

described in the table. I found that crossing-over takes place in all 3 chromosomes and all possible recombinants were produced. The frequencies of recombination in the hybrid females are not significantly different ($p > 0.30$) from the ones observed by Sturtevant⁴ in *D. simulans* females heterozygous for the same genetic markers. In addition, the banding pattern of the polytene chromosomes of *D. simulans* and *D. mauritiana* is homosequential in both species⁵. Therefore, it seems reasonable to assume that the frequency of crossing-over in hybrid females *simulans-mauritiana* and in *D. simulans* females might be the same for any chromosomal interval. The existence of crossing-over in the female hybrids *simulans-mauritiana* might furnish an experimental system for studying some specific problems, such as: How many genes are responsible for the male hybrid sterility? Where are they located in the genome? Also evolutionary questions of a more general kind can be studied in those hybrids, e.g. how much genetic information can we transfer from one species into another, and what is the effect on viability of such recombinant hybrids, which carry a certain part of the genome of one species in combination with a majority of genes from the other species.

The *simulans-mauritiana* hybrid males of the first generation are sterile, but after 3 generations of backcrossing the female hybrids with *D. mauritiana* males, I was able to get fertile males which had, in large part, a *mauritiana* genome, in combination with the yellow marker mutation or the white marker mutation from the parental *D. simulans* females ($y w$: yellow and white). It was possible to construct stocks from such males.

Recently, the potential of hybrids for studying regulation of gene expression during development has been pointed out⁶. It has been demonstrated, by means of interspecific hybrids between some species of Hawaiian *Drosophilidae*^{6,7}, that cis-acting and trans-acting genetic elements control the pattern of tissue specificity for certain enzymes. However, the mapping of such elements, as well as an estimate of their number could not be done, due to the sterility of the hybrids. Backcrosses between the female hybrids *simulans-mauritiana* with the parental species might constitute a useful tool for such a genetic analysis, and at the same time might yield information about the evolution of regulatory genetic systems.

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Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*¹

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Summary. Hypocotyls, cotyledons and leaves from 6 cultivars of *Solanum melongena* were cultured to induce callus formation and subsequently plantlet regeneration. Differences were observed among explants and cultivars. These differences were explained assuming that the explants reached different degrees of dedifferentiation in callus persisting also after numerous subcultures.

Regeneration of plantlets by cell cultures is a major goal when the interest is focused upon the genetic aspects. Since the initial reports on this subject^{3,4}, a great deal of work on regeneration has been devoted to finding an equilibrium between the components of the medium. Numerous studies

indicate that it is difficult to make extensive generalizations; in fact, an inductive treatment developed for a particular culture is not necessarily successful in other cultures. Consequently there is no doubt that organogenesis in vitro depends on a complex system of endogenous and

Table 1. Responses in callus induction from hypocotyl, cotyledon and leaf explants to different auxins; observations after 2 weeks

| Media | Hypocotyl | Cotyledon | Leaf |
|--------------------|--|--|---|
| LS+ 2,4-D 0.4 mg/l | Soft yellowish friable callus | Scarce development of yellowish friable callus | Scarce development of slightly compact callus |
| LS+ 2,4-D 2 mg/l | Scarce callus development | Scarce callus development and root formation | Scarce callus growth and profuse roots |
| LS+ NAA 1 mg/l | Friable callus, occasional development of roots and leaf regeneration from explant | Scarce callus production and occasional development of roots | Nodular callus and many hairy roots |
| LS+ IAA 2 mg/l | Rapid development of callus and roots | Rapid development of callus and roots | Rapid development of callus and roots |

exogenous interacting factors^{5,6}. For a better understanding of genetic and developmental control of differentiation we have cultured tissues from different explants of 6 cultivars of *Solanum melongena* and studied the regeneration pattern in subsequent subcultures.

Materials and methods. We tested 6 commercial cultivars of *Solanum melongena* from the 'Laboratorio analisi sementi' of the Institute of Agronomy of the University of Bologna: 3 inbred lines designated as B, E, I, and 3 hybrids C, D, R. Seeds were surface sterilized in a solution of Na hypochlorite 5% (w/v) for 30 min, rinsed 3 times in sterile water and germinated on a filter paper bridge soaked in LS medium without hormones⁷. From 20–30-day-old seedlings, hypocotyls, cotyledons and the first 2 leaves were excised and cut into small pieces (1–3 mg fresh weight) which were placed horizontally on 20 ml of LS culture medium solidified with 0.8% agar. Auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), indoleacetic acid (IAA) and naphthaleneacetic acid (NAA) were added to induce callus formation. For plantlet regeneration, 3 media were tested: LS without auxin, LS+gibberellin (GA₃) 2 mg/l, and LS+kinetin, 2 mg/l.

Callus cultures were held at 25 °C in the dark, while to obtain regeneration of plantlets callus was cultured under 4000 lx with 16/8 h light/dark cycles.

Results and discussion. The addition of different hormones to LS medium determined a similar response in the 6 cultivars while within each of them callus induction varied in the 3 explants (hypocotyl, cotyledon and leaf) in response to different media (table 1). The observations after 2 weeks of culture indicated that the addition of 0.4 mg/l 2,4-D to LS medium was optimal for callus induction. At this time callus was separated from the explant and subcultured on fresh medium of the same composition which appeared optimal also for maintaining callus growth.

When green spots appeared on the surface of the callus mass they were transferred on to media without auxin (LS). The addition of kinetin induced the formation of plantlets with compact nodular callus at the base, whereas in LS+GA₃ the plants had very thin shoots. The best result was given by the medium LS without hormones. In table 2 data are reported showing the response of cultivars to this medium; it was observed that they exhibited a different regenerative potential. In particular, hybrids showed a higher organogenetic potential than inbred lines; in fact, plants were obtained from the callus of all 3 hybrid explants. A recent study (unpublished data) carried out on 3 inbred lines and their hybrids demonstrated that genes with dominance and overdominance control the regenera-

tive potential. Our findings are in accordance with the conclusion by other authors who, studying organogenesis in alfalfa⁸ and in *Solanum*⁹ argued that the regenerative potential is under genetic control. No difference among cultivars was observed in regard to the chromosome number of plantlets regenerated: a preliminary analysis showed that they are mostly diploid, rarely aneuploid and polyploid.

Differences in regenerative potential were observed among the calluses from the 3 explants: in fact the results reported in table 2 indicate a higher regenerative potential in callus derived from leaf and cotyledon than in the callus from hypocotyl. The former explants are also able to maintain their potential through numerous subcultures. To give an explanation for the different regenerative potential exhibited by calluses isolated from different organs we must previously consider that: a) shoot formation is generally typical of cultures that are freshly isolated from hypocotyl and leaf segments⁶; b) different endogenous growth regulators may have an important role in regenerative ability. In previous work¹⁰ an isozymatic analysis of peroxidase patterns confirmed that developmental differences among organized tissues used as explants persist also in vitro during successive subcultures among calluses from various original tissues.

Taking into account the results as a whole we advance the hypothesis that different cell populations (explants) during callus establishment and successive subculturing, may keep the ability of producing peculiar substances concerning with differentiation; that is, the cell populations derived from the 3 explants retain a different gene expression in spite of the dedifferentiation process. The differences among tissues and cultivars we observed during successive subculturing should be investigated in the near future, also in relation to the chromosome number in the callus. There is still disagreement about the causes producing the decrease of organogenetic potential during subculturing; in fact the view that there is a correlation between cytogenetic variability and loss of morphogenetic capacity, advanced by several authors^{11–13} is contrasted by the view that epigenetic factors may cause a decrease in differentiation¹⁴.

Table 2. Plantlet regeneration in the calluses of three explants from 6 cultivars; medium LS without hormones

| Cultivar | Hypocotyl | Cotyledon | Leaf |
|----------|-----------|-----------|------|
| Inbreds | | | |
| B | — | ++ | + |
| E | — | + | ++ |
| I | — | ++ | + |
| Hybrids | | | |
| C | ++ | ++ | ++ |
| D | . | . | . |
| R | + | ++ | ++ |

—, No regeneration;

+, regeneration from the earlier subcultures;

++, regeneration also from the later subcultures;

., pieces of green callus.

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