus, it was possible to determine comparable general activity patterns according to different strengths of Zeitgeber.

All 5 Chiroptera-species tested show modulations of the activity pattern caused by different Zeitgeber conditions, as predicted by WEVER^{4,5}. The activity pattern of *Phyllostomus discolor* (Figure 1), recorded at 3 different Zeitgeber conditions, serves as an example showing the deformation of a unimodal pattern. The activity pattern of this bat is nearly symmetric when there is only a little difference between L (25 lx) and D (10^{-2} lx). With increasing strength of Zeitgeber, the maximum is evidently advanced, combined with a steeper increase of activity at the beginning of the dark period. The unimodal activity pattern of the African fruit bat, *Rousettus aegyptiacus*, also yields similar tendencies of pattern deformation.

The bimodal activity patterns of the neotropical *Molossus* species are affected by the increase of range of L:D oscillation, so that there is a gradual decrease of the second maximum, which appears just before the end of the dark period, and an increasing accentuation of the first maximum. This is illustrated for *M. molossus* in Figure 1, B. Whereas there is no complete change from alternans type to bigeminus type in the activity pattern of this species, *Molossus ater* clearly changes its activity pattern to bigeminus type. *Myotis myotis* advances the second maximum of its bigeminus pattern, appearing in the second third of the dark period with increasing L:D-intensity ratio. The main maximum, appearing just after beginning of the dark period, reaches the highest level at the greatest Zeitgeber strength.

There are also changes of the activity pattern if the average intensity of illumination of the LD 12:12 is reduced by decreased brightness during the L-phase (Figure 2). *M. molossus* shows an activity pattern of alternans type in LD 51: 4×10^{-4} lx, and an activity pattern of bigeminus type in LD 3×10^{-2} : 4×10^{-4} lx.

These results, though in remarkable agreement with the very explicit deductions of WEVER's oscillator model, cannot be taken as general proof for this model. Competing models⁶, however, have to be tested whether they are able to predict similar Zeitgeber-induced modulations of activity patterns. The results indicate in addition that 'the activity pattern' of a species does not exist. There are only patterns of species and individuals under certain conditions^{9,10}. In conclusion it is essential to have the same methods of registration and comparable Zeitgeber conditions in order to compare different activity patterns.

⁷ J. ASCHOFF, *Naturwissenschaften* 44, 361 (1957).

⁸ F. A. BAY, unpubl. Diss., Tübingen 1975.

⁹ J. ASCHOFF and K. HONMA, *Z. vergl. Physiol.* 42, 383 (1959).

¹⁰ H. G. ERKERT, *Oecologia* 14, 269 (1974).

Isoenzyme Patterns in Androgenic, Haploid *Datura meteloides* (Solanaceae)

R. SCOGIN

Rancho Santa Ana Botanic Garden, Claremont (California 91711, USA), 18 November 1975.

Summary. Leaf tissue isoenzyme patterns of androgenically derived, haploid plants of *Datura meteloides* were compared with patterns from diploid parental plants. No isoenzyme patterns unique to the haploid plants were detected among 5 enzymes studied.

Haploid angiosperm plants have been observed to occur sporadically through accident or experimental design¹; but only recently has tissue culturing of androgenic plants provided a consistent source of experimental, haploid angiosperm material^{2,3}. The potential utility of this technique for isolating auxotrophic angiosperm mutants, generating uniformly homozygous breeding stocks and identifying useful somatic genetic markers was quickly appreciated. Some of these prospects have already been realized; e.g., regeneration of diploid tobacco from colchicine treated, androgenically derived cells⁴ and the identification of malate dehydrogenase as a useful marker in somatic cell genetics⁵. The present report is a genetic comparison of androgenically derived haploid plants with their diploid parental stocks utilizing selected isoenzymes as genetic markers.

Anthers from plants of *Datura meteloides* A. DC. occurring naturally at the Rancho Santa Ana Botanic Garden, Claremont, CA, were aseptically cultured using reported procedures^{6,7}. The ploidy level of putative haploids was

established by chromosome counts of root tip cells (with the result that $2n = 12$) and by measuring stomate dimensions on haploid and parental plants, a parameter known to be sensitive to ploidy level⁸. The stomate dimension ratio of diploid to putative haploid plants was 1.4. Samples for electrophoresis were prepared by grinding fresh leaf material in distilled water, clearing by centrifugation ($5000 \times g$) and electrophoresing the opal-

¹ G. KIMBER and R. RILEY, *Bot. Rev.* 29, 480 (1963).

² J. P. NITSCH and C. NITSCH, *Science* 163, 85 (1969).

³ S. NARAYANSWAMY and L. P. CHANDY, *Ann. Bot., Lond* 35, 525 (1971).

⁴ L. G. BURK, G. R. GWYNN and J. F. CHAPLIN, *J. Heredity* 63, 355 (1972).

⁵ P. S. GANAPATHY and J. G. SCANDALIOS, *J. Heredity* 64, 186 (1973).

⁶ J. P. NITSCH, *Phytomorphology* 11, 2 (1961).

⁷ K. C. ENGRILD, I. LINDE-LAURSEN and A. LUNDQVIST, *Hereditas* 72, 331 (1972).

⁸ A. F. BLAKESLEE, *Am. Nat.* 75, 117 (1941).

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.

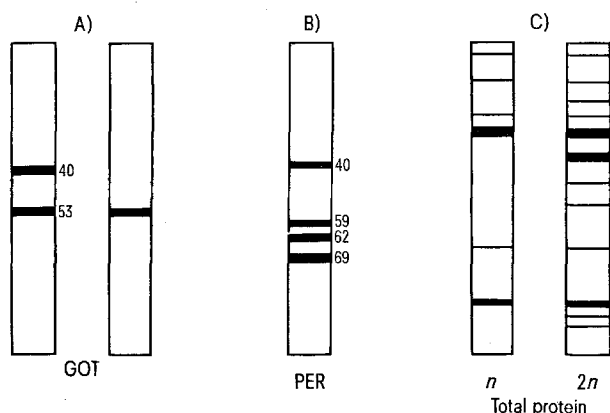
Glutamate oxaloacetate transaminase (GOT) patterns exhibited a polymorphism in haploid plants identical to that present in natural, diploid plant populations¹⁰ (Figure A). The occurrence of 2 bands in the GOT pattern of a haploid plant suggests that this enzyme is programmed by 2 loci (rather than multiple 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).