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Studies of the effects of toxic preparations of Helminthosporium sativum P.K. and B. on barley and wheat and perspectives of in vitro selection of these cereals

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SUMMARY
Callus, Culture Selection, Barley, Wheat, Helminthosporium sativum, Culture filtrate, Plant regeneration, Wheat embryoid, Mitochondria sensitivity.

The H. sativum filtrate medium inhibits the calli growth of wheat and barley, the root formation of calli and the embryo-survival of wheat, and the isolated mitochondria from calli of barley.

The methodology of these experiments on barley is developed further for an embryogenic strain of wheat.

INTRODUCTION
Helminthosporium sativum P.K. & B. is a major pathogen of cereals in some countries. LUDWIG (1957) has shown that cultures of this fungus contain toxic substances which inhibit the germination of barley seeds.

HARDING (1971) studied the effect of H. sativum on a number of varieties or lines belonging to 14 Triticum species. He found little relationship between aggressiveness of different isolates of the fungus towards wheat or barley seedlings and the level of toxicity exhibited by the corresponding culture filtrates; however a correlation of 0.73 was observed between seedling survival to inoculation by H. sativum and toxicity of the corresponding culture filtrates on Triticum species. It was suggested by LUDWIG (1957) that the toxic principles of culture filtrates of H. sativum affect susceptible hosts by conditioning them to invasion by the fungus.

Toxins present in filtered liquid medium colonized by H. sativum (culture filtrate toxins = CFT) inhibit the germination of barley seeds (LUDWIG, 1957; TANIGUCHI & WHITE, 1967; HARDING, 1971; DUTRECQ, 1977 a) and interfere with the elongation of wheat roots (TYAGI, 1963; HARDING, 1971) or wheat coleoptiles (HARDING, 1971) or with the growth of wheat embryos (DUTRECQ, 1979 c). As roots of barley or wheat were killed when treated for 90 min with CFT (DUTRECQ, 1977 b; DUTRECQ et al., 1978), a test based on root survival was developed with the purpose to evaluate rapidly the toxic potential of a given CFT. Using this test, we have shown a similar sensitivity of either barley or wheat roots to CFT (unpublished data).

HARDING (1971) pointed out the possibility of selecting resistant cultivars by treatment of seeds with the toxins involved. According to KONZAK (1959), the obtaining of induced mutants of cereal might be of interest in this respect. We have developed a similar line of research, by treating tissue cultures with CFT, with the prospect of selecting naturally occurring, or chemically induced mutants or variants. We report here over the problems which were encountered in this study and over the results obtained thus far.

EXPERIMENTS AND RESULTS
Toxic effects of CFT on barley

Using a cell line of barley, type GC1, kindly obtained from A. CATTOIR-REYNAERT (CATTOIR-REYNAERT & JACOBS, 1978), it was shown that CFT inhibits the growth of barley calluses and the multiplication of barley cells (DUTRECQ, 1978).
Figure 1A shows the effect of CFT concentrations on the fresh weight of barley calluses incubated for 1 month on a culture medium containing increasing amounts of toxins. At certain concentrations, a non-lethal inhibition was obtained which reverted when CFT-treated calluses were transferred to toxin-free medium. At some CFT concentration, the inhibition of cell multiplication in liquid medium could also be restored after 15 days, by transferring to normal medium (fig. 2).

The relationships between the results of the root survival test (root index) and either the barley callus growth test (callus index) or the barley cell multiplication test (cell suspension index) for different CFT appear in Table 1.

Effect of H. sativum toxins on barley callus mitochondria

TANIGUCHI & WHITE (1967) reported that helminthosporal, a toxin isolated from culture filtrates of H. sativum (De MAYO et al., 1961 ; SOMMEREYNs & CLOSSET, 1978), is an inhibitor of mitochondrial electron transport and oxidative phosphorylation in either sweet potato or wheat coleoptile.

As a guest in the Laboratory of Enzymology of Prof. GOFFEAU (Louvain-la-Neuve, Belgium), we have analyzed the action of the toxins of H. sativum on the respiration of mitochondria isolated from barley calluses (fig. 3). A partially purified CFT (TP), kindly received from Dr. BRQUET, was obtained by distillation of diethyl-ether extract from CFT ; the distillate was markedly enriched in helminthosporal and other UV-absorbing compounds, as revealed by thin layer chromatography (HUTSCHEMAKERS, personal communication) ; it inhibited the oxidation of both α-ketoglutarate and succinate by mitochondria prepared from barley calluses, type GC1.

Toxic effects of CFT on wheat calluses

The effect of CFT was assayed on primary or secondary wheat calluses and on wheat embryos.
Primary calluses (2-3 sub-cultures) formed numerous roots in the absence of 2,4D, exhibited a limited growth on culture medium containing 1 mg x 1^-1 2,4D and had no ability to regenerate plants. Figure 1B shows the effect of CFT concentrations on the growth (based on fresh weight) and rhizogenesis of wheat primary calluses. Growth and rhizogenesis of wheat calluses were inhibited at the same CFT concentration, while the lethal CFT dose for wheat calluses was 4-5 times higher than that for barley calluses. A line of wheat calluses derived from cv. « CAMA », able to regenerate rooted plantlets, was developed in our laboratory (DUTRECQ, 1979a). Sub-cultures of these calluses, grown on basal medium of CHENG and SMITH (1975) supplemented with 1 mg x 1^-1 2,4D, maintained their organogenetic ability for 26 months. The original callus was isolated within a group of calluses of 6 months of age (5 sub-cultures) derived from explants of an embryo coleoptile excised after 48 h pregermination.

Figure 4A shows a particular stage of this growing callus containing white organogenetic centers. Morphogenesis leading to the development of rooted green plants was obtained in a few cases from these whitish buds, after 2-4 sub-cultures on basal medium supplemented with 5 x 10 ^-7 M kinetin ; the possible formation of embryoids during sub-cultures of this callus line was discussed (DUTRECQ, 1979b). When induced on culture medium containing 2,4D + kinetin, a more generalized morphogenic development was obtained through the formation of calluses which generated green meristematic points.

Variable phenotypes (abnormal plants or tillers, chimeras, dwarfing, ear compacity) and differences in chromosome numbers in the cells of root tips were observed. From 13 plants regenerated from calluses 10-14 month old (8-10 sub-cultures), 4 plants had 42 chromosomes, 3 had 44, 42 + fragment, or 37 chromosomes, respectively. Normal plants had 42 chromosomes in root tip cells.

One set of regenerated plants yielded 12 flowering plants, but only one seed was obtained ; the mother plant involved had 2 roots with 44 chromosomes, 2 with 41 chromosomes and 3 with 42 chromosomes.

In cooperation with Dr. P. SEILLEUR (Department of Plant Biology of this Faculty), we obtained from another batch of 14 regenerated plants numerous seeds formed on 2 plants (example ear n° 19.4.1 : fig. 4E), and one seed formed on a third plant.

As shown in Table 2, primary wheat calluses, or sub-cultures with organogenetic capacity, were equally sensitive to CFT, while wheat embryos were 3 to 4-fold more resistant to CFT.

Primary calluses (2-3 sub-cultures) formed numerous roots in the absence of 2,4D, exhibited a limited growth on culture medium containing 1 mg x 1^-1 2,4D and had no ability to regenerate plants. Figure 1B shows the effect of CFT concentrations on the growth (based on fresh weight) and rhizogenesis of wheat primary calluses. Growth and rhizogenesis of wheat calluses were inhibited at the same CFT concentration, while the lethal CFT dose for wheat calluses was 4-5 times higher than that for barley calluses. A line of wheat calluses derived from cv. « CAMA », able to regenerate rooted plantlets, was developed in our laboratory (DUTRECQ, 1979a). Sub-cultures of these calluses, grown on basal medium of CHENG and SMITH (1975) supplemented with 1 mg x 1^-1 2,4D, maintained their organogenetic ability for 26 months. The original callus was isolated within a group of calluses of 6 months of age (5 sub-cultures) derived from explants of an embryo coleoptile excised after 48 h pregermination. Figure 4A shows a particular stage of this growing callus containing white organogenetic centers. Morphogenesis leading to the development of rooted green plants was obtained in a few cases from these whitish buds, after 2-4 sub-cultures on basal medium supplemented with 5 x 10 ^-7 M kinetin ; the possible formation of embryoids during sub-cultures of this callus line was discussed (DUTRECQ, 1979b). When induced on culture medium containing 2,4D + kinetin, a more generalized morphogenic development was obtained through the formation of calluses which generated green meristematic points. Variable phenotypes (abnormal plants or tillers, chimeras, dwarfing, ear compacity) and differences in chromosome numbers in the cells of root tips were observed. From 13 plants regenerated from calluses 10-14 month old (8-10 sub-cultures), 4 plants had 42 chromosomes, 3 had 41, 3 had 40, while 3 plants had 44, 42 + fragment, or 37 chromosomes, respectively. Normal plants had 42 chromosomes in root tip cells.

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As shown in Table 2, primary wheat calluses, or sub-cultures with organogenetic capacity, were equally sensitive to CFT, while wheat embryos were 3 to 4-fold more resistant than calluses.

Study of the factors which interfere in tests of selection of calluses for their resistance to CFT

Barley calluses

Most experiments were performed with calluses of barley, type GC1, which grow extensively on solid medium, but do not regenerate plantlets. Ethylmethane sulfonate (EMS), acridine orange and ethidium bromide were used as mutagens. The effect of mutagenic pretreatments, on the multiplication of barley cell clusters in the presence of CFT, are presented in Table 3. EMS had no effect, while material pretreated with acridine orange or ethidium bromide grew in the presence of CFT concentration which inhibited the non-mutagenized calluses.

The selection technique used was derived from that of GENGENBACH and GREEN (1975) with maize. Cell clusters were incubated for successive periods of 25 days on CFT
Effects of partially purified CFT on the oxidation of α-ketoglutarate and succinate by mitochondria from barley calluses, type GC1. Mitochondria were isolated as follows: 30-40 g of callus tissue were homogenized in a Potter homogenizer at 4°C, in 60-80 ml of extraction medium containing 0.5 M mannitol, 1 mM EDTA, 33.5 mM KH2PO4, 5 mM Tris-HCl, 3 mM cysteine and 750 mg bovine serum albumin (BSA), pH 7.2. The mitochondrial fraction sedimenting between 1,085 g x 3 min and 14,500 g x 5 min, was suspended in a minimal volume of reaction medium containing 0.5 M mannitol, 10 mM KH2PO4, 5 mM KCl, 5 mM MgCl2, and 75 p. 100 BSA, pH 7.2. Extraction and reaction media were made according to CEULEMANS (unpublished data). Oxidations were measured polarographically in a 3-ml temperature-controlled cell, using a Clark electrode. Cytochrome C 0.002 mM and 100-150 μl of mitochondria preparation were added to the reaction medium 1 min prior to addition of substrate (10 mM α-ketoglutarate or 10 mM succinate), followed by 1 mM pyruvate. Mitochondrial protein content was determined by the method of WADDELL (1957), using BSA as standard. The respiratory control (RC) were measured in the presence of 300 nmol ADP. The oxidation rates are indicated to adjacent lines in nmol O2 x min⁻¹ x mg⁻¹ protein. Partially purified CFT (TP) was added after two state 3-state 4 transitions for the α-ketoglutarate trace, and after 6 min incubation for the succinate trace. The quantity of TP added was expressed as O.D. 266 nm. Methanol treatment (MeOH : 5 μl) was included in control traces.

Études de CFT partielle purifié sur l'oxydation du céto glutarate ou du succinate par de mitochondries isolées de callots d'orge, type GC1. Les mitochondries sont isolées ainsi: 30 à 40 g de callots sont broyés dans un Potter à 4°C, dans 60 à 80 ml de milieu d'extraction (Mannitol 0,5 M, EDTA 1 mM, KH2PO4 33,5 mM, Tris-HCl 5 mM, cystéine, 3 mM et 750 mg de sérum albumine bovine (SAB), pH 7,2). La fraction mitochondriale sédimente entre 1,085 g pendant 3 min et 14,500 g pendant 5 min. Elle est renouvelée dans le milieu de réaction (Mannitol 0,5 M, KH2PO4 10 mM, KCl 5 mM, MgCl2 5 mM et 0,75 p. 100 de SAB, pH 7,2). Les milieux d'extraction et de réaction sont d'après CEULEMANS. Les oxydations sont mesurées polarographiquement en utilisant une électrode de type Clark dans une cellule de 3 ml thermostatée. Le cytochrome C 2 x 10⁻⁶ M et 100 à 150 μl de la préparation mitochondriale sont ajoutés au milieu réactionnel 1 min avant l'addition du substrat (10 mM de céto glutarate ou 10 mM succinate) suivi par 1 mM de pyruvate. Les protéines sont déterminées selon la méthode de WADDELL (1957), contre une gamme standard de SAB.

**TABLE 2**

Relationship between the results of the barley root survival test and the toxic effects of culture filtrates of *H. sativum* on wheat calluses or embryos.

<table>
<thead>
<tr>
<th>Type of tissue in culture</th>
<th>Criterion for toxicity measurement</th>
<th>Expt n°</th>
<th>Root index (°) = R.I.</th>
<th>Culture index (°) = C.I.</th>
<th>Levels of sensitivity to CFT (C.I. × R.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary calluses (2-3 sub-cultures) no organogenetic capacity</td>
<td>rhizogenesis and growth</td>
<td>55S</td>
<td>4.8 (°)</td>
<td>12.5 (°)</td>
<td>2.6</td>
</tr>
<tr>
<td>Secondary calluses (17-18 sub-cultures) organogenetic capacity</td>
<td>growth</td>
<td>XIV</td>
<td>5.2</td>
<td>11.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Excised embryos</td>
<td>embryo survival</td>
<td>XV</td>
<td>10.0</td>
<td>19.2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63S</td>
<td>3.8</td>
<td>30.0 (°)</td>
</tr>
</tbody>
</table>

(°) Experiments made with strain 15H of *H. sativum* (original strain: Dr Tinline); results are expressed as percent CFT in the growth medium, which corresponds either to complete inhibition (culture index) of rhizogenesis, callus growth or embryo survival, or to the LD 50 of the barley root survival test (root index).

(°) In this experiment, complete inhibition of embryo survival was not obtained at the concentration of CFT used (Dutrecq, 1979c).

(°) Voir (1) du tableau 1. L'effet toxique correspond soit à une inhibition totale (« culture index ») de la rhizogenèse, de la croissance des callus, de la survie des embryons, ou de la DL 50 du test de survie des racines d'orge (« root index »).

(°) Dans cette expérience, l'inhibition totale de la survie des embryons n'est pas obtenue à la concentration de CFT utilisée (Dutrecq, 1979c).
concentrations corresponding to 40, 50 and 70 p. 100 of the concentration which was lethal for non-mutagenized calluses.

By using the growth of calluses as criterion of resistance to various concentrations of CFT added to the medium, we obtained the results presented in Table 4. Some sub-calluses derived from calluses which were submitted to the selection pressure of CFT, grew on a higher CFT concentration than did sub-calluses derived from control calluses, not previously treated with CFT.

Stability of the resistance of « selected » barley calluses to CFT was measured during one year of sub-culturing calluses either with or without CFT; the results are presented in Table 5. The relative resistance of « selected »...
Table 3

Growth of mutagen-treated barley calluses in the presence or absence of CFT.

<table>
<thead>
<tr>
<th>Assay n°</th>
<th>Mutagenic treatment</th>
<th>CFT in the growth medium</th>
<th>GC1</th>
<th>GC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5% EMS, 2h</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-4} M acridine orange, 2h</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 x 10^{-4} M acridine orange, 2h</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>0.5% EMS, 4h</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10^{-4} M ethidium bromide, 4h</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>0.5% EMS, 3h</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10^{-4} M ethidium bromide, 3h</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(1) Mutagen-treated cell aggregates were incubated with the mutagen (either EMS, acridine orange or ethidium bromide), rinsed twice with fresh liquid medium, filtered on 100μm nylon filter and placed on solid medium containing or not CFT. Plates were observed for growth of the cell aggregates; ++ = normal growth, + = reduced growth, - = no growth.

Our results suggest that the sensitivity of « unselected » callus lines to CFT varied with time; these calluses were equally sensitive to CFT either at the beginning of the selection process (Table 1) or at the end of the stability study period, but showed an increased sensitivity at the end of the selection process, thus giving an apparent 12-fold increase of resistance of the « selected » calluses relative to the « unselected » ones. As GC1 calluses show chromosomal instability, it may be that different cell lines with different sensitivity of CFT were transferred in the course of sub-culturing the « unselected » calluses.

As shown in Table 4 and Figure 5, some « selected » calluses did not grow on a substrate containing 10% of uninoculated culture medium used to grow the fungus. This phenomenon could be due to intrinsic inhibition by this culture medium (fig. 6) or to toxin-dependence of the « selected » calluses involved.

The in vitro resistance of mitochondria isolated from « selected » calluses to partially purified toxin preparations was investigated (fig. 7). Sub-calluses from a « selec-
### TABLE 5

*Stability of the resistance of « selected » barley calluses to CFT.*
*Stabilité de la résistance au CFT des cais d'orge « sélectionnés ».*

| Days after the end of the selection process | Expt no | Root index \(= \text{RI} \) (*) || Concentration of culture medium \(= \text{C.I.} \) || « Unselected » calluses | « Selected » calluses | Relative resistance \(= \text{C.I.} \times \text{RI.}^{-1} \) |
|-------------------------------------------|--------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 28                                       | IX     | 3.7             | 10              | 0.2             | 0.05            | 1.4             | 0.38            | 7               |
| 90                                       | XII    | 4.7             | 10              | 0.3             | 0.06            | 1.8             | 0.38            | 6               |
| 145                                      | XIV    | 5.2             | 10              | 0.7             | 0.13            | 2.0             | 0.38            | 3               |
| 264                                      | XIX    | 3.8             | 10              | 0.8             | 0.21            | 1.6             | 0.42            | 2               |
| 277                                      | XXIIa  | 3.2             | –               | 1.2             | 0.38            | 1.2             | 0.38            | 1               |
| 377                                      | XXIIb  | 3.2             | –               | 0.8             | 0.25            | 1.2             | 0.38            | 1.5             |

(*) Results are expressed as percent CFT in the growth medium.
(†) « Root index » corresponds to the LD 50 of the barley root survival test.
(‡) Growth medium of calluses enriched with fresh culture medium used to cultivate the fungus (expressed as percent in the growth medium).
(§) « Callus index » corresponds to the minimal dose of CFT which completely inhibits the growth of « unselected » or « selected » barley calluses after 1 month of culture on a medium containing \(1 \text{ mg} \times 1^{-1} 2.4D\). This minimal dose varied for different sub-cultures of a single callus.
(‖) Corresponds to the ratio between the « callus index » of « selected » calluses and the callus index of « unselected » calluses.
(¶) Les résultats sont exprimés en % de CFT dans le milieu de croissance.
(‖) Le « Root index » correspond à la LD 50 du test de survie des racines d'orge.
(±) Milieu de croissance des cais enrichi avec du milieu de culture stérile utilisé pour la croissance du champignon (exprimé en % de milieu de croissance).
(‡) Le « callus index » correspond à la dose minimale de CFT qui inhibe complètement la croissance des cais d'orge « sélectionnés » ou « non sélectionnés » après 1 mois de culture sur un milieu contenant \(1 \text{ mg} \times 1^{-1} 2.4D\). Cette dose minimale varie pour différentes sous-cultures d'un même cal.
(‖) Correspond au rapport entre le « callus index » des cais « sélectionnés » et le « callus index » des cais « non sélectionnés ».

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**Figure 5**

*Growth of sub-calluses of « selected » calluses of barley placed on various concentrations of CFT. « Selected » barley calluses were 264 days old at the end of the « selection » process (see Table 5). O = plate containing control callus growth medium enriched with \(10\) % of unoinoculated culture medium used to grow the fungus; \(0.13, 0.25, 0.5, 1, 1.5\) = relative concentration of CFT, where 1 is the last CFT dilution (0.8 % of CFT + 9.2 % of unoinoculated culture medium) permitting the growth of « unselected » (C) calluses. C = control (« unselected » calluses); a = « selected » calluses which did not grow on control medium but grew on CFT medium; b = « selected » calluses which did not grow on any medium.

*Croissance de sous-cultures de cais d'orge « sélectionnés » sur plusieurs concentrations de CFT. Les cais sélectionnés ont 264 jours en fin du processus de sélection (voir tableau 5). O = boîte contenant le milieu témoin de croissance des cais enrichis avec \(10\) % de milieu non inoculé, utilisé pour la croissance du champignon; \(0.13, 0.25, 0.5, 1, 1.5\) sont les concentrations relatives de CFT, où 1 est la dernière dilution de CFT (0.8 % de CFT + 9.2 % de milieu de culture non inoculé) qui permet la croissance de cais « non sélectionnés » (c). C = témoin (cais « non sélectionnés »); a = cais « sélectionnés » qui ne poussent pas sur milieu témoin mais qui poussent sur milieu avec CFT; b : cais « sélectionnés » qui ne poussent sur aucun milieu.*
ted cell-line (102-237 B ; 102-236 B, ; 102-239 B) gave mitochondria which were twice less inhibited by the toxin preparation than those isolated from « unselected » calluses.

Wheat calluses

We have applied the experimental design described above to wheat calluses with a permanent organogenetic capacity. With wheat calluses pretreated with \( 3 \times 10^{-4} \) M ethidium bromide for 3 h, many sub-calluses grew on CFT concentrations which was lethal for untreated calluses. Plantlets were regenerated from embryoid structures within these resistant calluses. As resistance of pregerminated wheat embryos to CFT is higher than that of wheat calluses (Table 2), it might be that the apparent resistance of mutagenized calluses is due to the embryoid structures formed in these calluses. In order to test this proposition, experiments are now in progress, using CFT concentrations which are lethal for wheat embryos.

CONCLUSIONS

During the process of submitting sub-cultures from originally ethidium bromide mutagenized calluses to increasing CFT concentration, an apparent 12-fold increased resistance was observed, relative to that of control sub-cultures derived from wild-type calluses. However, this 12-fold increase in resistance was not found when the results were expressed relatively to the « root survival test », which
appears to be a very stable and reliable criterion to quantify the toxic effect of CFT.

Comparing directly the results of growth inhibition of « selected » sub-calluses to the results of the « root survival test » did not suggest a real increase in resistance of calluses to CFT during the « selection » process.

Therefore, we conclude that the evaluation of resistance to CFT within an experimental design which involves the maintenance of callus tissue in culture for a very long period should be considered with great caution, because of the possibility of changes in the relative sensitivity of the control lines not submitted to pre-treatment with CFT. We consider this situation to be due in part to the chromosomic instability of the cell lines used in our experiment.

We are now using a system based on wheat calluses, which are able to regenerate plantlet. These calluses are submitted to the selecting pressure of partially purified CFT, using the « root survival test » to evaluate the toxicity level of CFT, and primary calluses as control reference. In this way, we hope to solve the problems encountered so far with the GC1 barley calluses.

ACKNOWLEDGMENTS

I thank Prof. J. SEMAL for helpful discussions and for reviewing the manuscript, Dr. M. BRIQUET for kind advice in mitochondrial studies, Mr P. DERENNE, Chef de Section (Laboratoire de Cytogénétique, Station d’Amélioration des plantes, Gembloux) for his help in counting chromosome numbers and the « Institut pour l’Encouragement de la Recherche Scientifique dans l’Industrie et l’Agriculture » for financial support. The competent technical assistance of Mr Jean DEREACK is highly appreciated.

REFERENCES


