A peculiar structure of the flower in the homeotic barley mutant *tweaky spike*

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Many homeotic genes involved in flower development are also responsible for changes in number of flower elements. Most of these genes are investigated in *Arabidopsis thaliana*, but in other plant species such mutations are also known.

The barley mutant *tweaky spike (tw)* is a new homeotic barley mutant with lodicules converted to stamens and/or carpels in which homeosis is accompanied by alteration of the number of flower organs. Most intriguing is the fact that there are flowers not only with decreased, but also with increased total number of flower elements.

Mutant *tw* will contribute to the characterisation of floral development in barley and other monocots.

Key words: homeotic barley mutant, flower structure, lodicules conversion, number of flower elements

INTRODUCTION

In *Arabidopsis thaliana,* a favourite model plant for genetic studies, a large number of genes is known to control flowering. Many flower homeotic mutants have been isolated, and detailed studies have shown that in some of them homeosis is accompanied by changes in the number of flower elements. So, *Arabidopsis* mutants *AGAMOUS* [1], *CARPEL FACTORY* [2], *CLAVATA* [3, 4], *LEAFY* [5], *SUPERMAN* [6], *ULTRAPETALA* [7], *WIGGUM* [8] have an increased number of floral organs per flower, while the other mutants (*APETALA* [9], *FILAMENTOUS FLOWER* [10], *PISTILLATA* [1], *REVOLUTA* [11], *TOUSLED* [12], *UNUSUAL FLORAL ORGANS* [13], *WUSCHEL* [14]) have a reduced number of flower elements or formed mosaic organs in the flowers.

Mutations affecting plant development in monocots have also attracted attention, particularly in crop species such as maize, rice and barley. Numerous barley mutants with altered flower development are known [15–21], but only in few of them (for example, *laxatum-a; multiovary 7a*) homeotic transformations are accompanied by alteration in the number of flower organs [18–20]. Our investigations show that the list of such mutants can be supplemented by the barley mutant *tweaky spike*.

MATERIALS AND METHODS

Forty-eight ears of barley mutant *tw* (more than 500 flowers) were investigated. The ears were collected from a field of the Botanical Garden of Vilnius University on day 62 after seeding (only the first spike from each plant was used for flower analysis). Flowers were fixed in Carnoy's solution (3:1) and analysed on a stereozoom microscope (Motic). All parts of flowers were observed in detail after the lemma had been removed. The number of flower elements, their homeotic conversion and the number of mosaic organs were registered and statistical analysis was performed using the Excel and Statistic programs.

RESULTS

In our previous works with barley mutant *tw* [21, 22] homeotic conversion of lodicules to stamens and/or carpels was emphasised, but attention was also given to variation of the total number of flower elements. In the present work that phenomenon is examined in detail.

Normally each barley flower has two lodicules, three stamens and one carpel. First, conversion of lodicules and a changed number of flower elements was noted in 70.8% of flowers, but even 29.2% of flowers had the normal structure.

Besides, different alterations in the number of flower elements were noted (Fig. 1). Various developmental abnormalities were also observed for stamens or carpels: A – there is one stamen with appendage of undeveloped stamen; C – the flower has a carpel with four stigmas instead of two and a mixed structure – stamen with semi-developed carpel; D – the carpel has three stigmas, additional stamens are not fully developed; E – of four stamens two have a common stem; one can suppose that these are twin-double stamens as in A, but significantly much more developed; F – the flower has two carpels, but both are grown together, *i.e.* a twin-double nature of such carpels may be also supposed.

Thirdly, the main group of flowers had three stamens, but a significant part of flowers (17.1%) had two stamens. The number of stamens varied from 0 to 4 (Fig. 2 A). The number of carpels was even more uniform: 96.6% of flowers had the normal number of carpels – only one, but there were also flowers with two carpels (1.6%) or with no carpels at all (1.8%) (Fig. 2C)

Attention was focused on alterations of lodicules (Figs. 2B and 3). Most of *tw* flowers had lodicules converted to other structures. Only 29.2% of flowers had the 2IVL– normal number of lodicules (two) (Fig. 3). A significant part of flowers (9.3%) had no lodicules at all (Fig. 2 B). Interestingly, 1.8% of flowers had an increased number of lodicules (three).

However, a significant part (55.5%) of flowers had one or both lodicules homeotically converted to sta-

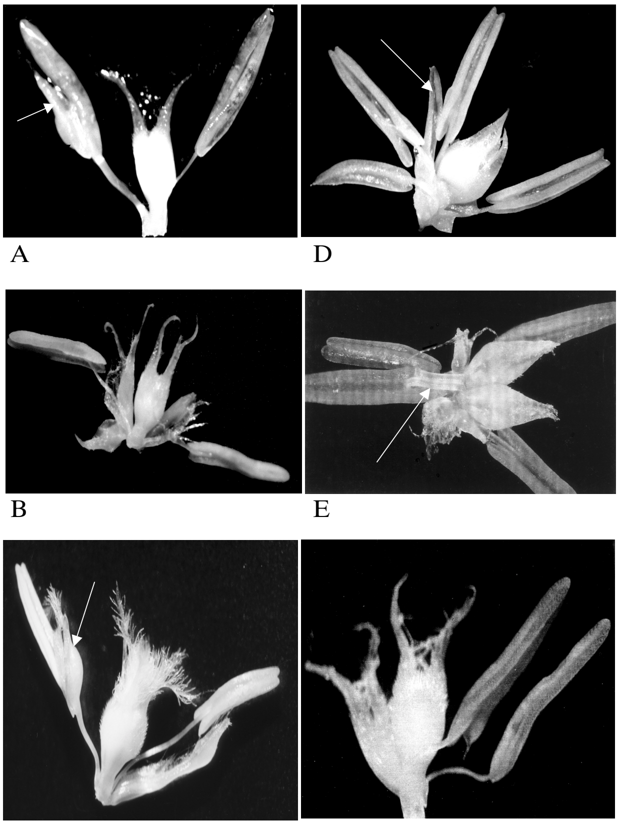

Fig. 1. Flowers of barley mutant *tw* with various number of flower parts. **A**. A flower consists of one carpel and two stamens. One of the stamens has an appendage (arrow). **B**. Total number of flower elements is normal (six), but the flower has two carpels, two stamens and two lodicules. **C**. Flower with one carpel (with four stigmas), two stamens and one lodicule semi-converted to stamen. One of the stamens has part of carpel (arrow). **D** and **E**.Flowers with an increased total number of flower elements: **D**. One carpel (with three stigmas), five stamens, one of them is converted from lodicule (arrow); **E**. Four stamens, two of them have a common stem (arrow), two normal carpels and two lodicules. **F**. Flower with two accreted carpels and two stamens

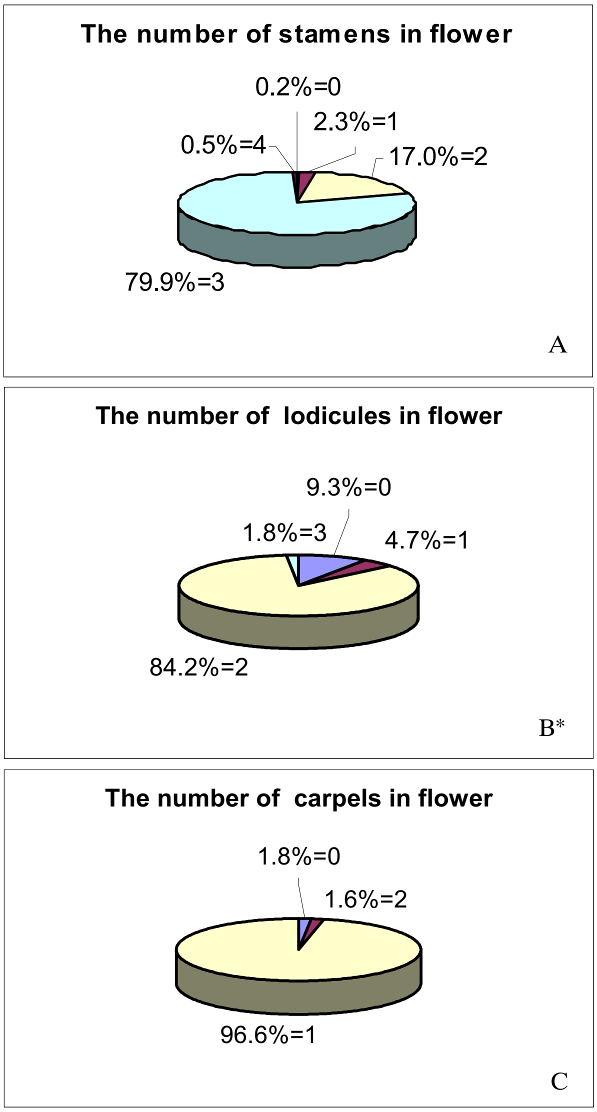

Fig. 2. The frequency of flowers (%) of barley mutant *tw* with various number of flower elements: 1, 2, 3, 4 – number of stamens (A), lodicules (B*) or carpels (C). B* – *84.2 = 2 – includes all flowers in which the original nature of homeotic conversion from normal number (two) of lodicules can be detected (see Fig. 3)

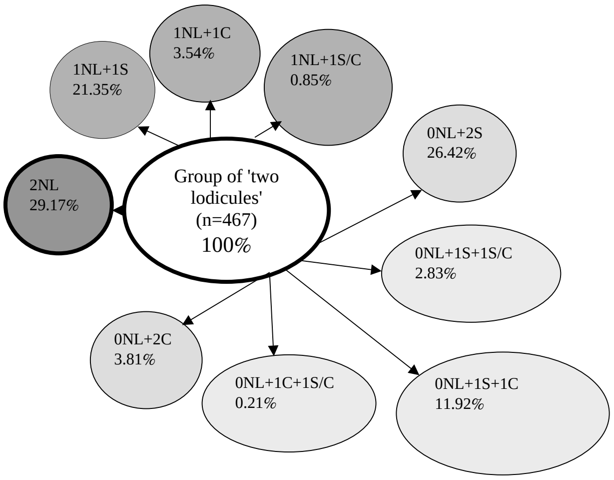

Fig. 3. Distribution of flowers with initially two lodicules proposed (84.2% from Fig. 2 B)

mens (S), carpels (C) or mosaic structures – stamen/carpel (S/C). Conversion of such lodicules to carpels or stamens could be easily detected, because they remained elements of lodicules. It was expressed to various extent, but in all cases it was possible to judge about the primary origin of such structures.

Most frequent (26.42%) was conversion of both lodicules to stamens (Fig. 3). The next frequent (21.35%) group had one of the lodicules was converted to stamen. Significant is also a group (11.92%) in which one lodicule was converted to stamen, and the other to carpel. Conversion of both lodicules to carpels was not so frequent, but it did take place (3.81%).

Thus, the most frequent type of conversion was from lodicules to stamens. In total, 467 flowers with 934 lodicules were investigated, and about half of them were converted to stamens (in Fig. 3–44% S and 3.5% S/C). About the same frequency of flowers had the both lodicules converted to other structures (Fig. 4). Lodicules converted to stamens differ in shape and in the anther tissue structure and form, but all of them were smaller

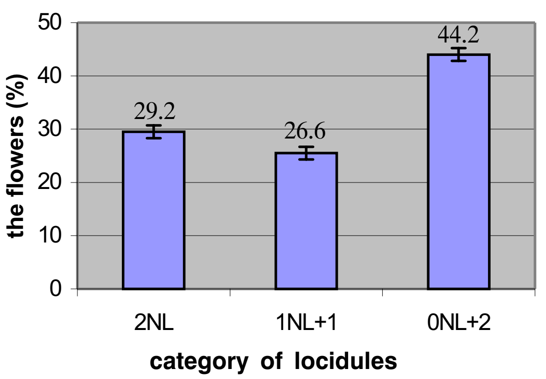

Fig. 4. Distribution of flowers with two normal lodicules (2NL), with one converted lodicule (1NL+1) or both homeotic converted lodicules (0NL+2)

than normal ones and had only one loculus instead of four in the typical case. Cross-sections showed that pollen was formed inside of the additional stamens, but all pollen grains were sterile. The additional carpels were smaller also and had no embryo sacks.

DISCUSSION

Although monocot flowers and inflorescences differ significantly from eudicots, a considerable progress in understanding the function of the floral identity genes was made after cloning the *silky1* gene of maize. In the *silky1* mutant the stamens in tassels develop as carpel-like structures, and the lodicules are replaced by palea-like organs [23]. This data suggest that lodicules are homologous to eudicot petals and that the ABCD model of organ identity genes can be applied to grass species. Lodicules were also transformed to palea/lemma-like structures in transgenic rice plants [24]. The feature of *tw* mutant is that the lodicules are converted to generative (pistils or stamens), but not to vegetative structures.

The same phenomenon is found in two other homeotic barley mutants, but their phenotype differs absolutely from that of the barley mutant *tw*. The *laxatum-a* mutant of barley has several distinct effects on plant phenotype, including a homeotic replacement of lodicules by stamens, or occasionally carpeloid stamens. The *laxatum-a* plants showed a strong tendency to produce more tillers, the ear length was increased [18]. In the *multiovary 7a* mutant of barley a variation in the number of carpel-like structures and lacking stamens were observed. The carpel-like structures looked abnormal, sometimes terminating in stamenlike structures. The ear was twisted and compacted with shortened awns [19].

Phenotypically, *tw* mutant is characterised by a gradient from a semisterile lower part of the spike to a ‘superdeveloped’ multirowed upper part of the spike with very well developed seeds [22]. We supposed that such phenotype might arise from hormonal imbalance. Abnormal size and/or morphology have been observed in mutants impaired in imbalance of auxin, gibberellins and ethylene [25–29].

The barley flower is asymmetrical, and no whorls are seen, so it is hard to say now in what position the new flower organs in the barley mutant *tw* appear. But it is quite possible that a defect in shoot apical meristem is involved in this developmental process [4, 7]. As noted in previous work, deviation from the norm in shoot apex formation and flower development appeared at the fifth stage of organogenesis in the top of the ear [30].

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YPATINGA HOMEOZINIO MIEŽIŲ MUTANTO *Tweaky spike* ŽIEDO STRUKTŪRA

Santrauka

Homeozės reiškinį, kai žiedo lodikulės virsta kitais organais – dulkinėmis ir/arba piestelėmis arba jų dalimis, miežių mutanto *tw* žieduose lydi ir žiedo dalių skaičiaus pokyčiai. Ištyrus 560 žiedų nustatyta, kad mutantui *tw* labiau būdingas žiedo elementų sumažėjimas (21,0%) negu padidėjimas (1,2%).

Išskyrus į atskirą grupę žiedus, turinčius po 6 žiedo elementus (n = 467), buvo kiekybiškai įvertintas homeozės reiškinys. Nustatyta, kad: 1) lodikulių virsmas vyksta nepriklausomai vienas nuo kito tiek žiede, tiek ir varpoje; 2) apie 30% žiedų turėjo abi normalias lodikules ir tiek pat žiedų turėjo abi lodikules, konvertuotas į dulkines. Šiek tiek mažiau (21%) žiedų viena lodikulė konvertavo į dulkinę, o kita liko normali. Į dulkines konvertuotos lodikulės paprastai turėjo vieną dulkializdį vietoj keturių; jose formuojasi sterilios žiedadulkės. Apie 10% tirtų žiedų turėjo įvairias anomalijas: kuokelius su papildomais priedais, kurių kilmė gali būti dulkinės ar piestelės (purkos) audinys; piesteles, turinčias ir vieną, ir tris–septynias purkas. Šis reiškinys yra būdingas daugeliui *Arabidopsis* homeozinių mutantų [1–14].

Homeozinio miežių mutanto *tw* tyrimai yra svarbūs tiriant žiedų vystymosi procesus tiek miežiuose, tiek ir kituose vienaskilčiuose.