183-32217

NASA TECHNICAL MEMORANDUM NASA TM-77263

PECULIARITIES IN FORMATION OF ARABIDOPSIS THALIANA (L.) HEYNH. GENERATIVE ORGANS UNDER SPACE FLIGHT CONDITIONS

Ye. L. Kordyum and I. I. Chernyayeva

Translation of "Osoblyvosti formuvannya henera+vvnykh orhaniv Arabidopsis thaliana (L.) Heynh. v umovakh kosmichnoho pol'yotu," Dopovidi Akademiyi nauk Ukrayinskoyi RSR, seriya B, no. 8, 1982, pp. 67-70.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 JULY 1983

STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA TM-77263		

4. Title and Subtitle	5. Report Date
PECULIARITIES IN FORMATION OF ARABIDOPSIS THALIANA (L.) HEYNH. GENERATIVE ORGANS UNDER SPACE FLIGHT CONDITIONS	July 1983
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Ye. L. Kordyum and I. I. Chernyayeva Ukrainian SSR Academy of Sciences Botanical Institute	10. Work Unit No.

9. Performing Organization Name and Address	11. Contract or Grant No.
Leo Kanner Associates Redwood City, CA 94063	NASW -3541
	13. Type of Report and Period Covered
	Translation
12. Sponsoring Agency Name and Address	14. Sponsoring Agency Code
National Aeronautics and Space Administration, Washington, D.C. 20546	

15. Supplementary Notes

Translation of "Osoblyvosti formuvannya henerativnykh orhaniv Arabidopsis thaliana (L.) Heynh. v umovakh kosmichnoho pol'yotu," Dopovidi Akademiyi nauk Ukrayinskoyi RSR, seriya B no. 8, 1982, pp. 67-70.

16. Abstract

Peculiarities in the formation of the andrecium and gynecium elements are described for Arabidopsis plants grown from the stages of two cotyledonous leaves in the Svitoblok-1 device on board the Salyut 6 orbital research station and in the laboratory. It is established that flower buds and flowers, normally formed in habitus, contain sterile elements of andrecium and gynecium whose degeneration occurs at different developmental stages of the Arabidopsis plants in the experiment under conditions of weightlessness.

17. Key Words (Selected by Author(s))	18. Distribution Statement
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19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22.
Unclassified	Unclassified	6	

PECULIARITIES IN FORMATION OF ARABIDOPSIS THALIANA (L.) HEYNH. GENERATIVE ORGANS UNDER SPACE FLIGHT CONDITIONS

Ye. L. Kordyum and I. I. Chernyayeva
Ukrainian SSR Academy of Sciences Botanical Institute

The possibility of using annual higher plants in complete ontogene-/67* sis (from seed to seed) under space flight conditions has been examined in some articles [1-5], but there has been no experimental confirmation.

Aboard the Salyut-6 orbiting scientific space station an experiment was done which involved growing Arabidopsis, an ephemeral of the family Cruciferae (Brassicaceae)--a convenient subject for radiobiological and space biological experimentation [6]--in a "Svitloblok-1" apparatus, which permitted growth of small higher plants under sterile, sealed conditions on an agarized growth medium, with light from 2 to 4 thousand lux[1]. Arabidopsis seedlings in the dicotyledonous phase were placed in the apparatus and put aboard the Salyut-6. The experiment lasted 65 days. For the first 16 days the plants were illuminated 14 hrs a day, for the last 51 days they were illuminated around-the-clock. The control plants were grown under laboratory conditions with the same lighting schedule.

At the end of the experiment, both the control and experimental plants were in the same stage of growth -- blossom and spadix fertilized -- and were no different as to average stem length, number of buds, flowers, and pods. The low light intensity produced a slowing of the generative phase. Arabidopsis is a long-day plant, its ontogenesis accomplished in 45-47 days if light intensity is 10 thousand lux and day length is no less than 16 hours.

For examination with the light microscope, buds, flowers, and pods of the Arabidopsis controls and experimental plants were fixed in 2.5%

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*Numbers in the margin indicate pagination in the foreign text.

1. Seeds produced at the USSR Academy of Sciences Institute of General Genetics.

glutaraldehyde, with postfixation in a 1% solution of OsO_4 and Navashin's mixture (chromacetoformol). The material was dried and embedded in /68 paraffin using the accepted cytologic method. Sections 10 μm thick were obtained with an MPS-2 microtome, stained with Heidenhain's iron hematoxylin and methyl green, and examined under an "NF" microscope. Sections were also stained using Feulgen's and Lugol's reactions and Sudan III; the latter two staining methods were used to ascertain the nature of nutrient stores in the pollen grain vegetative cells. The preparations were sketched using an RA-4 drawing apparatus and photographed with an "NF" photographic attachment.

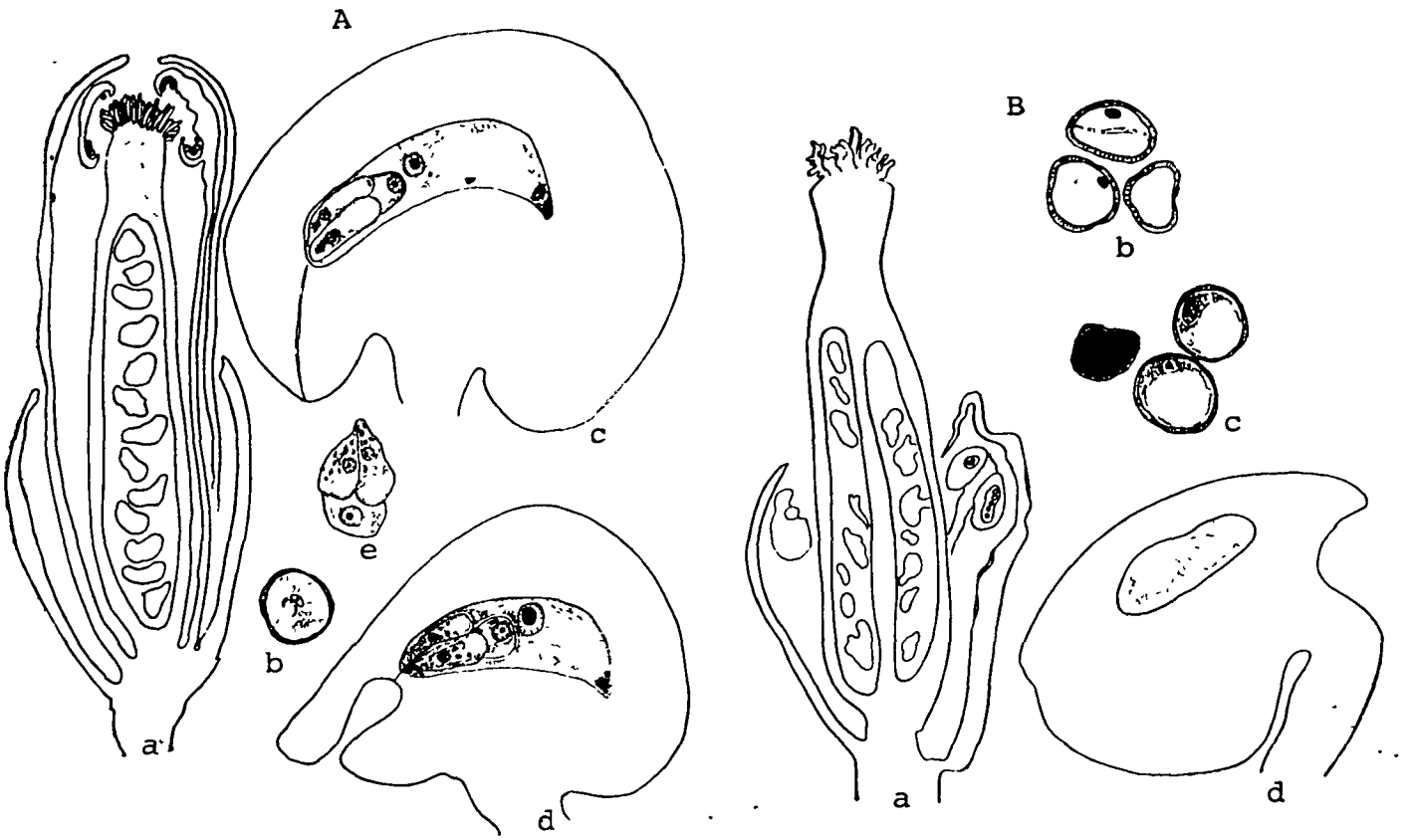
Microscopic examination of the Arabidopsis plant generative organs in the last stages of formation, as grown in the "Svitoblok-1" apparatus under laboratory conditions, revealed that the formation and development of gynecium and andrecium elements proceeded normally (see figure, A), as described in [7-8].

It was established, however, that the plants in the experimental population were not obligate self-pollinating, since, in addition to the normally first-order hermaphroditic flowers, potentially bisexual female ones had formed. These flowers differed from the hermaphroditic ones in external appearance. Before blossoming of the hermaphroditic ones, sepal length was no longer than 2/3 that of the white petals, which remained closed as the pollen burst out, ensuring contact between the pollen niduses of the long stamens, which had already opened, and the stigma, thus allowing self-pollination. In the potentially bisexual female flowers the petals did not exceed or only somewhat exceeded the sepals, so that the perianth surrounded no more than 2/3 the length of the ovule (the germ of which is seen on top), due to which cross-pollination is possible under natural conditions. When these flowers were artificially pollinated with the pollen of other plants, pods formed with complete seeds. The plants grown from these seeds were fertile. Thus this population of Arabidopsis was of gynomonoecious sexual type.

As opposed to the control plants, whose green pods contained seeds with germs at various stages of development, including differentiated, the experimental plants were found to have parthenocarpic pods with degenerating seed embryos. The buds and seeds apparently normally formed were replaced by sterile andrecium and gynecium elements whose degeneration began at various stages of generative organ development (see figure). It is remarkable that, as a rule, the flowers in the experimental material were externally similar to the potentially bisexual female flowers in the controls, i.e., the stigma and style and, generally, part of the germ were not surrounded by perianth, indicating the presence of a positive correlation between the degree of andrecium fertility and stamen filament length and size and location of the petal panicle, i.e., a morphological mechanism for ensuring pollination -- either self- or cross-pollination.

Andrecium element degeneration in the experimental plants began at the tetrad microspore or mononuclear microspore stages. Niduses rich in pollen were found to have bicellular or even tricellular pollen grains, but appeared non-viable from outward signs -- the absence in the vegetative cell cytoplasm of sufficient nutrient reserves, particularly of fat. The microsporangium walls, as in the controls, were made up of four layers of cells: epidermis, endothecium -- the future fibrous layer, middle layer, and secretory-type tapetum with binuclear cells; in the mature state -- epidermis and fibrous layer.

Gynecium element degeneration was found at the tetrad microspore, mono-, bi-, and tetranuclear stages, and in the formed embryonal sac. In none of the buds studied did the establishment or first developmental stages of the seed embryos differ from those in the controls. Two clear pathways of sterilization were later seen -- synchronous cessation /70 of macrospore or seed embryo embryonal sac and somatic cell development, and asynchronicity of embryonal sac and somatic element (primarily integument) element development, the first signs of developmental cessation appearing, as a rule, in the somatic elements. These primarily were the absence of integumentary tapetum differentiation, partial lysis of internal integument cells, and pathological vacuolization of integument cells. At the same time, the enriched seed embryos had morpho-



Arabidopsis thaliana (L.) Heynh.

A -- Control: a -- appearance of hermaphroditic flower in longitudinal section; b-e -- fragments of a; b -- tricellular pollen grain; c,d -- seed embryos from mature embryonal sacs; e -- ovum; B -- Experimental: a -- appearance of flower in longitudinal section; b-d -- fragments of a; b,c -- sterile microspores; d -- sterile seed embryo.

logically normally formed embryonal sacs with ov (egg cell and two synergids) and secondary nucleus of embryonal sac central cell. The mature seed embryos had a more or less campylotropous form, characteristic of seed embryos of this species, but they were smaller than in the controls. No fertile seed embryos were formed.

Thus the morphogenetic processes in the generative organs were more or less the same under weightless conditions, within the parameters used in this experiment, as normal. Generative organ sterilization was caused, most likely, by pathologically altered processes at the cell level, primarily cessation of cell division and differentiation, which have their specific structural manifestations in the andrecium and gynecium.

Based on comparative analysis of sterilization process features in the andrecium and gynecium elements in the experimental Arabidopsis plants and other angiospermous plants with various types of sterility under natural conditions, as well as possible causes of nondifferentiation of integumentary tapetum in seed embryos [9], it is assumed that the natural hormonal nature was disrupted in the generative phase of Arabidopsis plant development in this experiment under weightless conditions.

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