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Comparative analysis of genes affecting lodicule development in grasses

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Lodicules are grass-specific flower organs determined by B-class flower genes. Comparative analysis of the interaction of barley mutant *tweaky spike* with B-class barley mutant *lax-a* alleles and other known genes and mutants that affect lodicule development in barley, rice and maize allow to conclude that also other genes, not only of B and C classes, directly and indirectly determine lodicule expression. The barley mutant *tweaky spike* is among such genes and does not belong to B-class genes.

Key words: lodicule genetics, flower genes, barley mutants, *tweaky spike*, unusual inheritance

INTRODUCTION

Recently, much attention has been given to comparative genetics of flower development [1–6]. Comparative investigations of flower determination are based on dicot *Arabidopsis thaliana* and monocot grasses rice (*Oryza sativa* L.) and maize (*Zea mays* L.). The nearly complete genome annotation allows to use in those plants reverse genetic tools and to compare flower structure determination differences in both groups of plants [2–4, 7–9].

In general, the development of flower organs is explained by the model of ABC genes. The ABC model of flower development was originally described for eudicots, concretely *Arabidopsis thaliana* Heynh. and *Antirrhinum majus* L. [6, 7]. According to it, A class genes specify sepal fate in the first whorl; A plus B genes specify petals in the second whorl; B plus C genes give rise to stamens in the third whorl, and only C genes determine carpel development in the fourth whorl. This model has been expanded to incorporate two new classes the genes. The D class genes are responsible for ovule development, while the E class genes are expressed together with all other classes of genes. With the exception of the A class gene *APETALA2*, all the above-mentioned genes are member of the MADS-box family of transcription factors and act by forming dimers and complexes of a higher order [1].

Inflorescences and flowers of grass (Poaceae) species have characteristic structures differing distinct from those of eudicots. For instance, the normal flower of barley (and typically of other Poaceae plants) has two lodicules, three stamens and one carpel and is surrounded by two bract-organs – a palea and a lemma. Two empty glumes that are regarded as vestigial organs of two lower florets substended the apical floret in an alternative arrangement and two rudimentary glumes substended the empty glumes. The structure that contains the floret and the empty glumes is considered as a spikelet.

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Variation in flower structure has also place. So, the rice bisexual flowers have six stamens instead of three as in barley. Flowers of the monoecious plant maize have two lodicules only in male flowers. Female flowers are without lodicules [1, 7].

Investigations made on monocot plants such as rice (*Oryza sativa* L.) suggest that in general the ABCDE model could be extended to monocots. Recently it has been found that for the fourth whorl the decisive role belongs not a *AGAMOUS* gene orthologs, but to the *DROOPING LEAF* (*LD*) gene which belongs not to the *MADS* but to the *YABBY* family of transcription factors [8]. The view that the sepals have been lost during evolution is also discussed [1, 2].

Of course, interest to the lodicule determining genes comes first from evolutionary genetics and flower evolution in grasses. However, investigation of the lodicule genetics is also of practical interest. Recently, a relationship has been found between cleistogamy and lodicule structure, especially lodicule size which may be regulated genetically or with plant hormones. It seems promising by such mode to control the spread of GMO products in the environment [13, 14].

On the other hand, today it is obvious that not only B class genes influence lodicule development, and the interaction of genes belonging to different classes and their different mode of action are of interest.

In barley, a typical B class gene is *laxatum-a* (*lax-a*). In mutant alleles, *lax-a* lodicules are converted to stamens, and flowers have five stamens and no lodicule [15–18]. Contrary to *lax-a*, in the barley mutant *tweaky spike* (*tw*) only about half or even less flowers have lodicules converted to stamens, and other disturbances of normal flower development are also observed [19]. Subsequent investigations showed significant variations of flower structure in plants grown in different years and under different environmental conditions.

The interaction of *lax-a* and *tw* alleles in F_1 was investigated in our previous work [20], but analysis was restricted only to the F_1 generation of *lax-a* and *tw* hybrids, and it has been shown that *lax-a* and *tw* are in different loci. A complementation effect was

observed in all crosses [20]. Interest in renewing these investigations arose also from the variation in *tw* phenotype: we presumed that epigenetic mechanisms are one of the causes of that variation. The result of interaction between the different genes was supposed to depend also on these mechanisms. We hoped to choose a model for such investigation.

MATERIALS AND METHODS

The barley mutant *tw* used as the mother plant is of original origin induced by chemical mutagens in barley cv. 'Auksiniai II'. The latter was primarily obtained from the Lithuanian Institute of Agriculture and was used in the present work as a *Wild Type* (*WT*). The *laxatum* mutants used in the first experiment (2004) were of different genotypes *a, aa, ab, ac, ae, ag, b, c* and came from two different collections: *laxatum aa* (1572), *ab* (1573), *ac* (1574), *ag* (1575), *ae* (2041), *a* (2103), *a* (1775), *aa* (2276), *ab* (2277), *a1* (2278) – all from USA, Dacota; *tweaky and missing kernels* (1119), *tweaky N18* (111) – from Aberdeen, Idaho, USA (all that material was presented by the National Small Grains Res. Facility Barley Genetic Stocks Collection, Aberdeen, Idaho, USA (numbers in parentheses – numbers of accessions in that collection). The other part of *laxatum* mutants was from the Nordic Gene Bank (Alnarp, Sweden). There were *lax-a.01*, *a.54*, and *a.434*. For the second experiment (2005–2006), new lax-a alleles were introduced in the complementation test and F_2 analysis. All material was from Nordic Gene Bank. There were 11 new mutants: *lax-a.0.4, 08; .20; .37; .39; 208; 218; 222; 278; 286; 373; 450*. All mutants were induced by chemical and ionizing radiation mutagenesis in two barley cultivars 'Bonus' and 'Foma' and one – in cv. 'Kristina' (in general, the latter mutants have a number from >200; concrete initial cv. shown in Table 1).

All material was preliminarily planted for propagation; hybridization was made and the hybrid material was examined in the experimental field of Botanical Garden of Vilnius University.

For F_2 analysis, only part of F_1 material was sown in 2 m^2 plots. In the present work, the results of both experiments in F_2 generation are presented.

Flowers were fixed in Carnoy's solution (3: 1) and analysed on a stereozoom microscope (Motic). All parts of basic flowers were examined in detail after the lemma had been removed. The number of flower organs, their homeotic conversion and the number of mosaic organs were registered.

For phenotype analysis we used mature plants and their parts. Statistical analysis was performed using the Excel and Statistics programs.

Table 1. **Independent inheritance of barley *Laxatum-a* and *Tweaky spike* genes revealed by F_2 analysis**

Crossing		Number of plants in F_2 with phenotype[1]				
tw × *lax-a*	Phenotype in F_1	N	*tw*	*lax*	*lax/tw*	Phenotype ratio
2004						
× *lax-a* (2103)	N	296	90	65	18	16.4 : 5 : 3.6 : 1
× *lax-a* (1775)	N	199	71	41	13	15.3 : 5.5 : 3.2 : 1
× *lax-a.01*	N	110	56	26	13	8.46 : 4.3 : 2 : 1
× *lax-a.54*	N	183	52	34	8	29.9 : 6.5 : 4.3 : 1
× *lax-a.434*	N	133	34	55	5	26.6 : 6.8 : 11 : 1
Total	N	921	303	221	57	16.2 : 5.3 : 3.9 : 1
						χ^2 = 36.3; P < 0.01
2006						
× *lax-a.04*	N	128	37	3	3	42.7 : 12.3 : 1 : 1
× *lax-a.08*	N	142	45	27	9	15.8 : 5 : 3 : 1
× *lax-a.20*	N	248	82	25	12	20.7 : 6.8 : 2.1 : 1
× *lax-a.37*	N	198	61	44	20	9.9 : 3.1 : 2.2 : 1
× *lax-a.39*	N	279	90	42	24	11.6 : 3.8 : 1.8 : 1
× *lax-a.208*[x]	N	280	114	32	11	25.5 : 10.4 : 2.9 : 1
× *lax-a.218*[x]	N	188	72	18	8	23.5 : 9 : 2.3 : 1
× *lax-a.222*[x]	N	208	105	25	17	12.3 : 6.2 : 1.5 : 1
× *lax-a.278*[x]	N	178	61	19	6	29.7 : 10.2 : 3.2 : 1
× *lax-a.286*[x]	N	223	65	33	21	10.6 : 3.1 : 1.6 : 1
× *lax-a.373*[xx]	N	91	34	0	0	2.8 : 1
× *lax-a.450*	N	238	111	33	20	11.9 : 5.6 : 1.7 : 1
Total	**N**	**2401**	**877**	**301**	**151**	**15.9 : 5.8 : 2.0 : 1**
						χ^2 = 345.4; P < 0.01

[1] N – normal spike phenotype; *tw* – *teaky spike*; *lax*/*tw* – traits of both mutants ('double mutant' phenotype); as mother barley *tw* was used, crosses differ in *lax-a* alleles (as father); [x] – arose from cv. 'Foma'; [xx] – arose from cv. 'Kristina'; the rest in 2006 – from cv. 'Bonus'.

RESULTS AND DISCUSSION

Analysis of the new *lax-a* allelic mutants from the Nordic Gene bank and all of Swedish origin [15–17] confirmed fully our previous conclusion that *Tw* and *Lax-a* are two independent loci [20]. Complementation effect was observed in all test combinations of *tw* mutant with *lax-a* alleles (Table 1). This conclusion was especially strengthened by results of the F_2 (Table 1). In both series of hybridisation analysis of *tw* with various *lax-a* mutants, the dihybrid segregation mode was observed. Plant phenotypes in F_2 after hybridisation of *tw* with one of *lax-a*, namely l*ax-a.54*, are shown in Fig. 1.

Fig. 1. Segregation in F_2 to four phenotypes in the main group of hybrids *tw* × *lax*. Left: parents – *tw* (as mother plant) and *lax-a.54* (as father plant); right (from left): normal, *tw*, *lax* / *tw* and *lax* hybrids in F_2

The higher part of normal phenotype plants in the segregation ratio is easily explained by the worse survival of the mutant phenotypes. It is a well known phenomenon and is true also for *tw* or *lax* phenotypes. It is especially clear in double mutant *lax*/*tw* phenotypes (Table 1).

We observed no variation in the progenies of any newly tested *lax-a* alleles as could be expected from variations of flower structure of *tw* type mutants and from two hybrid combinations in F_1 *lax-a1* (accession № 2278, USA) and *lax-a.54* [20].

However, there were two *lax* mutants (both from USA), namely *lax-aa* (1572) and *lax-ab* (1573), in whose F_2 hybrid generation a significant variation was observed (Table 2 and Fig. 2). This variation is impossible to express in any Mendelian relation. Indeed, in variation includes traits that do not indicate a relationship to the development of flower organs such as six-row spikes. The other genes were also involved in the segregation process. F_1 plants were of normal phenotype.

Furthermore, surprising were also part of duplications from the USA collection: *lax-aa* (2276); *lax-ab* (2277); *lax-ac* (1574); *lax-ae* (2041), *lax-ag* (1575) and *lax-a1* (2278). The latter mutant was among the two that showed a relatively high variation (9.1%) of flower structure in our previous work [20]. Results from that group of *lax* mutants are shown separately (Table 3). As was expected, in F_1 all plants had the normal flower structure. However, in F_2 there was an unexpected fact: the ratio of progeny segregation was 3 : 1 as if for monohybrid crosses (Table 3). Such ratio is possible in two cases: if *tw* and the test *lax* loci are closely linked or even if intraallelic complementation and a composite locus (supergene) are present.

Table 2. **Barley genotypes showing exclusively high variation in F_2 and complementation effect in F_1**

Genotype of father plants *tw* ×	Phenotype in F_1	Plant phenotypes (%) in F2							
		N (6)[1]	*Semi-lax,* but long spikelets (11)	*Semi-lax* (8)	*tw*	*tw* multiple row (3, 4)	Six-row	Six-row *semi-lax* (7)	Six-row *lax* (9)
×*lax-aa* (1572)	N	51.2 ± 2.8	5.8 ± 1.1	4.0 ± 1.1	21.3 ± 2.3	4.6 ± 1.2	5.2 ± 1.2	5.8 ± 1.3	2.1 ± 0.8
×*lax-ab* (1573)	N	47.6 ± 2.3	2.6 ± 0.7	4.6 ± 1.0	18.3 ± 1.1	6.2 ± 1.1	16.7 ± 1.8	2.0 ± 0.7	2.0 ± 0.7

[1] Number in brackets: phenotype number is shown in Fig. 2.

Table 3. **Barley genotypes showing complementation effect in F_1 and monohybrid segregation in F_2**

Genotype of father plant	Phenotype of F_1	Plant number in F_2 with phenotypes		N / tw ratio	χ^2
		N	tw		
tw ×× *lax aa* (2276)	N	260	92	2.8 : 1	0.24
tw ×× *lax ab* (2277)	N	308	93	3.3 : 1	0.65
tw ×× *lax ac* (1574)	N	184	65	2.8 : 1	0.19
tw ×× *lax ae* (2041)	N	320	119	2.7 : 1	0.98
tw ×× *lax ag* (1575)	N	178	49	3.6 : 1	1.50
Total	**N**	**1250**	**418**	**2.99 : 1**	**0.003**
tw ×× *tweaky* N18	N	253	92 + 21	2.7 : 1	0.81
tw ×× *twmk2*	N	208	60 + 33	3.3 : 1	0.5
twmk ×× *tw2*	N	224	55	4.1 : 1	4.29
Total	**N**	**685**	**212**	**3.2 : 1**	**0.85**
tw ××lax a1 (2278)		258	104	2.5 : 1	2.68

[1] Phenotypes as father plant *tweaky N18* (as. № 111, USA); [2] *twmk – tweaky and missing kernels* – reciprocal crosses with *tw*; [3] several traits of twmk.

Fig. 2. Two crosses *tw* with *lax aa* (1572) or *lax ab* (1573) which revealed a high hybrid variation in F_2.
1 – *tw* (as mother plant); 12 – *lax ab* (1573) (as father plant); 2–11 – various hybrid phenotypes: 2–5 – *tw* type (2 – *super tw*, 3 and especially, 4 – as multiple row) – for all there is seen influence of hybrid state; 6 – seminormal; 7 – six-row, but two have distichous rows of awns as *lax*; 8–11 – various *lax* phenotype expression (10 – *super-lax*)

Unexpected results were obtain also for two mutants of the *tweaky* phenotype introduced in crosses with *tw* mutant (Table 3) (both mutants from the USA collection). There were *tweaky N18* and *tweaky and missing kernel*. With the latter, reciprocal crosses were made. In those combinations of crosses, two unexpected and controversial facts were fixed. First, in F_1 all plants were of normal phenotype with a normal flower structure, implying that these mutants must be attributed to different genes.

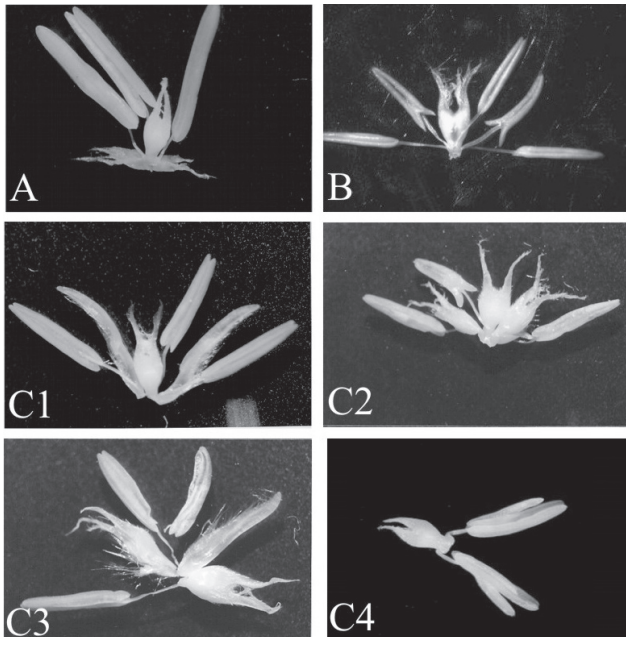

Fig. 3. Flower structure of normal barley plants (A), mutants *lax-a* (B) and variation in flower structure of mutant *tw* (C)
A – *WT*: 2 lodicules (L) + 3 stamens (S) + 1 carpel (C); B – 0L5S1C; C – 1 – 0L5S1C (it is easy to recognize normal S and converted S from L); 2 – 0L3S3C; 3 – 0L4S2C (one of them with appendage); 4 – very strong alteration of flower: 1C and two 'twin' S.

The second unexpected fact was segregation in F_2 as if it were a monohybrid cross. The ratio was about 3 : 1 (Table 3). Such results would be comprehensible if the test mutants were allelic.

Mutants of *tw* type have altered not only the structure of spike (Figs. 1 and 2), but also the form and structure of flower (Fig. 3, C1–4). The most frequent was conversion of both or one of the lodicules to stamens [19]. Conversion of lodicules to stamens is a typical phenomenon for B class mutants. In barley, it is *lax-a* (Table 4). In its flowers, conversion of lodicules to stamens is observed, and most flowers have no lodicules but five stamens and, as in a normal flower, only one carpel (Fig. 3, B) [15–18]. Typically *WT* genotype barley plants have two lodicules (L), three stamens (S) and one carpel (C) (Fig. 3, A).

In the beginning we supposed that *tw* mutants belong to mutations of B class genes. This conclusion was grounded on the fact that the most frequent is conversion of both lodicules to stamens (26.4%) and a flower has five stamens as *lax-a* mutants (Fig. 3, B). In the second place is conversion of one lodicule to stamen and a flower has four stamens [19]. However, many-year observations changed this preliminary opinion.

First, a significant variation in flower structure was observed even in the first work [19]. A significant part of flowers (about 30%) retained a normal structure (2L3S1C). Second, many-year observations fixed the gradient of alterations in spike and flower structure. It is not characteristic of B class mutants. If to divide the spike of *WT* genotype plants into three parts – upper, middle and lower, – the most developed is the middle part. It is seen also in Fig. 1. Third, *tw* mutant alleles vary in the expression of mutant phenotype. Mutant traits are best expressed in the *tw* allele. This mutant allele was also investigated in the present work. The other *tw* alleles such as tw_1, tw_2 and others, have a more slight expression of the mutant phenotype, including the gradient of spike and flower development.

Table 4. **Genes and mutations directly or indirectly acting on development of grass lodicules**

Gene class or expression type	Mutation or mode of *knock-out* mutation	Plant	Phenotype effect, alleles	Refs
B	*lax-a*	Barley	L → C, flower formula 0L5S1C; many alleles	15–18, 20
?	*mo6b*	"	S → C and L → sepal-like structures	21, 22
?	*tw*	"	Preferently L → S or rare L→ C, variation – about 40% of normal flowers, 11 alleles, different expression	19, 20
?	*tweaky N18*	"	Only 25% altered flowers, variation in flower structure	20
	tweaky and missing kernels	"	? only about 8% have sterile flowers	20
B/ort*AP3* = *OsMADS16*	*spw1*	Rice	L → to palea-like structures and S → C	23–26
B/AP3	*silky1(si1)*	Maize	L → lemma and palea-like structures	31
OsMADS2	RNAi	Rice	Expressed in L; L affected, S normal	23, 25
OsMADS45		"	Expressed in L	23, 25
C/OsMADS3 = ort*AG*	RNAi	"	Increased number of L, L → S	32–33
"	transgene	"	Ectopic expression – L → S	33
OsMADS58 = ort*AG*	RNAi	"	The same as for OsMADS1/lhs1	33
OsMADS1 = *SEP*	lhs1	"	Leafy lemma, palea and L, number of S reduced, occasionally extra C or floret	32, 34
"	RNAi	"	All 4 whorls, including L	32, 34, 38, 39, 12
LHS1 = A and C		"	Expressed in 1 and 4 whorls (according to 12)	12
B+C/*AG*	*si1+zag1*	Maize	L → to lemma and palea-like structures, additional lemmas and palea whorls	31
undetermined	*opb1,2*	Rice	Elongated L, partially glume identity, other flower alterations, mosaic L/S	30
	fib	"	Sometimes L → S or L / S mixed organs	27
	apo1	"	L increased at the expense of S	28, 29
Meristem				
OsLRK1 = ort*CLAVATA1*	RNA antisense	"	Increased number of L and other flower organs	44
"	*fon2, fon1(?)*	"	Increased number of L	26, 29, 46
Hormones				
Cytokinin	*log*	"	Number of flower organs, including L, decreased	47
Auxin	Physiological effect	"	Regulation of L size	49

Abbreviations: ort – orthologous; L – lodicule; S – stamen; C – carpel; full name of gene – see in text.

In the *tw* mutant, the gradient of spike and flower structure is well expressed (Fig. 1 and Fig. 2–1). The upper part of spike became many-rowed and the number of rows is irregular. The upper part of the spike we called the 'crown'. The crown is characteristic also of segregation products observed after hybridization of *tw* with *lax*. In hybrids, this trait is even strengthened, and it was even called a *super-tw* phenotype (Fig. 2: 3–5 and especially 2).

In the upper part of the spike, a higher degree of flower structure alterations is also observed. In this part of spike, flowers with an increased number of carpels are more frequent and also other, even drastic, alterations in flower organ number were fixed (shown in Fig. 3, C: 3–4 and in [20]).

Results of our previous [20] and present investigations confirm our view that the *tw* locus does not belong to B class genes and determines the flower structure in another way.

In barley, only a few mutants are known to affect the development of lodicules (Table 4), and only *lax-a* is attributed to B class gene mutations [18]. A large collection of *lax* mutations exists (*lax-b*, c and others), but they have no relation to the development of lodicules. They have a relation to *lax-a* only in some spikes. Genetic analysis has shown that they are inherited independently and belong to different loci [16, 17]. In rice, Lax genes such as *LAX PANICLE (LAX)* have also been revealed, and they encode a putative transcription factor with the plant-specific basic helix-loop-helix (bHLH) domain and are expressed at the boundary between the lateral and the apical meristems [8]. So, they have no direct relation to flower development, but may exert an indirect effect (see further).

A large collection of allelic *lax-a* mutants has also been created [15–17]. Nevertheless, most of them are characterized by variations in flower structure. Our experience with 11 allelic mutants of the *tweaky spike* locus shows a high variation in the number of flower organs. The other *tweaky (spike)* phenotype mutants are in genetic collections, but information about their flower structure is very sparse. The two mutants, *tweaky and*

missing kernels, *tweaky N18* have really altered, but only part of flowers. So, *tweaky N18* had about 25% of flowers with a changed number of lodicules and other flower organs, and the flower formula was as follows: 1L4S1C; 1S1C; 4S2C; 4S1C; there were even flowers with 3S0C and others [20]. So, we observed a variation of flower structure like in *tweaky spike* mutants [19, 20].

The mutant *tweaky and missing kernels* (our abbreviation *twmk*) fits into that group only according to spike structure. It resembles the mutant rice gene *LAX PANICLE*. Part of *twmk* flowers are really altered, but the alterations are of another type. About 8% of *twmk* flowers were sterile (without sexual organs) or even only with a glume (0L0S0C) (Table 4) [20]. Based on the complementation test, in our previous work we concluded that *tw*, *tweaky N18* and *tweaky and missing kernels* belong to different loci [20]. However, the results of F_2 cast doubt on this conclusion. But such results of segregation between *tw* × *tweaky N18* or *tw* × *tweaky and missing kernels* are fully real if different loci are closely linked or a composed *tweaky* locus exists, and intraallelic complementation is observed.

The other known barley mutant that affects the development of lodicules is *multiovary 6b (mo6b)* (Table 4). The phenotypic expression of *mo6b* is very close to those which in grasses are attributed to B class genes: stamens are converted to carpels and lodicules to sepal-like structures [21, 22]. The *mo6b* expression is very similar to the well examined rice mutant *SUPERWOMAN1 (SPW1)*. In the rice mutant *spw1*, lodicules are homeotically transformed to palea-like organs and stamens are converted to carpels. It has been shown that *SPW1* is identical to MADS-box gene *OsMADS16* and is attributed to class B gene as an ortholog to *APETALA3* [23–26]. However, in rice not only *SPW1*/*OsMADS16*, but also *OsMADS45* and *OsMADS2* genes are expressed in lodicules [23, 25].

The lodicule genetics is best examined in rice (Table4), and many different mutants influencing the development of lodicules are described. Not all of them are well characterized genetically, among them rice mutants *fib* (*fish bone*) [27], *apo1* (*aberrant panicle organization1*) [28, 29], *opb1* and *opb2* (*open beak 1, 2*) [30].

Beside B class genes such as rice *OsMADS16/SPW1* [23–26] or maize gene *silky1* [31], other BCE genes also influence the development of lodicules. Such effect was shown for rice *OsMADS3* (ortholog of *AGAMOUS* in *Arabidopsis*). It mainly regulates stamen identity, but prevents also development of lodicules [32], and in mutants of that gene lodicules are converted to stamens [25, 33]. This effect does not differ from that observed for several mutations in B class genes, for instance, barley *lax-a* [18].

The high interest to the other, E class, genes is fully comprehensible. The genes of E class in *Arabidopsis* are called *SEPALLATA (SEP)* and act on the whole amplitude of flower development in all the four whorls and in the ovule. The maize genome has eight and the rice genome five *SEPALLATA-like* genes. *SEP* genes act in complex with genes of the other classes (ABCD) [12, 34–37]. The development of lodicules is influenced by at least two E class genes [12, 37].

The best investigated gene is *OsMADS1*. Mutation in this gene was called *lhs1* (*leafy hull sterile*). In the *lhs1* mutant, the flower, lemma, palea and lodicules are leafy-like and the number of stamens is reduced, but occasionally an extra pistil or floret arise [32, 34]. Prasad and Vijayraghavan [38] have shown that the *knock-out* mutation of *OsLHS1* with RNAi affects all four floral whorls, although it is not expressed in lodicules [38, 39, 12 as review]. This fact supports the idea that the development of lodicules can be affected by genes specifying the other flower whorls and not only the second whorl. The same phenotypic effect as in *lhs1* mutants is produced also by the *knoc-kout* of two genes, *OsMADS3* and *OsMADS58*, in the RNAi technology. Both genes are orthologs to *AGAMOUS* [33].

The expression and presumed function of *LHS1* in lodicules, stamens and gynoecium are highly variable among grasses. The maize *ZmM8(LHS1a)* and *ZMM14(LHS1b)* are expressed in all three upper whorls of the upper floret of the spikelet, whereas the pearl millet (*Panicum glaucum L.*) *PgLHS1* is expressed only in lodicules and not in stamens and gynoecium [12, 34, 35].

As to the interaction of ABCE and other genes, several mutant phenotypes are a result of the interaction of double or even quadruple mutant genes. Such possibility is shown on different plants and genes. So, the quadruple *Arabidopsis* mutant *sep1sep2sep3sep4* is expressed as a phenocopy of the triple *abc* mutant, i.e. does not have petals, homologous to grass lodicules [40]. In the double maize mutant *silky1 (si1)* and *zag1*, the effect on the conversion of flower organs was significantly stronger than on the single mutant *si1*. Additional whorls of lemmas and paleas appeared in the double mutant (Table 4) [31].

The development of lodicules is also by determined other genes that do not belong to the ABCDE classes of flower genes. Such genes may be divided into two or even three groups.

To the first group may be attributed all genes specifying flower and shoot apical (SAM) meristems. These genes act upstream of ABCDE and their effects on the development of floral organs is quite clear. On the other hand, the feedback action of ABCDE genes on meristem development is also fixed. So, it has been shown that *LHS1* (*SEPALLATA-like*) gene, besides acting on the fourth whorl, also involved in specifying the spikelet meristem, independently determine the identity of lemmas and paleas [12]. Both functions of *LHS1* are separable [12, 34].

The effect of the *Arabidopsis* mutant *shepherd (shd)* on flower development was observed. The *WT* allele encodes an ortholog of GRP94, an ER-resident HSP90-like protein. The *shd* mutation expands SAM and the number of flower organs [41]. However, orthologous mutations are unknown in Poaceae plants, but barley and other grasses have an ortholog gene [42].

For several meristem genes, a direct action on the development of flower organs has been shown. Such effect was observed for the rice gene *OsLRK1* which encodes a protein with a high sequence homology to *Arabidopsis* CLAVATA1 (CLV1) protein. *Knock-out* mutation by antisense RNA increased the number of flower organs, including extra lodicules (Table 4) [44].

Mutations in the other SAM-expressed gene, *SHO1-3* (*SHOOT ORGANIZATION 1–3*), show also a pleiotropic effect on the development of flower organs [41, 42]. The same is also true for rice *fon* mutations. At least four independently inherited *fon* mutants are known in rice [45], but only *fon2* without any doubt [26, 46] and possibly *fon1* [26, 29] influence the development of lodicules.

It is possible that there are many other genes with a pleiotropic effect on the development of lodicules and they will be

discovered in the nearest future. It is guaranteed by an increased interest to lodicule genetics for biotechnological reasons (cleistogamy) [13, 14], especially because of the possibility to regulate the development of lodicules by plant hormones and mutations.

The other group of genes capable effect the development of flower organs, including lodicules, are plant growth cell division regulating hormones. For cytokinin, the direct action on the development of lodicules is supported by investigation of the rice mutant *log (lonely guy)*, defective in cytokinin synthesis. In the mutant, *log* affected not only SAM, but also flower development. The number of flower organs was decreased in it. The inner floral organs were affected stronger than others. There were flowers containing only one stamen, but no pistils (i. e. the 'lonely guy'). LOG encodes cytokinin-specific phosphoribohydrolase activating cytokinin by converting this nucleotide into a free-base form. LOG mRNA is specifically localizated in SAM tops, indicating that activation of cytokinins occurs in a specific developmental domain [47].

Direct data on the action of another plant growth hormone, auxin, on the number of lodicules are absent. However, the maize and rice mutant *barren inflorescence2 (bif2)* is very similar by their phenotypic expression to the *log* mutant. The flowers are also affected strongly. The *BIF2* is a co-ortholog for the *PINOID (PID)* gene which regulates auxin transport in *Arabidopsis*. In rice, *OsBIF2* is an ortholog of the *LAX PANICLE1 (LAX)* gene which was discussed above. Within rice spikelet, *OsBIF* is expressed in fertile upper florets, while the other two florets are weakly developed, sterile. It is a normal situation for *WT*. In the *osbif2* mutant, a rearrangement of the auxin pool takes place, and all three florets are well developed [48].

In our observation, the same phenomenon occurs in several hybrids (Fig. 2, N 8 and 9) between *tw* and *lax aa* (1572) or *lax ab* (1573) (see also Table 2). Therefore, rearrangement of the auxin pool may be expected in these barley hybrids. The latter finding is of particular interest because one of the possible ways to regulate the lodicule length and cleistogamy is auxin treatment [49].

The two peculiarities of *tw* are of interest in respect of materials discussed above. The first is variation in flower structure. Mutations in BC genes give a more definite phenotype. It seems from literature data that variation is characteristic of the genes belonging to the other groups. The second peculiarity is a gradient in spike and flower development. In the upper part, superdevelopment is observed. It is well seen even in Figs. 1 and 2. We supposed that the gradient came from the transport rearrangement of putative biologically active substances, maybe plant hormones. On barley tw mutants, plant hormones 3-indolylacetic acid (auxin), α-naphthaleneacetic acid (NAA), gibberellic acid and cytokinetin (6-furfurylaminopurine) were tested by spraying, but a slight return to a normal flower structure was observed only if NAA was used [50].

Of course, the choice of active substances also depends on luck, but a motivated supposition may be done that the list of the genes and substances that may influence the development of lodicules will increase significantly in future.

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LYGINAMIEJI GENŲ, KURIE VEIKIA LODIKULIŲ RAIDĄ MIGLINIUOSE AUGALUOSE, TYRIMAI

S a n t r a u k a

Lodikulės yra savitas miglinių šeimos augalų žiedo organas, kurį lemia B klasės žiedo raidos genai. Atlikti originalaus miežių mutanto *tweaky spike*, žinomo B klasės *lax-a* geno ir kitų *tweaky* mutantų palyginamieji tyrimai, taip pat apibendrinti literatūriniai duomenys apie miežių, ryžių ir kukurūzų genus, darančius įtaką lodikulių raidai. Taigi lodikulių raidai turi įtakos ne tik B ir C klasių genai, bet ir kiti genai, kurie veikia netiesiogiai reguliuodami meristemų raidą, hormonų sintezę.