Table I. Forward mutations induced by UV, MNNG and EMS

Mutagenic treatment	UV (3 min)	UV (3 min)	UV (6 min)	MNNG (30 min)	MNNG (60 min)	EMS (2 h)	EMS (2 h)
No. of colonies replicated	2820	1181	2299	3381	3002	2546	11,222
Survivors (%)	37	18	0.52	25	14	51	85
No. of mutants	19	4	7	18	6	9	32
Mutants (%)	0.67	0.34	0.30	0.53	0.20	0.35	0.29

All data are from independent experiments.

Table II. Types of biochemical mutants induced with UV, MNNG and EMS

Metabolite required	*p*-Aminobenzoic acid	Thiamine	Nicotinamide	Arginine	Carbon source	Unidentified	Total
UV	3	0	9	2	13	3	30
MNNG	4	3	3	2	8	4	24
EMS	3	4	13	6	15	–	41

puzzling is the fact that higher mutation frequencies were obtained with UV or MNNG in conditions of high survival. This phenomenon has been observed in other experiments and would deserve further study.

The comparison of the types of biochemical mutants induced with the 3 mutagens (Table II) does not reveal any marked difference in the mutation spectra. The lack of thiamine mutants after treatment with UV cannot be considered as significant owing to the fact that this type of mutant has been frequently obtained with UV in recent years[2,7].

However, the important feature lies in the finding of 4 arginine-requiring mutants after treatment with UV and MNNG. These mutants were shown to be NH_4^+-sensitive as EMS-induced arginine-requiring mutants described previously[4]. Furthermore, no arginine auxotroph was ever recovered in our laboratory, after treatment with EMS, on media containing a high concentration of NH_4Cl[8,9].

These results strongly suggest the specificity of mutations leading to an arginine requirement not to be related to the mutagenic agent used. This would mean that the specificity is determined at a very late stage in the mutation process, long after treatment with the mutagen, at the time of mutation expression.

It therefore seems that the culture medium is of crucial importance in that it either allows or does not allow the survival of the arginine auxotrophs. This specificity, however, is not absolute, since the 4 arginine auxotrophs isolated previously in other laboratories are insensitive to NH_4^+ ions. This problem will be discussed in another paper.

Résumé. Des mutations biochimiques ont été induites par le méthane sulfonate d'éthyl (EMS), la N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) et l'ultraviolet (UV) chez l'algue verte unicellulaire *Chlamydomonas reinhardi*. Des mutants auxotrophes pour l'arginine ont été isolés, après traitement par les 3 agents, sur un milieu contenant de l'extrait de levure comme unique source d'azote.

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Test-Tube Fertilization of Ovules in *Melandrium album* Mill. with Pollen Grains of *Datura stramonium* L.

The technique of test-tube fertilization provides new possibilities of overcoming incompatibilities in plants[1–4]. Little attention has been devoted to the study of the process of pollination and fertilization in those cases when pollen grains and ovules belong to species of different families. The present report contains the results of experiments carried out with the ovules of *Melandrium album* pollinated in vitro with pollen grains of *Datura stramonium*.

Female flower buds of *M. album* were bagged 3 days before pollination. Pistils were sterilized in saturated chlorine water for 15 min and then rinsed several times with autoclaved water. Later the ovary wall was removed

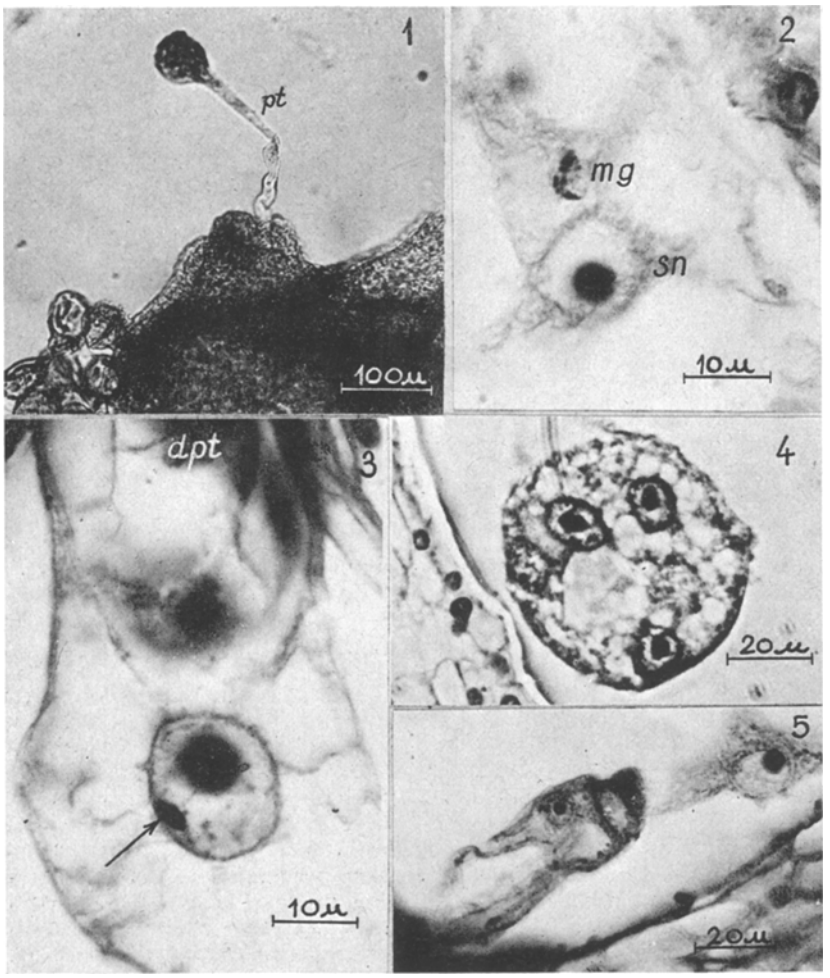

Fig. 1. The micropylar end of ovule after 4 h of culture. Pollen tube of *D. stramonium* is seen at the micropyle.

Fig. 2. A male gamete (mg) comes into contact with the secondary nucleus (sn).

Fig. 3. A male gamete inside the secondary nucleus; (dpt), discharged pollen tube at the micropylar end of the embryo sack.

Fig. 4. Part of the nuclear endosperm after 3 days of culture.

Fig. 5. Two-celled proembryo in a stage of degeneration.

and the ovules along with the placenta were inoculated on medium consisting of White's minerals[5] and 2% sucrose. The anthers of *D. stramonium* were excised at the stage of dehiscence. Pollen grains were scooped out from the anthers and dusted on the surface of the ovules which had been just implanted on the medium.

About 50–60% pollen grains started to germinate within 30 min and pollen tubes spread around the surface of the ovules. After 3–4 h of culture some pollen tubes were entering the micropyle (Figure 1). During the next 5–6 h a pollen tube reached the embryo sack, it burst and discharged its content into the region of synergids. As a rule the pollen tube disrupted one synergid, but in some cases both synergids were found to be completely destroyed. After 6–12 h the male gamete came into contact with the secondary nucleus (Figure 2), spreading upon its surface and penetrating inside it (Figure 3). The primary endosperm nucleus containing 1 big or 2 small nucleoli showed signs of growth and development. In many preparations, usually after 24–48 h of culture, about 4 endosperm nuclei were observed. In a few cases, after 3–4 days of culture, as many as 25 endosperm nuclei were found (Figure 4). It was difficult to find a distinct syngamy. However, in some cases the male gamete was observed inside the egg cell and, after 24 h of culture, a second nucleolus inside the egg nucleus was present. In 2 cases degenerating 2-celled proembryos were observed (Figure 5).

On the basis of the experiments presented above it is suggested that obtaining a more developed hybrid endosperm and embryo may depend on a higher ploidy of the female reproductive organs. Further efforts are being undertaken to test this suggestion.

Zusammenfassung. Samenanlagen von *Melandrium album* wurden in vitro mit Pollen von *Datura stramonium* bestäubt und befruchtet. In seltenen Fällen konnte ein zweikerniger Proembryo beobachtet werden. Das primäre Endosperm unfasste in der Regel 4 Zellen, nach 3–4 Tagen bis zu 25 Zellen.

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