

Bizarre lamina margins in *tae* mutant of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae)

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Summary: Phenotypic effects of *tae* mutation on leaves greatly differ from those produced by any known so far mutant genes involved in leaf development in *Arabidopsis thaliana*. This mutation does not affect the basic anatomical structure of the lamina, though it causes looser palisade and spongy chlorenchyma. *Tae* mutation causes local suppression of the lamina growing in width which is locally accompanied by 1) altered generating functioning of the sub-marginal meristem which results in a changed mesophyll development up to its absence in the lamina margin; 2) disharmonious growing in width of adaxial and abaxial sides of the lamina; 3) development of the secondary margin of the adaxial side of the lamina; and 4) local growing of the secondary margin into ectopic lobe(s), the latter being typically absent in *A. thaliana*.

Keywords: leaf anatomy, lamina, primary margin, secondary margin, ectopic lobe, *tae* mutant, *Arabidopsis thaliana*

The cells of the incipient leaf lose their indeterminate state which is inherent in the cells of the shoot apical meristem they have originated from. KNOX genes disabling is crucial for this process (TSUKAYA 2013). Regulation mechanism of such a KNOX gene disabling is disrupted in *taeniata* (*tae*) mutant of *Arabidopsis thaliana* as described briefly by LEBEDEVA et al. (2005). Prolonged expression of these genes in the developing leaf of the mutant is accompanied by ectopic outgrowths which look like lamina lobes, the latter being untypical of this species. Ectopic lamina lobes caused by ectopic expression of KNOX genes were revealed in *as1*, *as2* (SEMIARTI et al. 2001) and *bop-1* (HA et al. 2003) mutants of *A. thaliana*. The leaves of *tae* mutant differ from those of any other mutant described in narrower lamina, corrugated margin and occasional adventitious rosette shoots.

Laminas of the *tae* mutants were morphologically and anatomically scrutinized to elucidate phenotypic effects of the *tae* mutation on the leaf. The lamina margin was under special attention.

Materials and methods

Two *A. thaliana* lines from the collection of the Department of Genetics, Lomonosov Moscow State University, were used for the present investigation. The line of Blanes-M ecotype was sampled as a wild-type specimen. The *tae* mutant line was derived from the Blanes-M ecotype and passed through 5 back crossings with its parental line. Leaves of plants with initiating flowering scapes were fixed with 70% ethyl alcohol.

Light microscopy. Fixed leaves were dehydrated in gradual ethyl alcohol series, dealcoholized in gradual ethyl alcohol–xylol mixtures and twice in xylol and then embedded in paraffin wax. Transverse 10 µm thick microtome sections of the leaves were mounted on slides, deparaffinized, rehydrated and stained with Delafield's Haematoxylin and Carbol Fuchsin according to BARYKINA et al. (2004). The stained preparations were dehydrated and embedded in Canada Balm. Thus

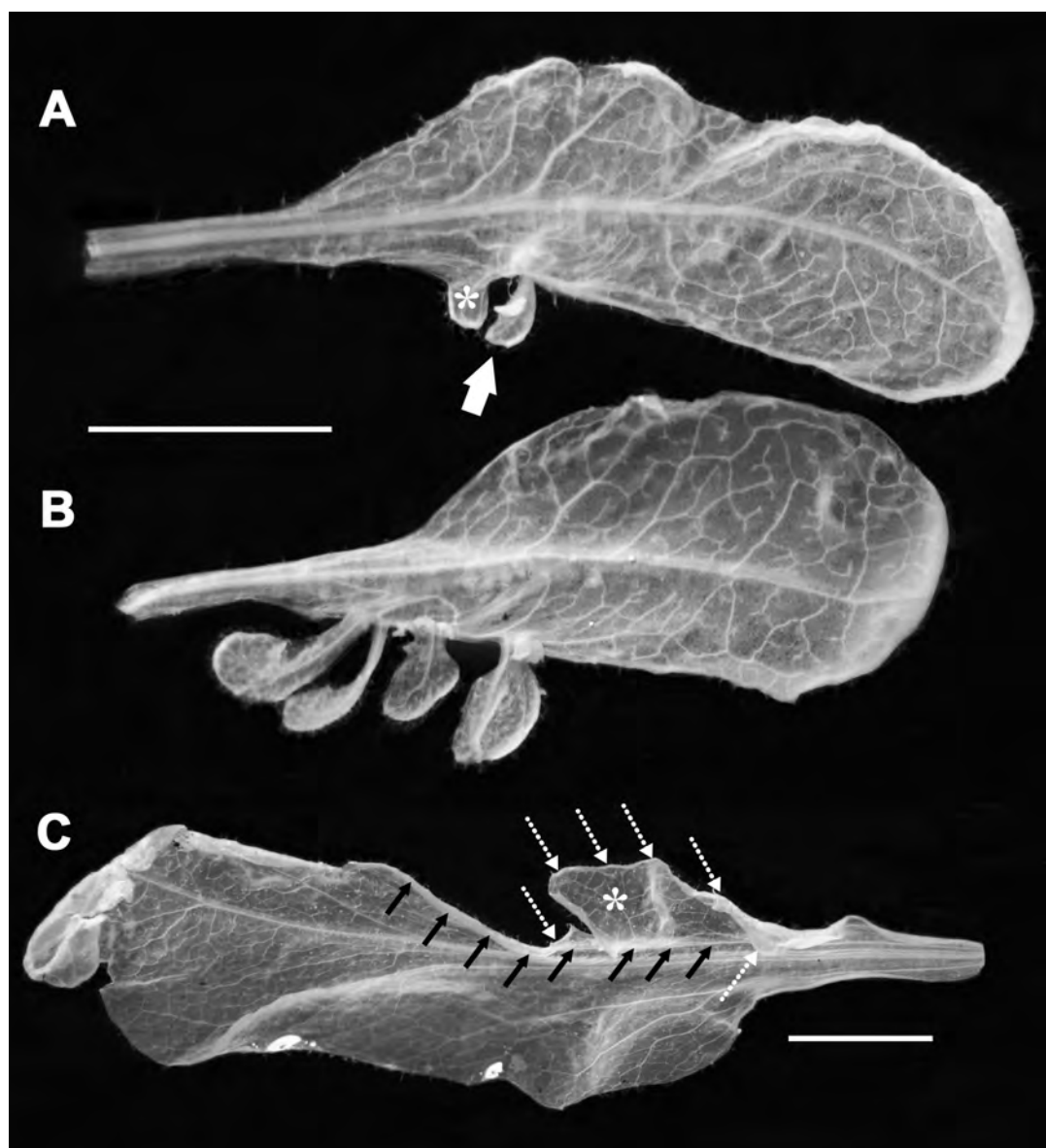


Figure 1. Leaf exterior of *tae* mutant from below, light microscopy. A – asymmetric lamina with ectopic lobe and adventitious rosette shoot; B – asymmetric lamina with a set of adventitious rosette shoots; C – asymmetric lamina with the primary and secondary margins. *asterisk* – ectopic lobe; *black arrows* – primary margin; *dotted arrows* – secondary margin; *white arrow* – rosette shoot. Scale bars = 1 cm.

prepared, they were examined under light microscope Univar (Reichert) equipped with digital camera Nikon Digital Sight DS-Vil.

SEM microscopy. The fixed leaves were dehydrated in gradual ethyl alcohol series, dealcoholized in ethyl alcohol–acetone and acetone series, critical-point dried in HCP-2 Drier (Hitachi) and mounted on stubs. Mounted material was coated with Pd–Au mixture in IB-3 Ion Coater (EIKO) and examined and photographed under scanning electron microscope Camscan-S2 (Cambridge Instruments). The work was carried out at the Electron Microscopy Laboratory of Lomonosov Moscow State University.

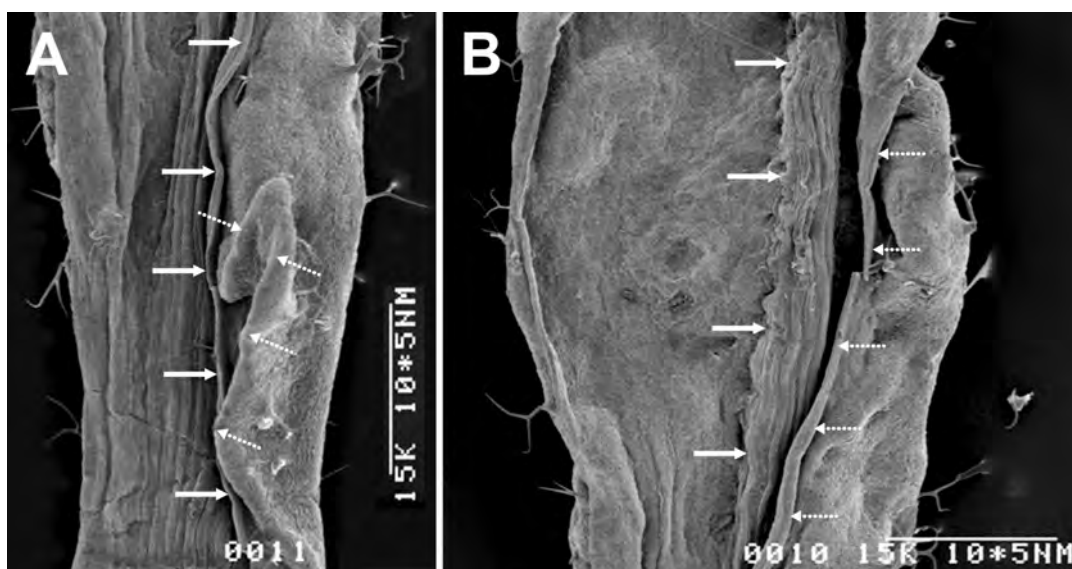


Figure 2. Lamina exterior of *tae* mutant from below, detail, SEM. A – secondary margin and ectopic lobe; B – abaxially turned primary margin and secondary margin. *dotted arrows* – secondary margin; *solid arrows* – primary margin.

Results

The laminae of the mutant are usually much narrower than those of wild-type plants. They range from linear to scapulate. The lamina base is often very asymmetric due to underdevelopment of one of the semi-laminae (Fig. 1A, B). Many petioles tend to grow wider and flatten to result in a gradual petiole–lamina transition. The margin of the underdeveloped semi-lamina is often abaxially turned (Fig. 1A, C). Such a margin is usually accompanied by the secondary one. The secondary margin is mostly a low crest parallel to the original margin (Figs 1C; 2B), but it locally grows into lobe(s) varying in their sizes and shapes (Figs 1A, C; 2A). The adventitious rosette shoots develop near the margin of underdeveloped semi-laminae of some leaves (Fig. 1A, B).

The laminae of mutants and wild-type plants considerably differ in their morphology, but they are very similar in their anatomy (Fig. 3A, B). The two are dorsiventral, amphistomatic, homobaric, double-face pubescent with T-shaped trichomes on large pedestals (Fig. 3F). The epidermal cells, especially the abaxial ones, have wavy anticlinal cell walls. Hereupon, these cells look greatly varying in their sizes in transverse sections of the lamina. The abaxial epidermal cells generally appear smaller than their adaxial counterparts (Fig. 3).

Adaxial palisade chlorenchyma is invariably single-layered and continuous in the midrib (Fig. 3A, B). The abaxial spongy one is always discontinuous in the midrib, where it is substituted by the ground tissue surrounding the single vascular bundle. The palisade cells of mutants are much shorter and much more loosely arranged than those of wild-type plants (Fig. 3A, B). The spongy chlorenchyma is also much looser in mutants than in wild-type plants.

Palisade and spongy chlorenchyma extend to the very lamina margin in wild-type plants (Fig. 3C). The margin is rounded and occupied by a subepidermal strand of dense chlorenchyma of uniform, evenly thin-walled cells which is hereinafter termed submarginal chlorenchyma. This chlorenchyma is here instead of collenchyma typical of dicotyledonous leaves (NAPP-ZINN

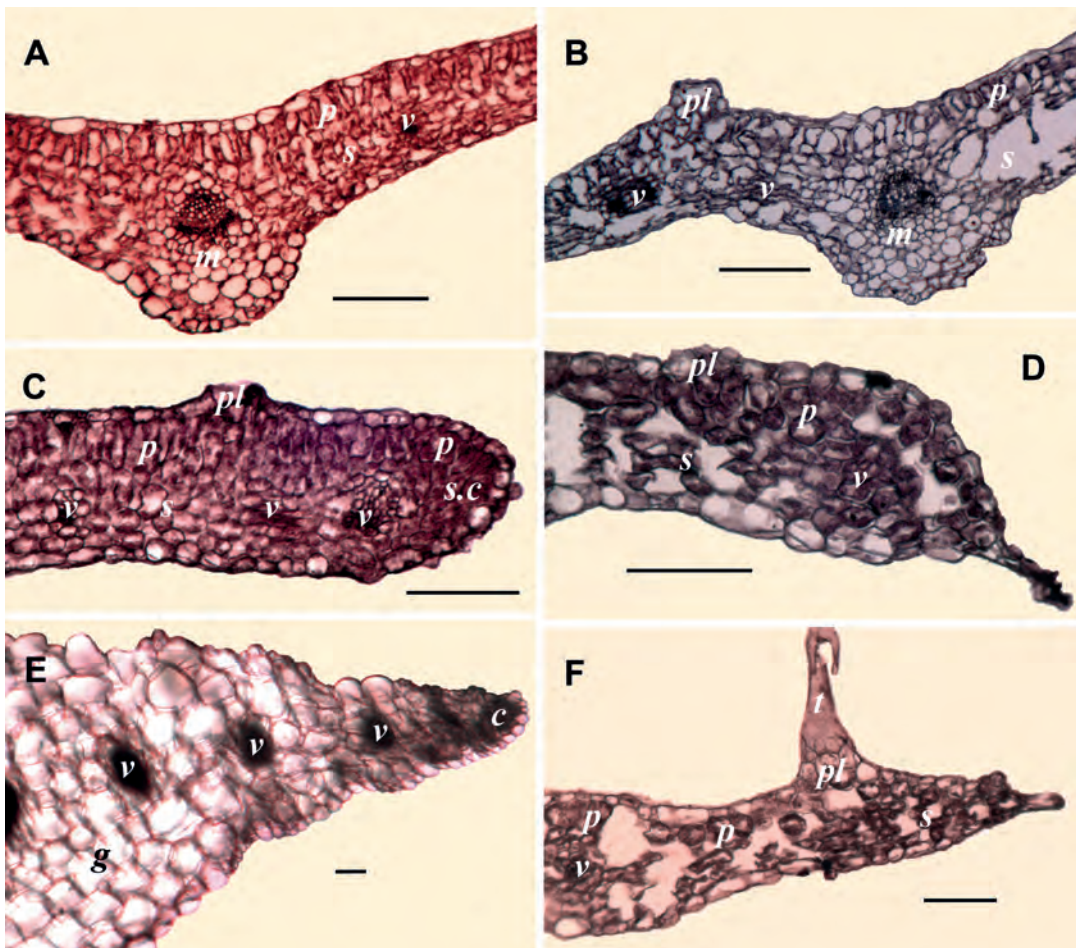


Figure 3. Lamina of wild-type plant (A, C, E) and *tae* mutant (B, D, F), transverse sections. A, B – the low third of midrib region; C, D, F – lamina margin; E – petiole margin. *c* – chlorenchyma; *g* – ground parenchyma; *m* – midrib; *p* – palisade chlorenchyma; *pl* – trichome pedestal; *s* – spongy chlorenchyma; *s.c* – submarginal chlorenchyma; *t* – trichome; *v* – veinlet. Scale bars = 50 μm.

1973). The petiole of wild-type plants is alate, its sharper margins have subepidermal dense chlorenchyma of uniform roundish cells (Fig. 3E).

Lamina margins of the mutants are diversified.

In some developed parts of the lamina, the margin is a little bit sharper than that in the wild-type plants (Fig. 3D). The palisade chlorenchyma almost extends to the very margin, but its cells are nearly isodiametric. They are distinguishable from the spongy chlorenchyma cells only by their tighter arrangement. There is no strand of submarginal chlorenchyma but a single layer of isodiametric chlorenchyma cells hardly discernible from adjoining cells of palisade and spongy chlorenchyma. The inner spongy chlorenchyma is very loose. In other developed lamina parts, the margin is acute and more like the petiole margin of wild-type plants (Fig. 3F). The palisade chlorenchyma is discontinuous near the margin. The submarginal chlorenchyma is completely substituted by the spongy counterpart.

The underdeveloped part of the lamina has mostly an acute margin and neither palisade nor spongy chlorenchyma there (Fig. 4A). The margin is often gradually thinned to 2 epidermises

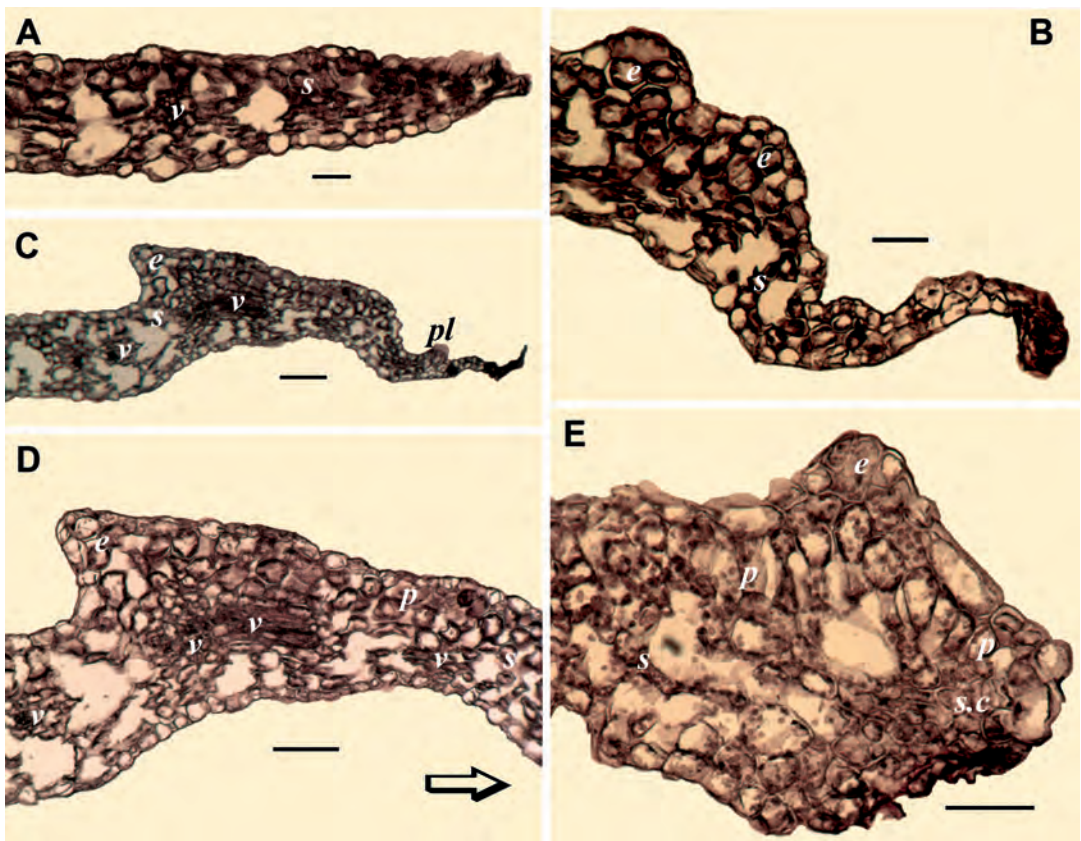


Figure 4. Margin of underdeveloped lamina part of *tae* mutant, transverse sections. A – acuminate margin without palisade chlorenchyma; B – primary margin thinning to 2 cell layers and 2 ectopic secondary margins; C – primary margin thinning to single cell layer and ectopic secondary margin; D – detail of former figure, secondary margin; E – thickened primary margin and secondary margin. *e* – ectopic secondary margin; *p* – palisade chlorenchyma; *pl* – trichome pedestal; *s* – spongy chlorenchyma; *s.c* – submarginal chlorenchyma; *v* – veinlet; *arrow* shows direction to the margin edge. Scale bars = 50 μ m.

without mesophyll in between (Fig. 4B) and even to a single layer of epidermal cells (Fig. 4C). Such a margin is generally turned abaxially (Figs 2; 4B–D). The margin of the underdeveloped part of the lamina is rarely similar to the lamina margin of wild-type plants, but it exceeds the latter one in thickness (Fig. 4E). There is a thick strand of submarginal chlorenchyma of isodiametric cells. Continuous palisade chlorenchyma adjoins the submarginal one in such a margin.

Adaxial secondary margin which accompanies the original margin (Fig. 2B) is median in relation to the lamina turn (Fig. 4C). If it is very low, the secondary margin looks like a trichome pedestal in transverse sections of the lamina. However, it differs from the pedestal in oblique paradermal divisions of subepidermal cells contributing to its development (Fig. 4B,E). Spongy-like but more dense chlorenchyma of uniform cells is in higher secondary margins (Fig. 4D). Some (parts of) secondary margin(s) consist(s) of only 2 epidermises and has/have no mesophyll (Fig. 5). The secondary margins grow locally into lobe(s) (Fig. 2A), the largest being dorsiventral. Two parallel secondary margins are rarely present on the adaxial side of the underdeveloped lamina part (Figs 4B; 5). They sometimes greatly differ in their thickness and anatomical structure (Fig. 5).

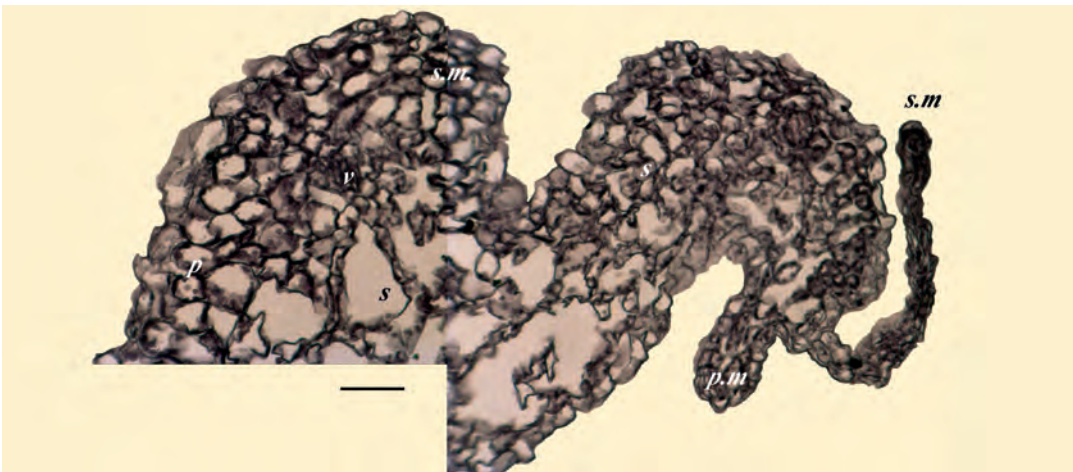


Figure 5. Margins of the underdeveloped part of the lamina of *tae* mutant, transverse section. *p* – palisade chlorenchyma; *p.m* – primary margin; *s* – spongy chlorenchyma; *s.m* – secondary margin; *v* – veinlet. Scale bar = 50 μ m.

Discussion

The results obtained enable phenotypic effects of *tae*, *as1*, *as2* and *bop1-1* mutations to be compared more comprehensively.

In both *as1* and *as2* mutants, ectopic expression of KNOX genes causes lamina lobes which are outgrowths of the primary margin on the place of lamina denticles (SEMIARTI et al. 2001). Such outgrowths also develop on the petiole at later developmental stages (SEMIARTI et al. l. c.). The primary margin never grows into lamina lobes in *tae* mutants investigated. Their lamina lobes originate from the secondary margin which is absent in *as1* and *as2* mutants. Though externally alike, the lamina lobes in these mutants are profoundly different intrinsically. These intrinsic structural differences of the lobes reflect different action of the genes *AS1* and *AS2* and *TAE* in leaf development.

Ectopic lamina-like outgrowths were also revealed on the adaxial side of lamina in *bop1-1* mutant (HA et al. 2003). These lobes resemble those in *tae* mutant in never originating from the lamina primary margin. However, they greatly differ from the ectopic lobes of *tae* mutant in developing near the midrib of the developed lamina and also on the petioles. The *BOP1* and *TAE* genes seem to determine differently leaf development.

The *tae* mutant differs from *as1*, *as2* and *bop1-1* in early ceasing the widening of the basal part of one of the semi-laminas. The widening of the dicotyledonous lamina is typically maintained by 2 meristems, viz. submarginal and plate meristem (ESAU 1965; DALE & MILTHORPE 1983). The plate meristem mostly contributes to lamina widening, whereas the submarginal meristem initiates this widening (FOSTER 1936) and gives rise to narrow lamina edge (GUÉDÈS 1979). Determination of layer structure of the lamina is the basic function of the submarginal meristem (ESAU 1965; DALE & MILTHORPE 1983; ICHIHASHI & TSUKAYA 2015). Figure 3 shows that the lamina layer structure is unaffected in *tae* mutant. Therefore, its submarginal meristem completely retains its basic function to determine typical lamina layer structure.

The generating function of the submarginal meristem is locally distorted. Directions of divisions of its cells seem to be violated to result in less layers of the plate meristem to be generated and in

margin thinning up to 2 epidermises without mesophyll in between and even to single-layered margin. The thicker margin and more-layered plate meristem is a very rare effect of *tae* mutation.

The *tae* mutation certainly greatly affects functioning of the plate meristem. It causes locally underdeveloped semi-lamina by means of precocious suppression of its widening there. It also causes looser mesophyll and discordant growing of adaxial and abaxial sides of the developing lamina. The adaxial side exceeds the abaxial one to result in abaxial turning of the lamina margin. Origin of the secondary margin on the adaxial side of the lamina near its abaxial turn might also be considered a result of disharmonious growing of adaxial and abaxial sides of the lamina. The secondary margin is mostly a low crest filled by spongy chlorenchyma, the latter being a little bit denser than its counterpart in the lamina *per se*. This margin locally grows into the lobe(s) of dorsiventral structure which is/are anatomically similar to the lamina.

Destruction or teratic bifurcation of early leaf rudiment are known to induce regeneration of its submarginal meristem (GOEBEL 1902; FIGDOR 1906; SNOW & SNOW 1941; SACHS 1969). The secondary margin and ectopic lobes on the adaxial side of the lamina of *tae* mutant are probably a result of reparative process(es) to compensate distorted functioning of the plate meristem and discordant interacting between the plate and the submarginal meristem. This assumption is indirectly confirmed by the secondary margin never being detected in the developed lamina parts. Prolonged expression of KNOX genes in leaves of *tae* mutant (LEBEDEVA et al. 2005) could promote such reparative-like processes.

The phenotypic effects of *tae* mutation on leaf development are profoundly original. They have no close analogues among the numerous well-known phenotypic effects of mutant genes involved in leaf development in *A. thaliana*.

Acknowledgements

We are greatly indebted to Dr Sergey R. Majorov for photographs of *A. thaliana* leaves (Fig. 1). We are grateful to Mr. Georgy N. Davidovich, the head, and also to the staff of the Electron Microscopy Laboratory of Lomonosov Moscow State University for opportunity to SEM investigation. The present investigation was supported by Russian Foundation for Basic Research (grant No. 16-04-00437).

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Autor(en)/Author(s): Fedotov Alexey P., Ezhova Tatiana A., Timonin Alexander C.

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