Table 3. Chromosome damage induced by *Paxillus* extracts in *Nigella* dry seeds, origin: lawn (200 metaphases analyzed in each case; small letters indicate independent experiments). 1) Extract at 20 °C; 2) heated; 3) evaporated.

| Treatment | | Chromosome aberrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Breaks (deletions) | Minutes | Gaps | Dicentrics | Rings | Total |
| 1 | a | 10 | 1 | 0 | 1 | 0 | 12 |
| | b | 3 | 0 | 1 | 0 | 0 | 4 |
| | Total | 13 | 1 | 1 | 1 | 0 | 16 |
| 2 | a | 8 | 1 | 0 | 3 | 0 | 12 |
| | b | 3 | 1 | 1 | 0 | 0 | 5 |
| | Total | 11 | 2 | 1 | 3 | 0 | 17 |
| 3 | | 3 | 2 | 1 | 3 | 1 | 10 |
| Control | | 1 | 0 | 0 | 0 | 0 | 1 |

few isochromatid gaps, which can be interpreted as prophasic lesions, all aberrations are of the chromosome type, i.e. induced in the $G_1$ phase, with a large proportion of chromosome deletions versus interchanges and intrachanges. No aberrations of the chromatid type were observed. The possibility of the accumulation of toxic and mutagenic/carcinogenic substances from parasitic or saprophytic fungi can be generally ruled out by careful examination of the cuticles, and also by the fact that only young carpophores were harvested.

There is also a possibility that the radioactivity accumulated in mushrooms a long time after the Tchernobyl disaster is responsible for the clastogenic effects. The radioactivity was measured several times in various mushroom species, including *Paxillus involutus*[10]. It never exceeded $50 \times 10^3$ Bq/kg dry weight for $Cs^{134}$ and $Cs^{137}$, and 2200 for $K^{40}$. These levels, although significantly increased, are far too low to explain the effect[9] ($1 \text{ Bq} = 27 \times 10^{-12}$ Ci). The variability of the response within the same habitat raises the problem of a possible metabolic transformation by mushroom tissues of a promutagen into an ultimate mutagen. Up to now, the nature of the substance(s) responsible for the chromosome damage remains unknown, and it should be isolated and identified in further research.

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## An evaluation of a somaclone of *Dioscorea floribunda* Mart & Gall

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*Summary.* 150 plants of *D. floribunda* representing a single clone were regenerated from a stem tissue culture and regenerants were subjected to cytological, phenotypic and biochemical analysis from the pre-transfer stage to three vegetative growth cycles in the field. The plants could be subdivided into three cytological categories, namely, diploid, mosaic and tetraploid. Diploids, mosaics and the one tetraploid showed diversity amongst themselves with respect to internode length, content of chlorophyll and diosgenin. No marked difference in the length and nature of the leaf or in the type of stoma was recorded. Possible causes of the observed variation are discussed.
*Key words. D. floribunda*; in vitro regeneration; somaclonal variation; diosgenin; mixoploidy.

Several plant species have been investigated for variability or stability following in vitro regeneration[1-7]. In *Dioscorea floribunda* Mart & Gall, yielding diosgenin, regeneration has also been obtained[8-10]. No attempt has, however, been made to analyse the regenerants cytologically, phenotypically and biochemically and hence the present study was undertaken.

*Materials and methods*

Plants of *D. floribunda* were regenerated from stem callus, originating from one source plant, on three media – 1) Murashige and Skoog's basal medium (MS)[11] supplemented with 4 mg/l of 2,4-D and 2 mg/l of BAP, 2) the same, supplemented with 4 mg/l of NAA and 2 mg/l of BAP and 3) modified White's medium (MWM)[12] supplemented with 4 mg/l of NAA, 3 mg/l of 2,4-D and 2 mg/l of BAP as reported earlier[10]. Regenerated plants with roots were transferred to pots after rhizome development, and thereafter to the field. Thirty-five such plants grown in the field for three vegetative growth cycles were utilized for this study.

A study of the following parameters was made starting from the pre-transfer stage and continuing for three vegetative growth cycles in the field. The parameters studied were: internode length, leaf size (maximum length), chlorophyll content, shape and number of stomata, flowering, somatic chromosome number and diosgenin content per plant. Each plant was subjected to analysis every year and the standard deviation was calculated for each parameter from ten measurements per year.

For the study of somatic chromosome numbers, pretreatment of shoot tips with paradichlorobenzene:oxyquinoline (1:1) followed by acetic ethanol (1:3) fixation and aceto-orcein staining was applied[13].

Analysis of diosgenin was made using thin layer chromatography followed by quantitative estimation at 486 nm in a spectrophotometer using a standard curve[14]. Chlorophyll content of each plant was measured in the leaves of the same stem nodes following the method by Arnon[15].

*Results and discussion*

*1. Number of chromosomes of source plant.* The shoot tip and the first 2–3 nodes from the apex of the source plant revealed cells with variable chromosome numbers. However, 70–80% of the cells contained 36 chromosomes, which is the diploid number of the plant ($2n = 36$)[16]. The remaining 20–30% of cells, where the number could be counted, had complements with 32, 34, 38 and even 72 chromosomes.

*2. Number of chromosomes of the regenerants.* A high degree of variation of the number of chromosomes of the


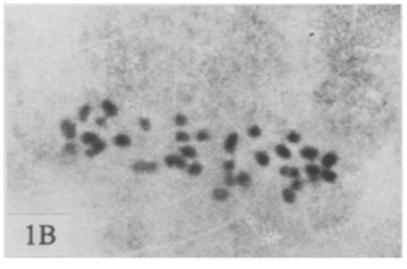


Figure 1. Mosaic regenerant and mitotic metaphase configuration from shoot tip of the same plant showing $2n = 36$.


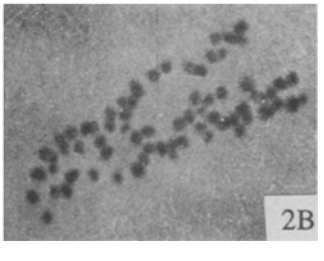


Figure 2. Tetraploid regenerant (a) and mitotic metaphase configuration from shoot tip of the same plant showing $4n = 72$ (b) chromosomes.

regenerants of a single clone was recorded. Out of such alterations, three categories could be delineated, namely diploids, mixoploids and tetraploids. The diploids had the highest frequency. Out of 150 plants regenerated, so far 35 have been analysed. Of these, 26 were diploid with 36 chromosomes in 90–95% cells, 8 were mixoploid with 36 chromosomes in 50–60% cells, and the rest with either 40, 52, and 72 chromosomes. There was one tetraploid plant with 72 chromosomes in 80% of the cells. The remaining cells had a variable chromosome number (figs 1 and 2). Among the mixoploids, there was a gradual shift towards the increase of diploids with age, especially in the third vegetative growth cycle. Clones of the three distinct cytological categories were further analysed in respect of other parameters.

*3. Length of the internode.* The internodal lengths from the 12th to the 20th node of the apex were measured. The diploids revealed a wide range of variation, that is, 0.5–15 cm compared to the parental range of 8–12 cm. The mixoploids and tetraploid, too, showed more or less similar variations. Only one mixoploid clone had very small internodes 0.5–4 ± 0.5 cm, and this plant acquired a trailing rather than climbing habit during first year of growth in the field (fig. 3). The climbing habit was, however, gradually restored in the field at a later stage.

*4. Leaf size.* The diploids, the mixoploids and the tetraploid were more or less homogeneous in respect of this character, measured on leaves from the 12th to 20th nodes from the apex. The range of variation was between 8.0 ± 2.1 and 15.2 ± 1.3 cm, which is the same as that of the parent plant. The leaf tip was acute and leaf spotting was absent in a few diploids (figs 4, 5 and 6).

*5. Leaf colour and content of chlorophyll.* All regenerated plants had dark green leaves except a few which had yellowish-green leaves. The chlorophyll content showed variability, but this was not necessarily correlated with diploidy, mosaicism and tetraploidy. In the diploids, there was a range of 0.46–0.65 µg chlorophyll/g fresh weight of leaves as compared to the parental range of 0.5 ± 0.05 µg chlorophyll/g fresh tissue. The tetraploid resembled diploids in the chlorophyll content. Mosaics showed a range between 0.4 and 0.51 µg chlorophyll/g fresh tissue (fig. 7).

*6. Stomatal characteristics.* The regenerated plants of all three categories had 16–21 stoma/mm$^2$ of leaves. The guard cells were elliptical and there were 8–9 plastids in each of them. However, two diploids showed an in-

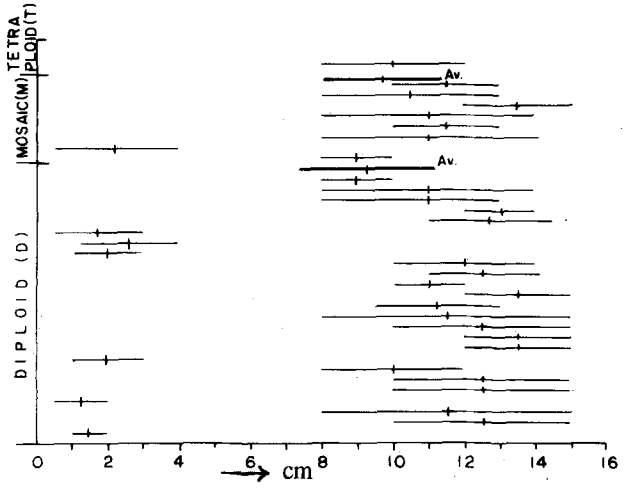


Figure 3. Average and variation of internode lengths in plants of groups with different ploidy at the third vegetative growth cycle in the field.

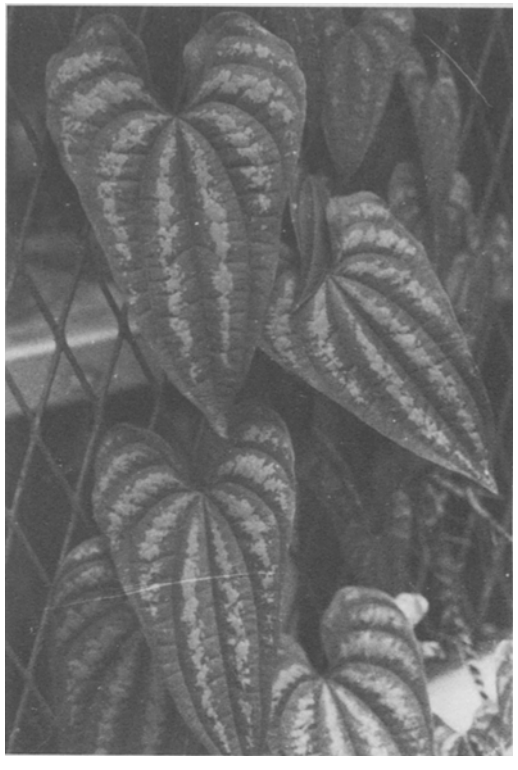

Figure 4. Leaves of diploid regenerants in the field with normal white spotting.

Figure 5. Leaves of diploid regenerants in the field without white spots.

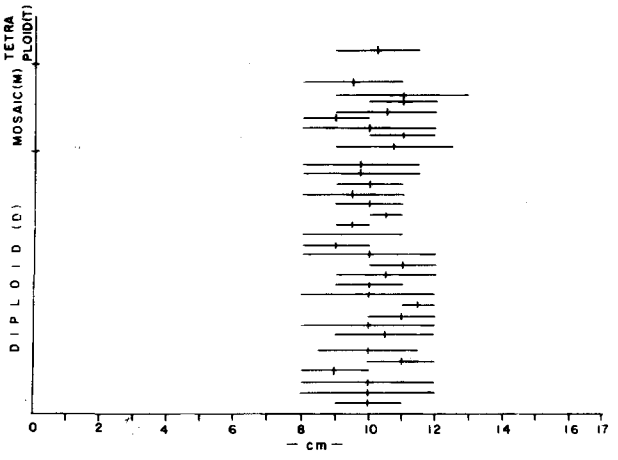


Figure 6. Average leaf lengths and their variation in plants of groups with different ploidy at the third vegetative growth cycle in the field.

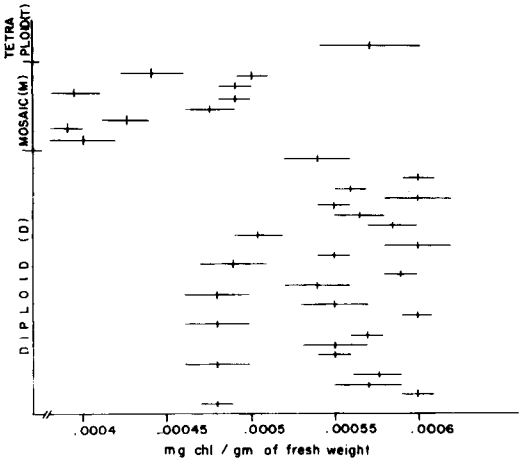


Figure 7. Chlorophyll contents with variation in plants of groups with different ploidy level at the third vegetative growth cycle in the field.

creased stomatal frequency of 27–28/mm$^2$ with round guard cells. This was evident in all three vegetative growth cycles in the field (figs 8 A and 8 B).

*7. Flowering.* Out of 150 regenerated plants transferred to the field, only two diploid individuals produced flowers in the third vegetative growth cycle. Flowers were female and without viable seeds. The original plant was also female.

*8. Diosgenin content.* The amount of diosgenin was different in 10 regenerated plants, and there were also differences in content between the source and the regenerants. The regenerants differed from each other in the amount of diosgenin irrespective of the chromosome number, in all three vegetative growth cycles (fig. 9). The content of diosgenin was 3.8% in one diploid plant as compared to 3.0% in the source plant. The tetraploid plant yielded a low content of diosgenin as compared to that of the diploid in the first vegetative growth cycle. Evidently, increased gene dosage does not necessarily stimulate diosgenin synthesis.

The reproduction of this species is by vegetative means. This precludes the influence of cross-pollination as a source of variation in the regenerants in the field. The vegetative characters and biochemical patterns, as reflected in diosgenin content, show wide variation in different regenerants which have diploid or mosaic chromosome numbers. As such, variability cannot be attributed to any numerical difference of chromosomes. Mosaicism is often considered as a source of variation in the regenerants[4, 17]. But in the present study, mosaics were more or less homogeneous and like the diploids in respect of several characters. Further, there was an increase of diploid cells with increasing age of the mosaics in the field. The characters like suppression of climbing habit at the initial phase with gradual reversion to normalcy may represent some form of physiological or non-genetic variation, as reported in other plant species[4]. The alteration

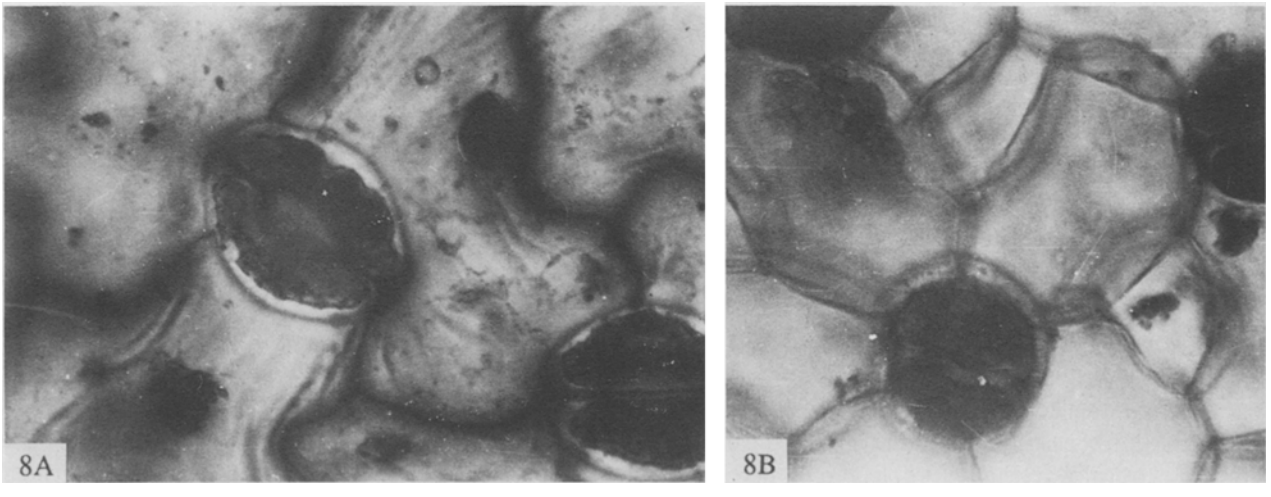


Figure 8. Elongated (a) and round shaped (b) stomata in regenerants.

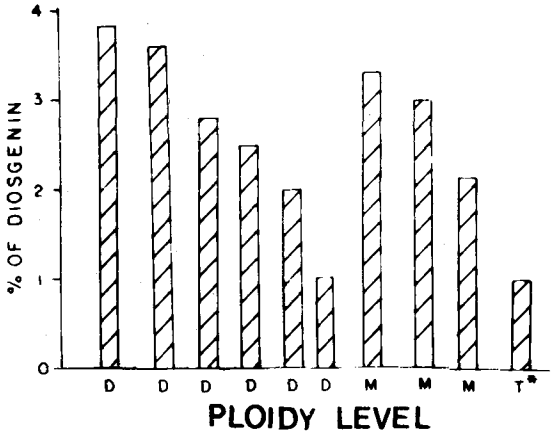


Figure 9. Percentages of diosgenin contents in plants with different ploidy at the third vegetative growth cycle in the field (D = diploid, M = mosaic, T = tetraploid; * represents diosgenin content in the first year of vegetative growth in the field).

in characters, such as the absence of white spotting in leaves or increased stomatal frequency appearing in two diploid individuals, may require further genetic tests to trace their origin.

There are several records of variants amongst regenerants in asexual species, but the genetic nature of such variability has not been fully clarified[4, 18–20]. In the present case, plants of *D. floribunda* were regenerated from stem tissue culture and variability could be documented in regenerants. The observed range of variations offers scope for an exploration of their economic potential.

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## Selection of high-level abamectin resistance from field-collected house flies, *Musca domestica*

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*Summary.* Abamectin is a novel, highly promising insecticide with activity against many pests. To determine if resistance to abamectin could occur, we collected house flies from several New York dairies and selected them in the laboratory. Resistance developed rapidly and to a high level (36 or > 60,000-fold, depending upon test technique and/or adjuvant) that could not be overcome by the synergists piperonyl butoxide or *S,S,S*-tributylphosphorotrithioate. There was no increase in (cross)resistance to crotoxyphos, dichlorvos, dimethoate, tetrachlorvinphos, permethrin, dieldrin or lindane following abamectin selection. Our results suggest the potential for abamectin resistance is high, at least in house flies, and that the judicious use of abamectin will be needed to prolong its usefulness as an insecticide.
*Key words.* Insecticide resistance; abamectin; ivermectin; house fly; selection.

Pesticide resistance is a severe problem that limits our ability to control pests of agricultural and medical importance. Historically, resistance problems have necessitated the use of new compounds, particularly those belonging to new pesticide classes. However, the time taken for resistance to develop to a new insecticide is extremely variable, ranging from only a few applications to decades of use. One of the most important factors that could