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## DESCRIPTION OF SOME CHARACTERISTICS OF FLOWERS AND SEEDS OF *ARABIDOPSIS THALIANA* – ECOTYPE LANDSBERG *ERECTA* AND MUTANT NW4

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### ABSTRACT

Flowers and seeds of Landsberg *erecta* (Ler) ecotype and NW4 mutant were studied by light microscopy and scanning electron microscopy to reveal characteristic features of their structure. The NW4 mutant flowers differ from Ler mainly in presence of two bract-like sepals with complicated vasculature and a variable number of secondary flowers. In the two outer whorls of NW4 flower, variable number of transformed stamen-, petal-, sepal- and style-like elements also occur. The NW4 mutant seeds are characterized by the absence of mucilage around the surface and a deviating seed coat morphology.

**KEY WORDS:** *Arabidopsis thaliana*, flower morphology, mucilage, mutants, seed structure, vascular system.

### INTRODUCTION

*Arabidopsis thaliana* is widely studied with respect to flower and seed structure and their development. Irish and Sussex (1990) examined Landsberg *erecta* (Ler) wild-type flowers, and some mutations affecting floral development. They suggested, that *Ap1* and *Ap2* genes determined the normal development of floral organs. Seed mutants were also investigated. Léon-Kloosterziel et al. (1994) described the disturbed seed structure of *transparent testa*, *glabra* (*ttg*), *glabrous2* (*gl2*), and *apetala2* (*ap2*) mutants.

In the presented study we investigated the *apetala* (*ap1*), *glabra* (*gl2*), *chlorina* (*chl*) NW4 mutant, which is derived from Ler. These mutant plants are characterized by the absence of trichomes on their yellow-green stems and leaves. Their flowers have no petals or, rarely, abnormally developed petals. The differences in flower and seed structure between Ler and NW4 are described in detail.

### MATERIAL AND METHODS

Seeds of Landsberg *erecta* (Ler) and NW4 mutant, obtained from The Nottingham *Arabidopsis* Stock Centre (Seed List 1992) were germinated under controlled growth conditions. 30 days after germination part of the plants (approximately 20 of each type) were fixed in 70% ethanol and cleared in 50% lactic acid. The remaining Ler and NW4 plants were grown until seeds maturation. Flowers arising from the terminal inflorescence have been investigated. Seeds were immersed in 2% glutaraldehyde for 16-20 hours at room temperature, dehydrated in graded series of ethanol, and dried in Critical Po-

int Drier Apparatus. The seeds were coated with a mixture of carbon/gold and observed with a scanning microscope (Nano-lab 7, Opton).

### RESULTS AND DISCUSSION

The Ler flower morphology has been extensively described and it is similar to that of other *A. thaliana* wild-types (Irish, Sussex 1990, Smyth et al. 1990, Trząski et al. 1995). However, we found 4 or 5 instead of 6 stamens in about 5% of examined flowers (Fig. 1A, B). There are several differences in shape and vasculature among one whorl sepals (Fig. 1C). The vasculature of adaxial sepal (Fig. 1C<sub>1</sub>) and abaxial sepal (Fig. 1C<sub>3</sub>) is more complicated than in lateral ones (Fig. 1C<sub>2</sub>, C<sub>4</sub>). Petals vasculature seems not to be very variable. Petals differ from each other in the tertiary ramifications number and distribution (Fig. 1D). It seems not to be a significant variability in petals vasculature as regards their position in the whorl. In general, patterns of sepals and petals vasculature are similar in all examined LER flowers.

The NW4 inflorescence-like flowers showed many abnormalities in the two outer whorls structure, while the number of inner whorls elements, e.g. stamens and carpels, was quite normal, like in Ler plants. We found more than 20 different floral diagrams among NW4 flowers (examples – Fig. 1E-L). Most of the flowers showed a transformation of abaxial and adaxial sepals into bract-like structures (Fig. 1M<sub>1-2</sub>). Flowers of NW4 mutant contained secondary flowers in their first and/or second whorl. One or two secondary flowers occurred in the position of lateral sepals (Fig. 1F, I, K, L). Distribution of secondary flowers in the second whorl was more variable.

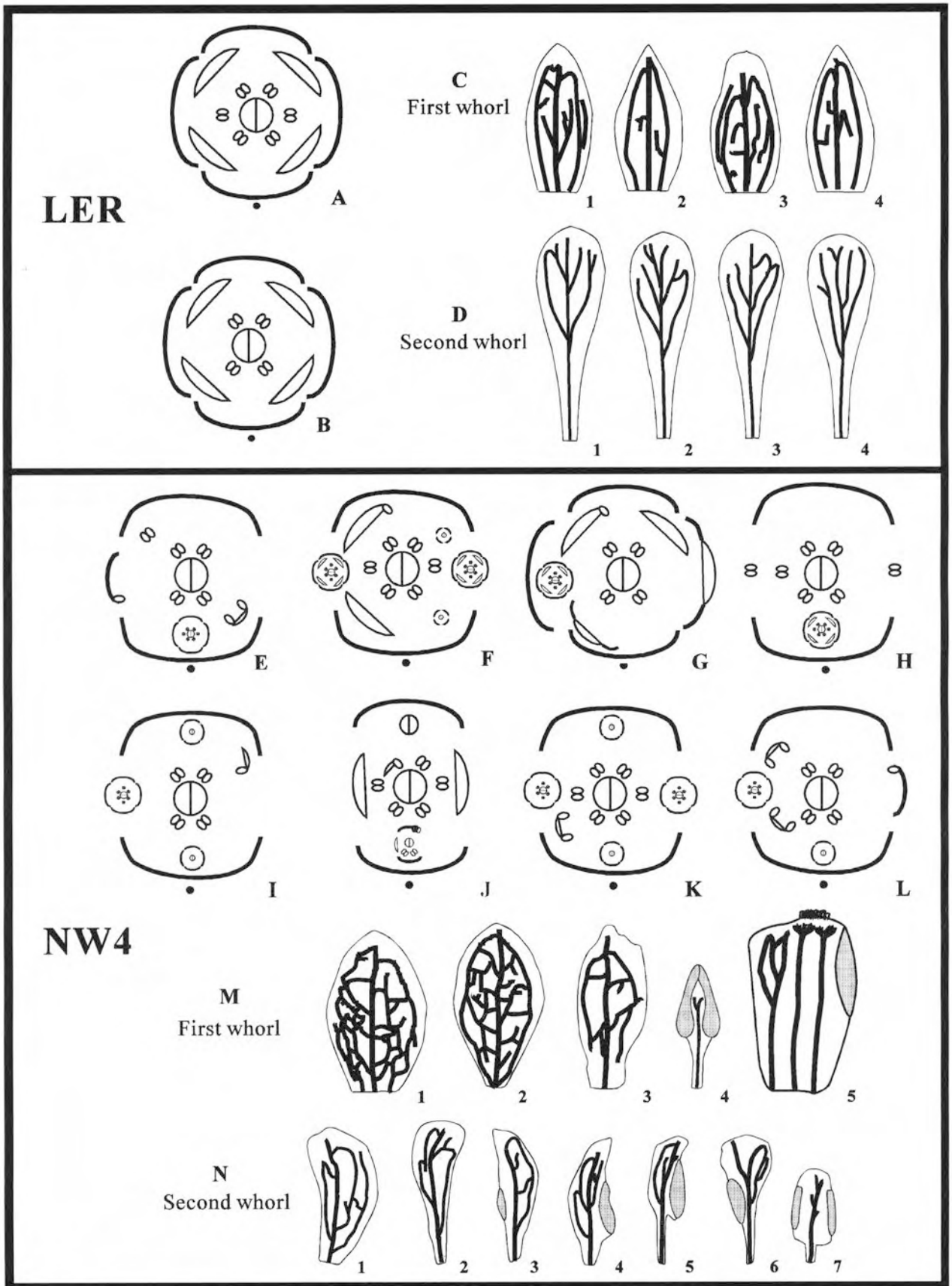


Fig. 1. Morphology of *A. thaliana* Landsberg erecta and NW4 floral organs.

A-D – Landsberg erecta. A, B – examples of floral diagrams, C, D – shape and venation of the two outer whorls elements in one flower. C<sub>1</sub> – adaxial sepal, C<sub>2</sub>, C<sub>4</sub> – lateral sepals, C<sub>3</sub> – abaxial sepal.

E-N – NW4. E-L – examples of floral diagrams. M-N – examples of shape and venation of the two outer whorls elements in the main flowers. M<sub>1</sub> – adaxial sepal, M<sub>2</sub> – adaxial sepal, M<sub>3-5</sub> – transformed elements in lateral position. N<sub>1-2</sub> – petal-like structures, N<sub>3-7</sub> – transformed elements with anther-like structures (marked by darken areas).

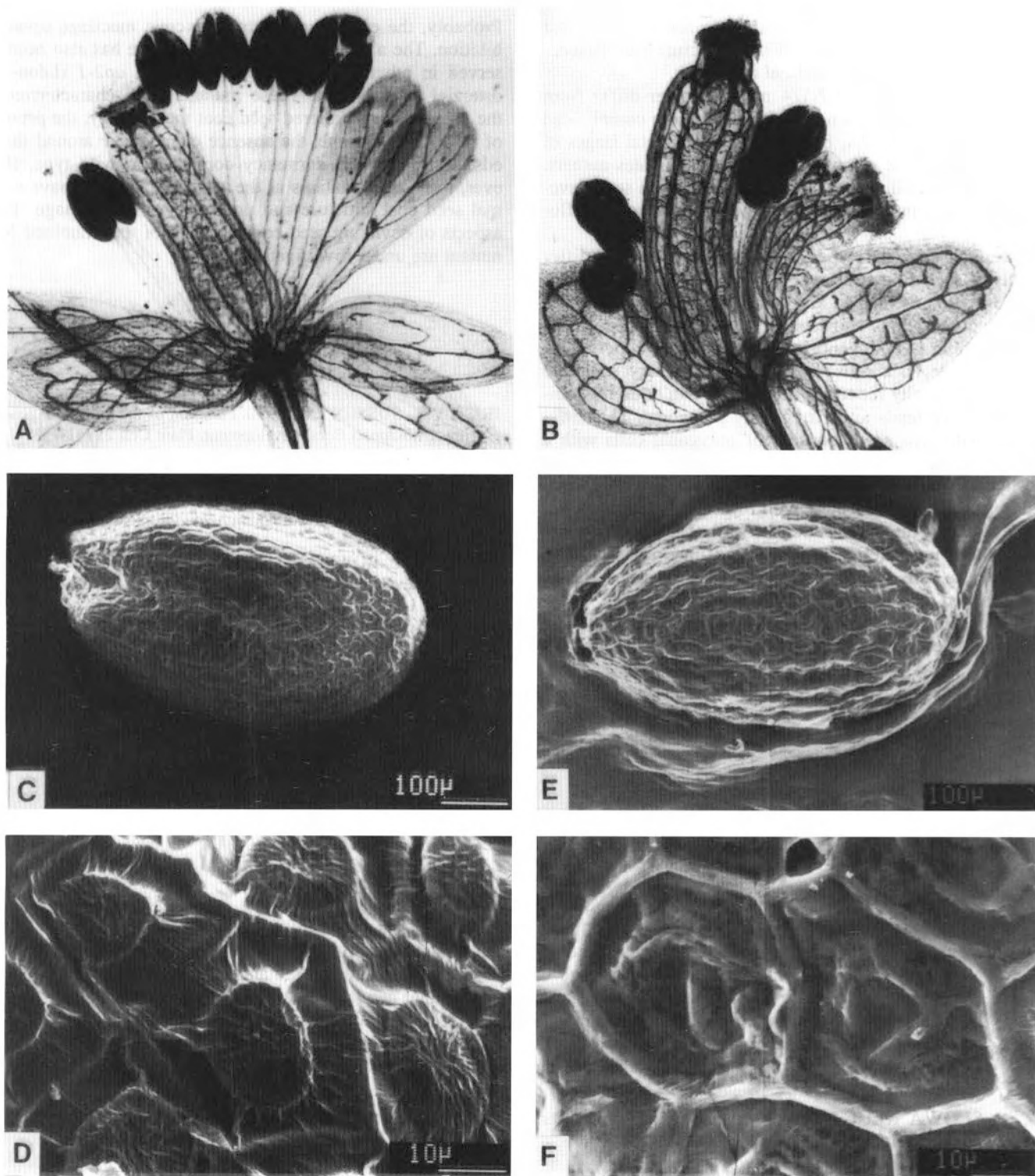


Fig. 2. Structure of Landsberg *erecta* and NW4 flowers and seeds.

A, B – cleared flowers: A – Landsberg *erecta*, B – NW4. C-E SEM structure of mature seeds: C-D – Landsberg *erecta*, E-F – NW4 mutant C – seed coat surface pattern of mature seeds, D – epidermal testa cells with a central elevation, E – reticulate pattern of mature seed, F – testa cells with collapsed outer periclinal cell wall.

Most of them were in axillary position of sepals, especially of the abaxial (Fig. 1E, H, I, J, K, L). We found secondary flowers also in the position of petals (Fig. 1F).

In some of NW4 flowers, petal-like or sepal-like structures with aberrant vasculature (Fig. 1M<sub>3</sub>), stamen-like structures or almost normally developed stamens (Fig. 1M<sub>4</sub>) or, rarely, style-like structures (Fig. 1M<sub>5</sub>) occurred in lateral position of the first whorl. In the second whorl of the main NW4 flowers the asymmetric stamen-like elements occurred (Fig. 1N<sub>3,6</sub>). Sym-

metric elements with two anther-like structures were rarely found (Fig. 1N<sub>7</sub>). About 30 % of secondary NW4 flowers contained petals, similar to those of immature Ler flowers. In contrast, petals or petal-like structures in the main flowers occurred rarely (Fig. 1N<sub>1,2</sub>).

Earlier investigations showed that *apetala* mutants had a regular flower structure. For example, the flowers of single homozygous *apl* mutant contained four secondary flowers instead of petals. Moreover, all the secondary flowers contained

tertiary flowers (Irish, Sussex 1990). It is not unlikely, that the high variability of the NW4 flower structure was connected with the presence of *gl1* and *chl* mutations.

The two inner whorls of NW4 mutant flower differ from those of Ler mainly in stamens length and more complicated style vasculature (FIG. 2A, B). In all developmental stages of the NW4 flowers, the stigma distinctly predominates anthers. However, stigma pollination and, subsequently, the seed development proceeds in main flowers as well as in secondary flowers.

The seeds of both genotypes are similar in shape, but differ in details of seed coat structure. However, some of the NW4 seeds are irregularly shaped and smaller in size than those of Ler. SEM observations show clearly that seed surface topography is similar in both genotypes, but differs in cuticular relief of the outer testa cells. Seeds have a primary sculpture that appears basically reticulate (Fig. 2C, E). In both, the individual cell shape tends to be irregularly polygonal. The surface of Ler wild type seeds consists of polygonal cells with a central elevation, the columella (Fig. 2D). These cells after initial hydration excrete a layer of mucilage. It can be visualized by staining the mucilage with ruthenium red. Upon imbibition of water, the outer periclinal wall of testa cells collapses, resulting in excretion of the stored mucilage. In NW4 seeds, the testa cells is affected because the elevations are absent or reduced (Fig. 2F).

The absence or reduction (collapsing) of mucilage may be a result of the deviating seed coat observed in NW4 mutant.

Probably, the elevating structures excrete mucilage upon imbibition. The absence or collapsed mucilage has also been observed in other Ler mutants *ats*, *ttg*, *gl2*, *ap2-1* (Léon-Kloosterziel et al. 1994). These mutants were characterized by the presence of an altered seed coat morphology, the presence of an abnormal shape, the absence of mucilage around the seeds, and a reduced dormancy compared to wild type. However, the *glabra* mutants at the *gl-1* and *gl-3* loci have a normal seed coat structure and produce normal mucilage. These aspects of deviating seed coat structure of the examined NW4 mutant are under investigation.

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#### OPIS NIEKTÓRYCH CECH KWIATÓW I NASION *ARABIDOPSIS THALIANA* – EKOTYP LANDSBERG *ERECTA* I MUTANT NW4

#### STRESZCZENIE

Przeprowadzono badania nad strukturą kwiatów i nasion u ekotypu Landsberg *erecta* (Ler) oraz mutantu NW4, z wykorzystaniem mikroskopu świetlnego oraz skaningowego. Kwiaty mutantu NW4 różnią się od Ler głównie obecnością liściokształtnych działek o skomplikowanej nerwacji oraz obecnością zmiennej liczby kwiatów drugorzędowych. W obu zewnętrznych okółkach kwiatu NW4 występuje także zmienna liczba elementów płatko-, działko- i słupkopodobnych. Nasiona mutantu NW4 charakteryzują się brakiem powierzchniowej otoczki śluzowej oraz zmienioną morfologią łupiny nasiennej.

SŁOWA KLUCZOWE: *Arabidopsis thaliana*, morfologia kwiatów, otoczka śluzowa, mutanty, struktura nasion, nerwacja.