GENETIC MODIFICATION OF THE WAXY CHARACTER IN BARLEY UNDER THE ACTION OF EXOGENOUS DNA OF THE WILD VARIETY

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SUMMARY

Genetic transformation was studied on spring barley mutants carrying the recessive mutant allele of waxy locus.

Analysis of the pollen grains of 38 control plants that were not subjected to any treatment showed that in the whole sample there was no mutant grain that stained black with iodine. It is also indicative of the genotypical purity of the original waxy plants.

After mechanical damage (puncture in the top and in the side of a grain) there were 5 pollen grains of wild type among 124000 mutant grains. Injection of grains with 2 μl distilled water led to the emergence of pollen grains that stained black at a frequency similar to that after puncture. The overall frequency of wild barley pollen grains for all control variants was 2.2 \cdot 10^{-5}.

The frequencies of wild-type pollen grains were practically the same after injections of DNA from E. coli, extensively deproteinized highly polymeric DNA from barley leaves or slightly deproteinized low polymeric DNA from barley endospermal material.

There was no marked increase of the wild pollen frequency after the injection of slightly deproteinized low polymeric DNA from the endospermal material (2.2 \cdot 10^{-5} in the control versus 3.7 \cdot 10^{-5} in the experiment).

The analysis of the material for the amount of altered pollen grains in individual plants also unequivocally demonstrated significant differences between the control and the experiment. In the first four variants there was no plant having over three altered pollen grains among the 2500 mutant grains examined. In the variants with injections of barley DNA differing in the extent of deproteinization and in polymerisation only in one case (of low polymeric slightly deproteinized DNA) there were no plants with many altered pollen grains. In all the other variants there were plants having much pollen of wild type.

The largest number of plants with a great many affected pollen grains occurred

Abbreviation: SSC, 0.15 M NaCl--0.015 M Na citrate.
Recent successful attempts at genotypical modifications in human tissue cells in culture under the action of exogenous DNA\textsuperscript{11} have focused the attention of many researchers on the problem of genetic transformation in higher organisms. This is not only a principal fundamental problem for the understanding of the molecular basis of heredity; its solution is likely to offer new opportunities for tackling practical problems of “genetic engineering”, particularly in plant breeding. Transformation studies in plants are also important, in as much as to date genetic transformation has been demonstrated and sufficiently well understood only in microorganisms; in plants no case of genetic transformation has yet been reliably established. The most thorough studies in plants were carried out by LEDOUX \textit{et al.}\textsuperscript{10} and Hess\textsuperscript{*}. LEDOUX studied in detail the possibility of penetration of exogenous donor DNA into the recipient’s organism and its further fate\textsuperscript{9}. In a number of plants (barley, Arabidopsis, \textit{etc.}), labelled DNA absorbed by germinating seeds passed into a stem and generative organs. Moreover, it was shown in LEDOUX’s laboratory that exogenous bacterial DNA was capable of penetration into the seedling cells’ nuclei and integration there with chromosomal DNA. It was convincingly demonstrated by sedimentation analysis of DNA, and by experiments with its melting and ultrasonic degradation, that exogenous DNA was a constituent part of the plant DNA connected with the latter by covalent bonds.

The further fate of the donor DNA is not known. It is not clear how exogenous DNA replicates\textsuperscript{1,10} and there is no evidence of the transcription and translation of foreign information in the genome of higher plants. The tomato crown gall is the only model in which transcription of exogenous DNA into RNA\textsuperscript{18} and its translation into protein\textsuperscript{18} have been unambiguously demonstrated. No convincing examples of the phenotypical expression of new genetic information are as yet available. The evidence of LEDOUX \textit{et al.} as regards the production of prototrophic Arabidopsis plants from auxotrophs under the influence of exogenous DNA has met with much criticism\textsuperscript{10}. Unfortunately, the evidence of Hess on the transformation of anthocyanic colour in several Petunia plants\textsuperscript{8} is not conclusive either. However, clear-cut negative results were obtained by Coe and Caspar\textsuperscript{3} and Carlsson\textsuperscript{2}.

The most popular method used in transformation studies in plants is that of feeding exogenous DNA to the root system of the recipient’s seedlings or rubbing bacteria and bacterial DNA over the leaf. But it is not yet clear what degree of purification and polymerisation of DNA is most effective.

The aim of the present work was to find out whether various DNAs have specific actions on such recessive mutant character as lack of amyllose in starch grains. The experiments were carried out during 1971–73.

MATERIALS AND METHODS

\textit{Characteristics of barley lines used}

\textit{Waxy} mutant seeds were kindly supplied to Professor N. V. Turbin by Professor

\footnote{See review by Soyfer and Turbin\textsuperscript{17}.}
A. Gustafsson in Sweden and sown on the experimental station of the Institute of Genetics and Cytology of the Academy of Sciences of BSSR. The spikes of the plants to be injected with DNA were isolated before flowering. Donor DNA was obtained from the wild barley variety "Yuzhny" planted on the experimental field of the Poltavian Agricultural Station.

**Preparation of the material for extraction of DNA**

Endosperm ("milk") was squeezed out of the grains at the milk maturity stage and transferred into buffer of the following composition: \(0.15 \text{ M NaCl; } 0.01 \text{ M EDTA; } 0.015 \text{ M sodium citrate; } 0.05 \text{ M Tris buffer; } 0.1\% \text{ triton X-100 (pH 8.2)}\). In all, 200 g of endospermal material was recovered. The leaves were separated from the plants, and their DNA was also extracted.

**Extraction of DNA from the endospermal material**

The material obtained from the plant at the milk maturity stage was grated in a mortar till the mass appeared homogeneous. \(\frac{1}{3}\) volume of \(0.15 \text{ M NaCl-0.1 M EDTA (pH 8.0)}\), sodium dodecylsulphate (Serva, Heidelberg, W. Germany) at a final concentration of \(1\%\) and pronase (Calbiochem, B grade, 2 mg/ml) were added. Incubation lasted 2 h at 37°. Nucleic acids were sedimented with two volumes of ethanol. This material, wound round a glass rod, was dissolved in \(0.15 \text{ M NaCl-0.1 M EDTA}\). RNase (Calbiochem, A grade) at a final concentration of 50 \(\mu\text{g/ml}\) and \(1\%\) sodium dodecylsulphate were added. After 60 min incubation at 37° pronase was added to the solution, and incubation continued at 37° for 2 h more.

DNA was sedimented with ethanol and dissolved in NaCl-EDTA. The treatment with RNase (30 min) and pronase (2 h) was repeated. The sedimented material was dissolved in \(0.1 \times \text{ SSC (pH 8.2)}\). This preparation of DNA was considered to be a slightly deproteinized fraction of highly polymeric DNA. The part of the DNA which remained polymeric after the first deproteinization by detergents and treatment with RNase (wound round the rod), but then lost its polymerisation and could be sedimented only by centrifugation, was considered to be a low polymeric, slightly deproteinized fraction of DNA. For more extensive deproteinization, NaCl was added to highly polymeric DNA to a final concentration of \(1 \text{ M}\), and the preparation was deproteinized in the mixture chloroform: isoamyl alcohol (24:1 by volume). For this, the mixture was shaken on a shuttle apparatus at 4° for 40 min, then centrifuged at 4° for 1 h at 2000 rev./min. An aqueous saline fraction was decanted and DNA was sedimented with ethanol. After solution in \(0.3 \text{ M sodium acetate-0.01 M EDTA}\), the DNA was sedimented in cold isopropanol and dissolved in \(0.1 \times \text{ SSC}\). The overall yield of DNA was approximately 1 mg per 15 g of raw material. DNA from *E. coli* had \(\text{absorbance}_{260/280} = 1.80-2.14\), \(\text{absorbance}_{280/290} = 1.77-2.00\). Extensively deproteinized highly polymeric DNA from barley leaves and endospermal material had \(\text{absorbance}_{280/290} = 2.10-2.34\), \(\text{absorbance}_{280/290} = 1.67-1.76\).

**Extraction of DNA from leaves**

The material, cut very small with scissors, was poured with liquid nitrogen and ground to powder in a mortar. Two volumes of buffer \((0.015 \text{ M NaCl, } 0.01 \text{ M EDTA, } 0.015 \text{ M sodium citrate, } 0.05 \text{ M Tris, } 0.1\% \text{ triton X-100, pH 8.2)}\) and dodecylsulphate were added to the final concentration of 5%, and the solution was incu-
bated for an hour at 55°. NaCl to a final concentration of 1 M and two volumes of chloroform: isoamyl alcohol (24:1) were added. The mixture was shaken for 1 h on a shuttle apparatus at 4°. The suspension was then run for 1 h in a refrigerated centrifuge at 2000 rev./min.

The aqueous saline layer was decanted into two volumes of chilled 96% ethanol, and the mixture was stored for 1 h at 4°. The medusa formed was dissolved in 0.1× SSC (pH 8.2) and incubated for 1 h at 24° with RNAase (Calbiochem, A grade) at a final concentration of 50 μg/ml, followed by a 2-h incubation with pronase at a final concentration of 200 μg/ml. There were three repeats of the whole deproteinization cycle. The medusa formed after sedimentation with ethanol was dissolved in 0.3 M sodium acetate–0.01 M EDTA and sedimented with chilled isopropanol. The medusa was dissolved in 0.1× SSC and sedimented with chilled ethanol. The dried material was washed with 70%, then 80% ethanol and dissolved in 0.1× SSC.

Extraction of DNA from Escherichia coli

Bacteria grown in medium M-9 with the addition of casein hydrolysate (5 mg/ml) to the titre of 1.6·10^8 cells/ml were sedimented by centrifugation. The sediment was resuspended in 0.15 M NaCl–0.1 M EDTA (pH 8.0), then lysozyme (Oline factory, U.S.S.R.) was added to the final concentration of 500 μg/ml. The mixture was incubated for 50 min at 37°. p-Aminosalicylic acid (final concentration of 6%) and an equal volume of 80% freshly distilled phenol were added to the lysate. Deproteinization took 1 h at room temperature in a shuttle apparatus. The lysate was centrifuged at 3500 rev./min for 30 min. The aqueous fraction was transferred to a centrifuge tube and extracted with ether. DNA was sedimented with ethanol, then dissolved in 0.1× SSC. The solution was treated by RNAase (Calbiochem, USA, at a final concentration of 50 μg/ml) at 37° for 1 h. After a repeat of the phenolic deproteinization, ether extraction and ethanol sedimentation, the DNA obtained was dissolved in 0.1× SSC. After sedimentation with isopropanol the DNA was transferred into 1× SSC.

RESULTS

Spring barley mutant carrying a recessive mutant allele at the waxy locus was the subject of investigation. Pollen grain starch of wild barley consists of amylose and amylopectin and is stained blue to black by diluted iodine solution in potassium iodide. Plants carrying the recessive allele have no amylose, so their pollen is stained red-brown. The recessive mutation of waxy locus of spring barley is located in the first chromosome, and its emergence in microsporogenesis is indicated by the colour of the pollen^4.

The reasons for the choice of the above mutant as a recipient were as follows. First, the frequency of spontaneous wx→Wx reversions is very low, equal to 10^{-6}–10^{-7} (refs. 4, 20). Second, with this subject, a large amount of material (pollen grains) may be analyzed. Third, the scoring of changes is done at a haploid level, which accelerates analysis of the material in the next generation and sharply increases resolution of the genetic analysis (almost to the level of microbial genetics). Fourth, individual (pollen) cells, and not multicellular organs, are analyzed. However, it should be emphasized that induction of changes for a certain gene (marker) makes additional difficulties.
Injection of DNA into waxy barley grains at a milk maturity stage

Our procedure of injection of exogenous DNA was different from those described in the literature, for we used a special syringe designed by Y. B. Titov and V. N. Soyfer for the injection of exogenous DNA into grains at a milk maturity stage. At early stages of embryogenesis, endosperm is known to be a symplastic heterokaryon without cell walls, and the embryo to consist of a relatively small amount of cells. We believe that injection of DNA into the symplast facilitates the passage of exogenous DNA to the embryo and its inclusion into the embryo cells. According to the literature, introduction of mutagenic substances into grains at a milk maturity stage markedly increases the mutation frequency.

About 1 μg DNA (1–2 μl solution, depending upon DNA concentration) per grain was injected. There were the following variants of injections: (1) control without any treatment; (2) mechanical injury (puncture of a grain without injection of a solution; (3) injection of 2 μl distilled water; (4) injection of extensively deproteinized highly polymeric DNA from E. coli; (5) injection of extensively deproteinized highly polymeric DNA from wild barley leaves; (6) injection of extensively deproteinized highly polymeric DNA from the endospermal material (“milk”) of wild barley; (7) injection of slightly deproteinized low polymeric DNA from the endospermal material (“milk”) of wild barley; (8) injection of slightly deproteinized highly polymeric DNA from the endospermal material (“milk”) of wild barley.

Before the injection, DNA dissolved in 0.1 × SSC was sedimented with chilled 96% ethanol and dissolved in distilled water. The DNA absorption spectrum was obtained on spectrophotometers SF-4A, and Pye Unicam, SP 800 (England).

Injections were made under the aseptic condition: the needle of the syringe and the operator's hands were regularly cleaned with alcohol. As a result, 95% of all spikes injected with the DNA solutions yielded normal grains. A total of 163 plants were treated; 5510 injections were made. Injected spikes were again covered with isolators and left to grow in the field till full maturity.

Examination of the pollen of plants grown from the injected grains

The next year all treated grains were sown in Poltava. The plants had not yet reached a pollen maturity stage (pollen sacs were closed) when 20 anthers (one per floret) were sampled from every main spike. Anthers of each spike were fixed in 1 ml 70% alcohol in vials. The vials with the fixed pollen were brought to Minsk to the Laboratory of Experimental Mutagenesis of the Institute of Genetics and Cytology of the Academy of Sciences of BSSR for a cytological examination. The anthers in each vial were carefully grated with a glass rod, then two drops of the suspension were put on a slide. The slide was heated on a specially designed oven till the drop was completely dry. Then a drop of potassium iodide solution (75 mg I₂, 250 mg KI, 50 ml distilled water) was added, tapped with a cover glass, and by pressing the cover glass with a finger the pollen grains were squashed slightly. The slide was searched microscopically for black pollen grains. Not less than two slides were prepared from each vial and analyzed. On an average, 1240 pollen grains were analyzed on each slide. The pollen of 1639 plants, 3261 slides and over 4 million pollen grains were analyzed.

The results of the injection of wild-type DNA into waxy barley grains at a milk maturity stage are presented in Table I. The plants with grains injected with DNA were designated I₀. Their seed generation was designated I₁. By sowing the seeds, we
TABLE I
RESULTS OF INJECTION OF DNA FROM NORMAL INTO \textit{waxy} BARLEY GRAINS

<table>
<thead>
<tr>
<th>Variant of expt.</th>
<th>Number of plants injected with wild barley DNA ($I_0$)</th>
<th>Examined Plants grown from the injected pollen seeds ($I_1$)</th>
<th>Pollen grains ($I_2$)</th>
<th>Found-Wild barley pollen grains</th>
<th>Total Frequency ($\times 10^{-3}$)</th>
<th>Excess over the control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Without treatment</td>
<td>13</td>
<td>38</td>
<td>72</td>
<td>89,280</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>2 Mechanical injury</td>
<td>20</td>
<td>55</td>
<td>100</td>
<td>124,000</td>
<td>6</td>
<td>4.1</td>
</tr>
<tr>
<td>3 Injection of distilled water</td>
<td>14</td>
<td>234</td>
<td>466</td>
<td>577,540</td>
<td>11</td>
<td>1.9</td>
</tr>
<tr>
<td>4 Extensively deproteinized highly polymeric DNA from \textit{E. coli}</td>
<td>28</td>
<td>383</td>
<td>774</td>
<td>939,600</td>
<td>20</td>
<td>2.1</td>
</tr>
<tr>
<td>5 Extensively deproteinized highly polymeric DNA from barley leaves</td>
<td>27</td>
<td>327</td>
<td>656</td>
<td>813,440</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td>6 Extensively deproteinized highly polymeric DNA from barley endospermal material (milk)</td>
<td>19</td>
<td>276</td>
<td>543</td>
<td>673,320</td>
<td>46</td>
<td>6.8</td>
</tr>
<tr>
<td>7 Slightly deproteinized low polymeric DNA from barley endospermal material (milk)</td>
<td>23</td>
<td>130</td>
<td>260</td>
<td>322,400</td>
<td>12</td>
<td>3.7</td>
</tr>
<tr>
<td>8 Slightly deproteinized highly polymeric DNA from barley endospermal material (milk)</td>
<td>14</td>
<td>196</td>
<td>390</td>
<td>483,000</td>
<td>2501</td>
<td>510</td>
</tr>
<tr>
<td>Total</td>
<td>158</td>
<td>1639</td>
<td>3261</td>
<td>4043,480</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

obtained plants with generative organs, including pollen grains, which are the next generation; we designated them $I_2$.

The examination of the pollen grains of 38 control plants not subjected to any treatment showed that in the whole sample of mutant pollen grains none was stained black with iodine. Also, it is indicative of the genotypical purity of the original \textit{waxy} plants.

For our purposes, it was extremely important to know exactly the spontaneous frequency of reversions from \textit{waxy}. Estimations were made in a large series of experiments carried out in 1969-70 when 3 million pollen grains were studied microscopically\textsuperscript{20}. There was only one pollen grain of wild type among them (reversion frequency was $3 \times 10^{-7}$). This estimate is in agreement with those of \textsc{Eriksson}\textsuperscript{4,5}.

After mechanical damage (puncture in the top and in the side of the grain) among 124,000 mutant grains there were 5 pollen grains of wild type. Injection of grains with 2 \(\mu\)l distilled water led to the emergence of pollen grains stainable (black) at a frequency similar to that after puncture.

The overall frequency of wild-type pollen grains for all control variants (without treatment, only mechanical injury and injection of distilled water) was $2.2 \times 10^{-5}$.

After the injections of DNA from \textit{E. coli}, extensively deproteinized highly polymeric DNA from barley leaves and slightly deproteinized low polymeric DNA from the endospermal material the frequencies of wild-type pollen grains were practically the same.
After the injections with donor DNA from *E. coli* and with extensively deproteinized DNA from barley leaves there was no increase in the frequency of appearance of normal pollen grains as compared with the control.

A slight increase in the frequency of wild-type grains was observed in the series of experiments with highly polymeric extensively deproteinized DNA from the endospermal material (up to $6.8 \cdot 10^{-4}$).

Thus, there was no significant increase in the frequency of wild barley characters in any of the three variants of the experiments with highly polymeric extensively deproteinized DNA. Nor was there any marked increase in the wild-type pollen frequency after the injection of slightly deproteinized low polymeric DNA from the endospermal material ($2.2 \cdot 10^{-6}$ in the control *versus* $3.7 \cdot 10^{-6}$ in the experiment).

The possibility of plant character modification by DNA injections was shown by the experiments with slightly deproteinized highly polymeric DNA from the endospermal material. In these experiments, wild-type pollen grains occurred at a frequency 230 times higher than in the control.

**Examination of the wild-type pollen frequency in individual plants**

The overall examination of the pollen of all plants in one variant is insufficient in itself for any definite conclusions to be drawn as regards the role of injected exogenous DNA. It should be extended by the data on the frequency of wild-type pollen grains on the slides examined. It is reasonable to suggest that, under the non-specific influence of the injections, few pollen grains would be affected.

It would have been an enormous labour to study microscopically all the pollen obtained. So we restricted ourselves to a representative sample, that is we chose one anther from each floret, put all the anthers of the same spike into one vial and agitated the contents. We then examined about 2500 pollen grains (of the average of 15000–20000 pollen grains in a vial.

Table II shows the results of the frequency scoring of the wild-type pollen grains in individual plants. Again, the variant with the injection of highly polymeric DNA from the endospermal material not subjected to deproteinization by chloroform: isoamyl alcohol yielded the largest number of plants with affected pollen. One plant in this variant had only black pollen on the first two slides examined. Although both before and after DNA injections the flowers and spikes of $I_0$ and $I_1$ generations were kept rigorously isolated, we checked all pollen collected from this plant for the possibility of accidental transfer of wild-type material. Should there be seeds or pollen of wild barley, all pollen of $I_2$ plants would be black. All fixed pollen of this plant was studied microscopically. Table III shows that each of the 13 slides examined (except the third) had a slight admixture of pollen of the mutant phenotype (0.2–1.0%).

So we ensured that the plant containing 75 mutant pollen grains was mutant not as a result of contamination but by origin.

It is hard today to understand the origin of a small amount of red pollen among completely altered wild pollen. Presumably, one or two of the fixed anthers preserve their mutant genotypes. But it is also highly likely that each anther contained several unaltered (mutant) pollen grains. The question may be answered after the later generations are grown from the seeds and their pollen examined.
TABLE II

DISTRIBUTION OF WILD BARLEY POLLEN GRAINS IN PLANTS GROWN FROM WAXY BARLEY GRAINS INJECTED WITH EXOGENOUS DNA

<table>
<thead>
<tr>
<th>Variant of expt.</th>
<th>Number of plants containing waxy barley pollen grains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One grain per plant</td>
</tr>
<tr>
<td>1 Without treatment</td>
<td>-</td>
</tr>
<tr>
<td>2 Mechanical injury without any injection</td>
<td>4</td>
</tr>
<tr>
<td>3 Injection of distilled water</td>
<td>6</td>
</tr>
<tr>
<td>4 Injection of extensively deproteinized highly polymeric DNA from E. coli</td>
<td>17</td>
</tr>
<tr>
<td>5 Injection of extensively deproteinized highly polymeric DNA from barley leaves</td>
<td>12</td>
</tr>
<tr>
<td>6 Injection of extensively deproteinized highly polymeric DNA from barley endospermal material</td>
<td>10</td>
</tr>
<tr>
<td>7 Injection of slightly deproteinized low polymeric DNA from barley endospermal material</td>
<td>10</td>
</tr>
<tr>
<td>8 Injection of slightly deproteinized highly polymeric DNA from barley endospermal material</td>
<td>11</td>
</tr>
</tbody>
</table>

TABLE III

EXAMINATION OF PLANT 506/19 POLLEN

<table>
<thead>
<tr>
<th>Slide No.</th>
<th>Number of mutant pollen grains</th>
<th>Percentage of wild barley pollen grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>99.0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>99.6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>99.8</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>99.1</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>99.4</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>99.7</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>99.4</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>99.1</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>99.8</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>99.5</td>
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<tr>
<td>12</td>
<td>9</td>
<td>99.3</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>99.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>75</strong></td>
<td><strong>Av. = 99.5%</strong></td>
</tr>
</tbody>
</table>

DISCUSSION

To sum up, the evidence obtained suggests that as a result of injection of wild barley DNA into mutant plant grains at a milk maturity stage the waxy character of barley is modified. The character studied in this work, as a subject of investigation, is free from the drawbacks of the subjects of Ledoux and Hess. The aim advantage of our subject is that, while examining pollen grains, we discovered alterations in the
individual cells. Maybe due to this, an event insignificant in its frequency, such as the appearance of eight cells of wild type among 577,000 mutant cells could be registered.

We suggested elsewhere\(^1\) that the genetically surprising findings of Hess on the colour of Petunia flowers, and of Ledoux on Arabidopsis auxotrophic mutants that all progeny of the treated plants turned out to be altered (or transformed, in these authors' terminology), may be a mere consequence of the inadequate test for the type of transformation.

Through the possibility of working with individual cells, we showed that mechanical action and injection of distilled water changed the reversion level. The reversion frequency increased approximately tenfold as compared with the spontaneous level (if we assume the latter to be \(10^{-6}\)). A similar frequency of altered pollen was observed in the experiments with the injection of DNA from \(E.\ coli\) and low polymeric DNA from the endospermal material of wild barley. Hence, in these variants the influence of DNA on the variability of the character examined was non-specific. In other variants, alterations may be described as specific.

In the variant with the injection of extensively deproteinized highly polymeric DNA from the endospermal material, along with an increase in the overall frequency of altered pollen (3.1-fold increase over the control) there was a plant yielding 34 wild-type pollen grains on two slides.

The influence of exogenous DNA of wild barley is most clear-cut in the experiment with the injection of slightly deproteinized highly polymeric DNA from the milk. As many as three plants in this variant had a large amount of altered pollen.

Taking into account that only about 5-10% parts of all pollen grains recovered from one spike underwent microscopic examination, the several pollen grains on two slides may be the result of modifications of dozens and even hundreds of wild pollen grains of one plant. A pollen sac can contain from several dozens to several hundreds of pollen grains. So far it is not understood whether cells of one flower or a few cells of every anther of a plant underwent changes. It will become much clearer when all seeds are sown and the pollen of the progeny is examined carefully.

The question to be solved is what proportion of embryonic cells may be affected by the injection of DNA into grains at a milk maturity stage? At this stage the apical meristem of the embryo comprises several cells. The degree of their competence, the probability of DNA penetration into competent cells, the fate of the injected DNA—all these questions are still open.

It is possible that the injected DNA is intensively attacked by cellular nucleases. The problem has not yet been adequately studied in plants, though it is well understood in bacteria\(^2\). No doubt, DNA surrounded by a protein coat is protected against attack by nucleases, which may explain our result, \(i.e.\) that slightly deproteinized DNA brought into being many more affected cells than did extensively deproteinized DNA. We continue to work in this direction.

REFERENCES


