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Response of barley immunodeficient mutants *tweaky spike* to salicylic acid in field conditions

V. Vaitkûnienë[1],

L. Balèiûnienë[2],

A. Varnaitë[2],

V. Ranèelis[1]

[1] *Department of Botany and Genetics, M. K. Èiurlionio 21, Vilnius University, 03001 Vilnius*
[2] *Botanical Garden of Vilnius University, Kairënø 43, Vilnius*
E-mail: Aurelija.Varnaite@gf.vu.lt

Salicylic acid (SA) serves as a signaling molecule for activation of several plant defense responses including systemic acquired resistance to pathogens. The action of SA was tested in field conditions on six barley allelic mutants *tweaky spike* (*tw*) and two mutants *branched ear* (*be*) induced in two different cltuvars, 'Auksiniai II' and 'Auksiniai 3' whese, were also tested as *WT*. Immunodeficiency of *tw* type mutants to fungal pathogens is manifested by increased susceptibility to *Ustilago nuda* and *Claviceps purpurea*, as well as by an increased frequency of moldy germinating grains. Field conditions were unfavorable to *C. purpurea* and *U. nuda*, but even in these conditions susceptibility of the *tw* mutant to *U. nuda* increased and the positive action of SA was observed. Susceptibility to *Puccinia hordei* and *Drechslera teres* is determined not by the genotype of the test mutants, but by the basic *WT* genotype of initial cultivars. Cv. 'Auksiniai II' was exclusively sensitive to *D. teres.* Seed-treatment of cv. 'Auksiniai II' and of the mutant *tw* increased plant resistance to that pathogen. Both the initial cultivars as well as all mutants arisen from them were resistant to powdery mildew (*Blumeria graminis*).

Key words: induced immunity response, salicylic acid, barley mutants, immunodeficiency

INTRODUCTION

Plants have evolved a number of mechanisms to defend themselves against environmental stresses such as pathogen invasion. Local and systemic accumulation of salicylic acid (SA) is an important requirement for the activation of several plant defence responses including systemic acquired resistance (SAR). SA serves as a systemic signal, which is transduced over long distances from the inoculated leaf to uninoculated leaves and other parts of the plant [1–4]. SA is sufficient for induction of so-called *pathogenesis related* (*PR*) genes [5–7].

Application of exogenous SA induces a range of defence genes, many of which encode also PR-proteins [5]. Treatment with exogenous SA increased expression of *PR-2* gene 2- to 11-fold [6]. Application of exogenous SA correlates with an increased resistance of plants to the pathogen [8]. Accumulation of SA and induction of *PR*-genes is also observed in plant responses to various abiotic and biotic stresses, even after attacks by insects. Exogenous SA increases also resistance to abiotic stresses [6, 7, 9–15].

The role of SA as a key signalling molecule in SAR induction was determined by two approaches, using transgenic plants or mutants with altered response to pathogen infection. Plants are unable to transduce the SA signal due to a mutation in the *Non-expressor of PR1* (*NPR1*) / *no immunity 1* (*NIM1*) gene. Mutants in *NPR1* gene are hypersusceptible to pathogens and exhibit no induction of SAR [16–19]. SAR induction is also blocked in plants with transgene *NahG* from virulent bacteria. Transgenic plants expressing *NahG* gene encode the SA degrading enzyme salicylate hydroxylase unable to accumulate SA and are compromised in SAR [19]. The two key ways of SAR signalling, SA or jasmonic acid + ethylene, were discovered by using plant mutants [20, 21], as well as negative regulators of SAR [22, 23] and an *NPR1*-independent way of SA signalling [24]. The complicated and not fully understood mechanism of SA action shows also introduction of *R*-genes in the SA way. Overexpression of the tomato *R*-gene *Prf* leads to enhanced resistance to a number of normally virulent bacterial and viral pathogens. These plants have a level of SA, comparable to that of plants induced for SAR, and constitutively express *PR*-genes [25]. Recently a new gene family has been discovered, which encodes TGA transcription factors interacting with NPR1 [26, 27]. The activated com-

plex NPR1 + TGA binds to the SA response element of *PR*-genes. Redox changes, induced by SA, enhance DNA-binding activity of TGA1 [28]; *npr-1*-like phenotype reduces induction of *PR*-genes after treatment with SA analogue and enhances disease symptoms after infection with avirulent bacterial pathogens, which are not observed after infection of *WT* type [27]. The latter case shows also that mutants can be effectively used for evaluation of exogenous inducers of plant resistance to pathogens. Such chemical inducers are detected and used for research work [1, 18, 22, 29].

One of the characteristics of the barley recessive pleiotropic homeotic mutants *tw* (*tweaky spike*) is immunodeficiency. The main characteristic of *tw* mutants is altered flower structure: its lodicules are converted to stamens or pistils [30]. However, immunodeficiency is also one of the common characteristics of *tw* mutants. Plants infected by *Ustilago nuda* were observed in field conditions exceptionally only among *tw* plants. Another manifestation of a higher susceptibility of *tw* mutants to fungal infection is a higher frequency of moldy germinating grains [31]. These characteristics are permanent, reproducible and still observed.

In the present work, in field conditions the action of SA on the frequency of diseases caused by several pathogens such as *Ustilago nuda, Claviceps purpurea, Drechslera teres, Blumeria graminis* and *Puccinia hordei* were studied.

MATERIALS AND METHODS

All the barley mutants tested in the present work are of original origin, induced by chemical mutagens in cv. 'Auksiniai II' (*tw*, tw_1 and tw_2) and in cv. 'Auksiniai 3' (tw_7, tw_8, tw_{11}, be_1, be_2). The latter two barley mutants were chosen for comparison and are of another type – *branched ear*. The initial *WT* seed material of cv. 'Auksiniai II' and 'Auksiniai 3' was obtained from the Lithuanian Institute of Agriculture (Dotnuva). All material tested in this work for many years has been planted in the Botanical Garden of Vilnius University without pesticides. Both barley cultivars were grown under the same conditions as barley mutants.

Susceptibility of plants to pathogens. The frequency of plants affected by *Drechslera teres* (Sacc.) Shoem. (syn. *Helmintosporium teres* Sacc.), *Puccinia hordei* G. H. Otth, (syn. *Puccinia simplex* (Koern.)

Table 1. **Number of plants treated with salicylic acid (SA) and tested in field conditions**

Grain-treatment with SA mM	Tested material									
	AII	*tw*	tw_1	tw_2	A3	tw_7	tw_8	tw_{11}	be_1	be_2
Not sprayed with SA										
0	259	247	251	267	276	260	287	245	238	268
0.05	253	235	301	357	267	268	248	278	258	308
0.25	270	318	268	259	281	270	283	270	227	227
0.50	224	364	315	248	291	288	288	272	215	234
1.00	284	283	208	245	261	224	264	266	227	220
0.05 mM SA sprayed once										
0	310	343	237	277	246	239	213	248	229	253
0.05	318	288	296	330	250	273	284	228	218	221
0.25	246	279	328	254	250	243	203	255	241	252
0.50	298	259	231	328	270	273	279	250	218	257
1.00	360	264	319	276	271	264	262	304	253	235
0.05 mM SA sprayed twice										
0	305	305	269	333	315	257	250	225	163	227
0.05	290	286	284	317	294	250	243	257	251	261
0.25	259	289	337	233	266	157	279	252	227	195
0.50	236	235	259	335	270	254	236	256	192	232
1.00	289	306	291	275	247	174	232	256	197	219
0.05 mM SA sprayed three times										
0	306	304	302	380	245	198	239	141	186	245
0.05	310	262	269	365	256	178	266	233	202	217
0.25	310	299	253	305	219	204	195	217	197	250
0.50	332	321	329	332	245	241	235	273	219	277
1.00	260	271	231	286	290	259	294	240	235	247

AII – WT / initial cv. 'Auksiniai II'
A3 – WT / initial cv. 'Auksiniai 3'

Erikss. Et Henn.), *Blumeria graminis f.* sp. *hordei* (syn. *Erysiphe graminis* DC ex Merat.), *Ustilago nuda* (Jens.) Rostr., *Claviceps purpurea* (Fr.: Fr.) Tul. was determined by a standard method [32, 33]. All determinations were made on the same plant material when plants reached wax ripeness, and lasted one week, beginning from 19 July 2002. It allows also to determine interactions among the pathogens observed on revertant material [34]. In order to escape recurrence in referring to the plant number which for all tested fungal diseases was the same, it is shown in general form in Table 1 separately.

SA treatment in field conditions. Susceptibility of mutants of *tw* and *be* type as well as of the initial cultivars 'Auksiniai II' and 'Auksiniai 3' (as *WT*) to fungal diseases was determined in different conditions of SA treatment. Grains were soaked in SA (Sigma) solutions of 0; 0.05; 0.25; 0.50 and 1.00 mM concentrations for 12 h, and then were planted in the experimental field of the Botanical Garden. Part of the plants were not sprayed with SA, while the other plants were sprayed once, twice or three times with the same 0.05 mM SA. The choice of SA concentrations used for seed-soaking and plant spraying in field was based on summarised data of other works [1–5, 9, 17, 18, 20, 21, 29]. The same SA concentrations were also used in several recently published works [6, 10]. The dates of spraying were 23 May, 04 June, 16 June. The pH 6.5 of SA solutions was regulated with KOH.

Statistical analysis. The significance of differences between the means was analyzed by Student's t test.

RESULTS AND DISCUSSION

The action of SA was examined on the most harmful and common fungal pathogens such as ergot (*Claviceps purpurea*), smut (*Ustilago nuda*), powdery mildew (*Blumeria graminis* f. sp. *hordei*), leaf rust (*Puc-*

Table 2. **Action of salicylic acid (SA) in field conditions on smut (*Ustilago nuda*) in barley *tw* mutants of different origin**

Grain-treatment with SA mM	% of ill plants									
	WT AII	*tw* from AII			*WT* A3	Mutants from A3				
		tw	tw_1	tw_2		tw_7	tw_8	tw_{11}	be_1	be_2
Not sprayed with SA										
0	0	4.05	0	0	0	0	0	0.40	0	0
0.05	0	0.43 [2b]	0.33	0.56	0	0	0	0.36	0	0
0.25	0	0[2b]	1.12	0	0	0	0	0	0	0
0.50	0	1.10[1a]	0	0	0	0	0	0.74	0	0
1.00	0	1.77	0.48	0	0	1.34	0.38	0	0	0
0.05 mM SA sprayed once										
0	0	0[2]	0	0	0	0	0	0	0	0
0.05	0	1.39[a]	0	0.30	0	0	0	0	0	0
0.25	0	0.72[1]	0	0	0	0	0	0	0	0
0.50	0	3.86[b]	0	0	0	0	0	0.40	0	0
1.00	0	0[2]	0.31	0	0	1.52[1a]	0	0	0	0
0.05 mM SA sprayed twice										
0	0	0.33[2]	0	0.30	0	0	0	0	0	0
0.05	0	1.05[1]	0	0	0	0	0	0	0	0
0.25	0	2.10[a]	0	0	0	0	0	0	0	0
0.50	0	4.68[b]	0	0.90	0	0	0	0	0	0
1.00	0	4.56[c]	0	0	0	0.57	0.43	0	0	0
0.05 mM SA sprayed three times										
0	0	1.97	0	0	0	0	0	0	0.54	0
0.05	0	2.21	0.30	0.27	0	0	0	0.43	0	0
0.25	0.32	3.01	0.40	0	0	0	0	0.46	0	0
0.50	0	1.56	0.30	0	0	0	0	0	0	0
1.00	0	2.21	0	0	0	0	0	0	0	0

1, a – P < 0.05; 2, b – P < 0.01; 3, c – P < 0.001.
1, 2, 3 – compared with plants absolutely untreated (unsoaked and unsprayed) with SA
a, b, c – compared with SA sprayed plants, but from seed-material untreated (0) with SA
AII – WT / initial cv. 'Auksiniai II'
A3 – WT / initial cv. 'Auksiniai 3'

cinia hordei), net blotch (*Drechslera teres*). However, the pecularity of the present work is that the frequency of diseases was determined in natural conditions of infection, *i.e.* in field. The meteorological and other environmental conditions have a very strong influence on pathogen infection in field conditions. So, despite the fact that ergot (*Claviceps purpurea*) is among harmful pathogens, in conditions of 2002 only few ergotic plants were observed, and this fact did not allow us to investigate action of SA on *C. purpurea*.

The situation was almost the same with the other pathogen, *Ustilago nuda* (Table 2), whose infection capacity, like that of *C. purpurea*, depends on plant flowering conditions [33]. Investigation of both the initial cultivars 'Auksiniai II' and 'Auksiniai 3' and the mutants of *be* and *tw* types confirmed that conditions in 2002 were not favourable for *U. nuda*, with one exception – mutant *tw*. The conditions of 2002 manifested more clearly an exceptional susceptibility of this *tw* mutant to *U. nuda*. It was the only mutant for which a considerable frequency of smutted plants was observed among control untreated plants (Table 2). It allowed also to find a protective effect of SA against *U. nuda* in that mutant, if seed material was treated with SA. Spraying with SA was less effective than seed material treatment alone.

Unexpected was SA action on the frequency of plants affected by barley leaf rust (*Puccinia hordei*) (Table 3). First, the level of affected plants depended not on mutant plants tested but on the initial plant genotype from which those mutants arose. One group was composed of cv. 'Auksiniai II' itself and all its mutants (*tw*, tw_1, tw_2), while the other group was lead by cv. 'Auksiniai 3', and it comprised all mutants arisen from this cultivar without differentiating between *tweaky spike* and *branched ear.* The difference between these two groups is expressed very clearly. In the group of cv. 'Auksiniai II' the higher level of leaf rust-affected plants was urgently deter-

Table3. **Action of salicylic acid (SA) in field conditions on leaf rust (*Puccinia hordei*) in barley *tw* mutants of different origin**

Grain-treatment with SA mM	% of ill plants									
	WT AII	*tw* from AII			*WT* A3	Mutants from A3				
		tw	tw_1	tw_2		tw_7	tw_8	tw_{11}	be_1	be_2
Not sprayed with SA										
0	5.50	5.33	3.95	6.93	2.00	0.75	0.20	3.50	2.00	1.00
0.05	9.90^{3c}	8.83	9.45^{2}	7.75	0^{1a}	0	0	0^{2b}	0^{1}	0.61
0.25	6.00	6.80	4.48	8.95	0^{1a}	0	0	0^{2b}	0^{1}	0
0.50	1.18^{2b}	8.18	2.88	1.35^{3}	0^{1a}	0	0	0^{2b}	0^{1}	0
1.00	0.30^{3c}	1.53^{1a}	5.68	5.95	0^{1a}	0	0	0^{2b}	0^{1}	0
0.05 mM SA sprayed once										
0	1.73^{3}	2.15	3.95	1.08^{3}	0.50	3.00	1.50	2.15	1.25	11.85^{3}
0.05	8.70^{c}	4.58	1.53	2.53^{1}	0.50	2.50	8.25^{3c}	2.25	0.75	0^{c}
0.25	5.78^{a}	4.40	3.73	1.25^{3}	0^{1}	0.50^{a}	0.25	0^{2a}	5.00^{a}	2.00^{c}
0.50	1.25^{2}	4.38	0.98^{1a}	5.18^{b}	3.85^{b}	0.55^{a}	1.50	0.25^{2a}	0.25	0^{c}
1.00	4.20	10.35^{1c}	1.90	1.85^{2}	0^{1}	0^{b}	0^{1a}	0^{2a}	0^{1}	0^{c}
0.05 mM SA sprayed twice										
0	2.45	3.83	4.70	3.45	0^{1}	0	0	0^{2}	0^{1}	0
0.05	3.50	2.80	0.70^{1b}	2.88^{1}	0^{1}	0	0	0^{2}	0^{1}	0
0.25	1.45^{1}	2.73	0.25^{2c}	0.75^{3a}	0^{1}	0	0	0^{2}	0^{1}	0
0.50	1.13^{2}	9.50^{b}	3.28	0.95^{3a}	0^{1}	0	0	0^{2}	0^{1}	0
1.00	1.50^{1}	3.62	4.10	1.25^{3}	0^{1}	0	0	0^{2}	0^{1}	0
0.05 mM SA sprayed three times										
0	1.50^{1}	0.43^{3}	0.70^{1}	1.48^{3}	0^{1}	0	0	0^{2}	0.13^{1}	0
0.05	0.03^{3a}	0.50^{3}	3.00^{a}	0^{3a}	14.25^{3c}	6.00^{2c}	3.60^{2c}	0.25^{2}	1.50	2.35^{a}
0.25	3.03	2.95^{a}	0.88^{1}	0.58^{3}	0^{1}	0	1.75	0^{2}	0^{1}	0.25
0.50	0.33^{3}	6.00^{c}	3.13^{a}	1.25^{3}	0^{1}	4.50^{2c}	0	0^{2}	0^{1}	0
1.00	4.85^{a}	5.50^{c}	2.50	7.00^{c}	0^{1}	0.50	0	0.15^{2}	0^{1}	0

1, a – P < 0.05; 2, b – P < 0.01; 3, c – P < 0.001.
1, 2, 3 – compared with plants absolutely untreated (unsoaked and unsprayed) with SA
a, b, c – compared with SA sprayed plants, but from seed-material untreated (0) with SA
AII – WT / initial cv. 'Auksiniai II'
A3 – WT / initial cv. 'Auksiniai 3

Table 4. **Action of salicylic acid (SA) in field conditions on net blotch (*Drechslera teres*) in barley *tw* mutants of different origin**

Grain-treatment with SA mM	% of ill plants									
	WT AII	*tw* from AII			*WT* A3	Mutants from A3				
		tw	tw_1	tw_2		tw_7	tw_8	tw_{11}	be_1	be_2
Not sprayed with SA										
0	28.40	15.08	11.10	14.00	3.15	5.85	10.15	7.35	5.20	8.45
0.05	13.08[3c]	8.38[1a]	6.75	6.98[2b]	7.04[1a]	6.22	7.03	8.26	7.03	6.06
0.25	8.68[3c]	7.30[2b]	9.15	11.15	6.69	4.44	6.20	8.00	4.20	5.06
0.50	9.75[3c]	5.45[3c]	8.10	7.65[1a]	4.21	5.50	6.15	5.16	4.21	6.33
1.00	6.70[3c]	7.15[2b]	11.83	10.18	4.55	8.82	5.62[1a]	7.00	5.98	6.26
0.05 mM SA sprayed once										
0	8.05[3]	8.80	9.70	9.45	7.70	8.90	9.00	10.45	8.20	7.45
0.05	10.43[3]	12.33[2]	8.05	10.65	5.30	6.65	9.00	7.60	9.05	3.30[2a]
0.25	13.50[3a]	9.90	8.35	9.28	5.65	4.85	7.25	6.70	6.95	11.25
0.50	9.45[3]	8.25	9.25	9.28	9.40	10.85	9.35	10.95	4.35	4.50
1.00	9.70[3]	9.73	10.53	9.60	6.62	6.68	5.40[1]	7.00	6.50	5.83
0.05 mM SA sprayed twice										
0	10.23[3]	7.98	13.75	9.58	5.54	7.00	5.39	5.25	7.17	4.07
0.05	9.90[3]	10.03[2]	11.98	10.48	3.33	7.96	5.61	6.30	4.14	7.39
0.25	11.48[3]	10.80	6.08[b]	8.28	4.25	6.50	6.71	6.87	5.01	9.02[a]
0.50	4.28[3c]	2.98[3b]	10.95	7.58[1]	4.72	8.32	6.52	7.03	4.42	5.04
1.00	4.05[3c]	2.75[3b]	10.56	7.00[2]	5.00	6.23	8.46	6.90	5.23	5.25
0.05 mM SA sprayed three times										
0	7.05[3]	4.45[3]	10.13	5.99[2]	5.93	6.66	5.85	7.71	2.85	5.00
0.05	4.78[3]	7.63	6.35	7.07[2]	7.55	8.50	6.30	7.35	5.50	5.90
0.25	11.73[3]	11.18[b]	10.88	8.78	3.00	5.10	6.90	6.25	4.90	4.80
0.50	11.00[3]	10.30[b]	6.63	10.85[a]	5.70	5.45	6.40	6.70	4.20	4.07[1]
1.00	9.90[3]	7.55	4.90[2a]	6.20[2]	4.40	4.80	7.95	4.60	0.50[2a]	3.00[2]

1, a – P < 0.05; 2, b – P < 0.01; 3, c – P < 0.001.
1, 2, 3 – compared with plants absolutely untreated (unsoaked and unsprayed) with SA
a, b, c – compared with SA sprayed plants, but from seed-material untreated (0) with SA
AII – WT / initial cv. 'Auksiniai II'
A3 – WT / initial cv. 'Auksiniai 3'

mined by initial *WT* genotype of cv. 'Auksiniai II' from which the mutants *tw*, tw_1, and tw_2 arose.

Second, a different reaction of both groups to 0.05 mM SA spraying was also evident. If plants were sprayed with SA twice, leaf rust among the plants of the second (cv. 'Auksiniai 3') group disappeared completely, while in the first (cv. 'Auksiniai II') group not all leaf rust plants disappeared. This fact may suggest that both plant groups represent the different ways of resistance regulation and signalling to *P. hordei*. It is perspective to compare the response of both groups of the basic genotypes also to the JA + ethylene pathway [20, 21, 24]. Our suggestion regarding the presence of different signalling pathways in different basic genotypes is supported by the fact that SA-treatment of seed-material of the second group (cv. 'Auksiniai 3', tw_7 – tw_{11}, be_1, be_2) effectively reduced the frequency of leaf rust to zero (Table 3). A long period of time separates seeds from mature plants in which the frequency of rusted plants can be fixed, and such reduction may be explained only by SAR induction [2–4].

Thirdly, various mutants differ noticeably even within the same basic genotype group. Especially it is clear for tw_{11} compared to other *tw* type mutants arisen from cv. 'Auksiniai 3'. Differences are observed also among two *be* type mutants.

The effect of SA spray on *Puccinia hordei* development was irregular (Table 3).

Susceptibility to *Drechslera teres* also depended mainly on the basic genotype of initial cultivars from which the mutants arose (Table 4). As a whole, the *tw* type mutants developed from cv. 'Auksiniai II' were more frequently affected by *D. teres* than *tw* type mutants from *cv*. 'Auksiniai 3', although variations within the groups were also observed. The higher susceptibility of *tw* mutants from cv. 'Auksiniai II' is in agreement with the exclusively high susceptibility of the initial cv. 'Auksiniai II' to *D. teres*. On the exclusively high background of cv. 'Auksiniai II' plants af-

Table 5. **Comparison of the two groups *tw* mutants of different origin according effectivity of salicylic acid (SA) action against powdery mildew (*Blumeria graminis*) in field conditions**

Grain-treatment with SA mM	% of ill plants									
	WT AII	*tw* from AII			*WT* A3	Mutants from A3				
		tw	tw_1	tw_2		tw_7	tw_8	tw_{11}	be_1	be_2
Not sprayed with SA										
0	0.88	0.55	0.70	0.25	0.95	0.25	0.10	0.15	0.50	0.30
0.05	1.25	0.88	0.93	0.45	0.04	0.11	0.16	0.23	0.18	0.15
0.25	0.45	3.48[1a]	2.93[1a]	1.53	0	0.47	0.03	0	0.06	0.31
0.50	5.45[1a]	0.88	0.55	0.35	0.18	0.11	0.12	0	0.18	0.17
1.00	0.08	0.33	0	0.38	0.03	0.05	0.03	0.02	0.08	0.05
0.05 mM SA sprayed once										
0	0	0.13	0.20	0.30	1.05	0.50	0.45	0.30	0.30	0.60
0.05	0.13	0.73	0.65	0.15	0.10	0.55	0.60	0.40	1.00	0
0.25	0.63	0.13	0.20	0.13	0	0.15	0.35	0.05	0.10	0.20
0.50	0.20	0.65	0.38	0.40	1.60	0.25	0.05	0.05	0.50	0.30
1.00	0.83	0.43	0.58	0.08	0.29	0.23	0	0.30	0.15	0.33
0.05 mM SA sprayed twice										
0	0.83	0.85	0.65	0.93	0	0	0	0.13	0	0.75
0.05	0.40	1.28	2.05	1.20	0.25	0.10	0.11	0.10	0.11	0.14
0.25	0.30	1.23	0.15	0.38	0.13	0.02	0.05	0.11	0.10	0.25
0.50	0.05	1.75	0.30	0.10	0	0.15	0.04	0.11	0.30	0.31
1.00	0.08	1.35	0	0.15	5.00[2c]	0.20	0	0.02	0.15	0.20
0.05 mM SA sprayed three-times										
0	0.15	0.30	0.93	1.33	0.79	1.17	0.25	0.81	0.20	0.38
0.05	0.53	0.15	0.53	0.07	0.45	2.55[1]	0.55	0.90	1.85[a]	1.25
0.25	0.55	0.75	0.08	0.05[a]	0.30	0.40	0.70	2.20[1]	0.95	1.50
0.50	0.18	0.65	0.46	0.08	1.05	1.45	1.80[1]	0.85	1.75	1.60
1.00	0.15	0.45	0.10	0.30	0.60	0.30	0.30	0.80	0.40	0.40

1, a – P < 0.05; 2, b – P < 0.01; 3, c – P < 0.001.
1, 2, 3 – compared with plants absolutely untreated (unsoaked and unsprayed) with SA
a, b, c – compared with SA sprayed plants, but from seed-material untreated (0) with SA
AII – WT / initial cv. 'Auksiniai II'
A3 – WT / initial cv. 'Auksiniai 3'

fected by *D. teres*, all *tw* type mutants were even more resistant to net blotch. The barley cv. 'Auksiniai 3' was relatively resistant to *D. teres*, and all mutants arisen from it (including both *be*) were more susceptible to *D. teres* than plants of the initial genotype cv. 'Auksiniai 3'.

The protective effect of SA was observed only on cv. 'Auksiniai II' and *tw*, and treatment of seed material with 0.05–1.00 mM solutions in combination with spraying of plants twice with 0.05 mM SA solution decreased the frequency of plants affected by *D. teres* to a level observed in barley cv. 'Auksiniai 3'. The same effect, even more pronounced, was observed also on the barley mutant *tw*. The protective action of SA was observed also for both *be* mutants if massive treatment had been used (seed material soaked in 1.00 mM SA plus 0.05 mM SA spraying three times). The same was noted also for two *tw* type mutants, tw_1 and tw_2, belonging to cv. 'Auksiniai II' group. In other cases, the effect of SA was irregular (Table 4).

According to data of 2002, both barley initial cultivars 'Auksiniai II' and 'Auksiniai 3' used as *WT* were resistant to powdery mildew (*Blumeria graminis*) (Table 5). Only <1% of plants were affected by powdery mildew, about equally of both cultivars. This is in agreement with the fact that cv. 'Auksiniai 3' has in his genome genes *Mla6*, *Mla14* [35]. These genes determine race-specific resistance to powdery mildew [36] and encode R-proteins [37, 38]. All mutants, independently of type and initial cultivar, were slightly less affected by *B. graminis*, however, the difference was statistically insignificant. On the background of such a low level of *B. graminis* affected plants, it is difficult to observe the protective effect of SA. In several, but irregular, cases SA treatment even increased the frequency of affected plants.

A comparison of development of different pathogens (*Claviceps purpurea*, *Ustilago nuda*, *Blumeria graminis* f. sp. *hordei*, *Puccinia hordei* and *Drechslera teres*) in field conditions allowed us to conclude that

despite that the field conditions exert a strong influence on the development of pathogens and the conditions in 2002 were unfavourable for *Claviceps purpurea*, *Ustilago nuda* and *Blumeria graminis*, interesting observations were made even regarding *B. graminis* and *U. nuda*. It was found that most mutants were not more susceptible to those pathogens than *WT*. One exception was mutant *tw*, which is very sensitive to *U. nuda* even in such unfavourable conditions for pathogen development. This mutant is perspective for investigation of resistance induction to *U. nuda* with various chemical inducers including SA, because *U. nuda* was the only pathogen resistance to which SA increased very clearly, especially after seed treatment of *tw*.

Investigation of *Puccinia hordei* and *Drechslera teres* has led us to another finding: susceptibility or resistance to both those pathogens is determined not by the genotype of mutants but by the initial genotype of *WT* cultivars from which the mutants were obtained.

On the other hand, the exclusive susceptibility of barley cv. 'Auksiniai II' to net blotch (*Drechslera teres*) helped us to find a positive effect of SA on resistance induction to *D. teres*. Increase of resistance was shown also by the mutant *tw* whose *WT* is cv. 'Auksiniai II'. However, a protective effect against *D. teres* was observed only after seed treatment. Additional spraying with 0.05 mM SA increased resistance to *D. teres* only on the background of a high susceptibility of cv. 'Auksiniai II', but the effect did not exceed the effect of seed treatment with SA alone.

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V. Vaitkûnienë, L. Balèiûnienë, A. Varnaitë, V. Ranèelis

IMUNODEFICITINIØ MIEÞIØ MUTANTØ *TWEAKY SPIKE* ATSAKAS Á SALICILO RÛGÐTÁ

S a n t r a u k a

Salicilo rûgðtis (SR) yra apsauginë ir kartu signalinë medþiaga, dël kurios perneðamo signalo augalas ágyja sisteminá imunitetà. Lauko sàlygomis buvo iðtirtas SR poveikis mieþiø aleliniams mutantams *tweaky spike* (*tw*), kuriems trûksta imuniteto. Imuniteto stoka pasireiðkia padidintu jautrumu *Ustilago nuda* ir *Claviceps purpurea,* taip pat padidëjusiu grûdø pelijimu. Nustatytas skalsëmis (*Claviceps purpurea*), kûlëmis (*Ustilago nuda*), lapø rûdimis (*Puccinia hordei*), lapø dryþlige (*Drechslera teres*) ir miltlige (*Blumeria graminis* f. sp. *hordei*) susirgusiø augalø daþnis. Augalø ligotumas labai priklausë nuo aplinkos sàlygø. 2002 m. skalsiø visai neaptikta. Palyginti maþai augalø sirgo miltlige ir kûlëmis. Vis tik *tw* mutanto jautrumas *U. nuda* ir padidëjæs atsparumas paveikus SR pasireiðkë visomis sàlygomis. Jautrumas *Puccinia hordei* ir *Drechslera teres* buvo nulemtas mutantø, pradinio bazinio genotipo – veislës, ið kurios ðie mutantai buvo iðskirti. Ypaè jautri *D. teres* buvo mieþiø veislë ‘Auksiniai II’. Veislës ‘Auksiniai II’ ir mutanto *tw* sëklø mirkymas SR tirpaluose padidino augalø atsparumà lapø dryþligei.