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Quality of seed material of barley *tw* type mutants according to susceptibility to micromycetes after treatment of previous generation with salicylic acid

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A peculiarity of the *tweaky spike* (*tw*) allelic barley mutants is an increased frequency of moldy germinating grains. In the present work, this peculiarity has also been established for the *branched ear* (*be*) barley mutants. Unexpectedly, it has been determined that seed material quality can be improved by treating the previous plant generation with salicylic acid. This effect of salicylic acid is new, and employment of such mutants as *tw* or *be* enables to research immunoresistance induction by other inducers.

Key words: induced immunoresistance, salicylic acid, effect on next generation, usage of mutants

INTRODUCTION

Plant genetic resourses can be evaluated in various respects. Not only economically valuable characters, but also traits interesting and important for research purposes are notable for gene pool programmes. Valuable genetic sources for specialized investigations are natural or induced mutants. The use of induced barley mutants *tweaky spike* (*tw*) for investigation of flower development genetics was discussed in previous works [1, 2]. These mutants are also promising for evaluation of immunoresistance induced by various chemical means, because barley *tw* type mutants are susceptible to *Ustilago nuda* (Jens.) Rostr. and *Claviceps purpurea* (*Fr.:Fr.*) in field conditions and, what is more important, for the use of *tw* mutants as a test-system for induced immunoresistance. The *tw* type mutants are characterized by an increased frequency of moldy germinating grains, and the effect is very well reproducible [3].

This peculiarity of *tw* type mutants allows us to investigate the effect of immunoresistance inducers not only directly in the year of treatment [7], but also to evaluate the quality of seed material in the next generation after treatment by the inducer. It could be supposed to be a plausible advantage of the barley *tw* type mutants, and this expectation was examined in the present work. As the inducer of immunoresistance, salicylic acid (SA) was used as one of the most common and most widely used inducer of acquired immunoresistance in plants [4–6, 8].

The present investigation is a direct continuation of the previous work [7] in which the results of SA_1 generation were discussed and the harvested seed material was analyzed as SA_2.

MATERIALS AND METHODS

All 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* (*be*). 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 the work had been planted for many years in the Botanical Garden of Vilnius University without pesticides. Both barley cultivars were grown under the same conditions as the barley mutants.

SA_1 treatment in field conditions for seed-material of SA_2. The treatment with SA was combined. At first, 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 planted in an experimental field of the Botanical Garden. Then part of the plants were grown without spraying with SA, while the other part of plants was sprayed once, twice or three times with the same

0.05 mM concentration of SA. The choice of SA concentrations used for seed soaking and plant spraying in the field was grounded on the summarized data of the other works [4–6, 8, 9]. The dates of spraying were 23 May, 04 June and 16 June 2002, respectively. The pH 6.5 of SA solutions was obtained using KOH. Seed material from all specimens was harvested separately. Plants grown in the year of SA treatment were designated as SA_1, while the seed material harvested in this experiment was designated as SA_2.

Evaluation of the frequency of moldy grains in SA_2. All manipulations were made in sterile conditions. Laboratory flasks and water for seed germination were sterilized, but the seed material was planted in Petri dishes without sterilization to evaluate the natural infection and sensitivity of germinating grains. Barley grains were germinated for six days on six layers of filter paper in Petri dishes in a thermostat at 24 °C in the dark. In each Petri dish ten grains were placed. In total, 200 grains (20 Petri dishes) for cv. 'Auksiniai II', *tw*, tw_1 and 100 grains for other material were examined. Germination capacity (G), root length (L) and the frequency of moldy grains were determined. Root length in each sample was determined for 30 germinating grains after six days of germination.

Statistical analysis. The significance of differences between the means was analyzed by Student's t test according to [10].

RESULTS AND DISCUSSION

The higher frequency of moldy germinating grains is one of the manifestations of barley *tw* type allelic

Table 1. **Seed-material quality in SA_2 according to moldy germinating grain frequency in progenies of barley *tw* type mutants and initial cv. 'Auksiniai II' (AII) in SA_1 exposed to salicylic acid (SA)**

Grain treatment in SA_1/ SA mM	% of moldy germinating grains in SA_2			
	AII	*tw*	tw_1	tw_2
SA_1 not sprayed with SA				
0	11.6 ± 2.3	18.0 ± 2.7	18.4 ± 2.8	19.2 ± 2.8
0.05	10.5 ± 2.2	16.5 ± 2.6	17.5 ± 2.7/L_{1a}	18.0 ± 2.7
0.25	10.0 ± 2.1	15.0 ± 2.5	16.0 ± 2.6	14.5 ± 2.5
0.50	8.0 ± 1.9/L_{2a}	16.4 ± 2.6	14.8 ± 2.5/G_{1a}	12.8 ± 2.4/L_{1a}
1.00	8.0 ± 1.9/L_{1a}	13.6 ± 2.4	10.8 ± 2.0^{1a}	12.0 ± 2.3^{1a}
SA_1 sprayed once with 0.05 mM SA				
0	11.5 ± 2.3	17.0 ± 2.7/L_3	19.0 ± 2.8L_1	19.5 ± 2.8
0.05	12.0 ± 2.3/L_{2a}	20.0 ± 2.8/L_2	17.5 ± 2.7	18.7 ± 2.8
0.25	11.0 ± 2.2	20.7 ± 2.9/L_3	16.5 ± 2.6	18.5 ± 2.8
0.50	10.0 ± 2.1	17.0 ± 2.7/$G_{3a}L_b$	15.3 ± 2.6/L_c	13.3 ± 2.4
1.00	10.0 ± 2.1/G_{1a}	15.0 ± 2.5/L_c	14.0 ± 2.5^2/G_{2b} L_b	12.0 ± 2.3^{1a}/L_b
SA_1 sprayed twice with 0.05 mM SA				
0	10.0 ± 2.1	19.0 ± 2.8/G_2	21.5 ± 2.9	26.5 ± 3.1
0.05	9.5 ± 2.1/G_{1b}	17.5 ± 2.7	21.0 ± 2.9	23.5 ± 3.0
0.25	9.5 ± 2.1/G_{1b}	17.0 ± 2.7G_a	19.0 ± 2.8/G_{2c} L_b	18.0 ± 2.7^a/G_a
0.50	9.5 ± 2.1	15.0 ± 2.5L_2	17.0 ± 2.7/G_a L_b	15.0 ± 2.5^b
1.00	8.5 ± 2.0	13.0 ± 2.4G_a L_2	16.0 ± 2.6/L_b	14.0 ± 2.5^b
SA_1 sprayed three times with 0.05 mM SA				
0	8.5 ± 2.0	17.0 ± 2.7	13.5 ± 2.4	12.5 ± 2.5
0.05	8.5 ± 2.0	15.5 ± 2.6/G_2	14.5 ± 2.5	15.0 ± 2.5/L_b
0.25	8.5 ± 2.0/L_b	12.0 ± 2.3	13.5 ± 2.4	11.5 ± 2.3^1/L_c
0.50	7.5 ± 1.9	12.5 ± 2.3	11.5 ± 2.3^1	12.0 ± 2.3^1/L_a
1.00	6.0 ± 1.7^1/L_c	12.5 ± 2.3	9.5 ± 2.1^2	8.5 ± 2.0^2

1, a – P < 0.05; 2,b – P < 0.01; 3, c– P < 0.001; 1, 2, 3 – compared with germinating grains from plants in SA_1 absolutely untreated (unsoaked and unsprayed) with SA (**0**); a, b, c – compared with germinating grains from plants in SA_1 sprayed with 0.05 mM SA, but unsoaked in SA (0); **L** – length of roots and **G** – germination capacity, where $L_{1,2,3}$, $L_{a,b,c,}$ $G_{1,2,3,}$ $G_{a,b,c}$ are respective mean of P in comparison to 1,2,3 or a,b,c; $L_{1,2,3}$,$L_{a,b,c}$, or $G_{1,2,3}$,$G_{a,b,}$ in italic – the value is decreased; AII – *WT*/initial cv. 'Auksiniai II'.

Table 2. **Seed-material quality in SA_2 according to mold germinating grain frequency in progenies of barley *tw* type mutants and initial cv. 'Auksiniai 3' (A3) exposed in SA_1 to salicylic acid (SA)**

Grain treatment in SA_1/ SA mM	% of moldy germinating grains in SA_2					
	A3	tw_7	tw_8	tw_{11}	be_1	be_2
SA_1 not sprayed with SA						
0	10.0 ± 3.0	23.0 ± 4.2	22.0 ± 4.2	24.0 ± 4.3	22.0 ± 4.2	17.0 ± 3.8
0.05	13.0 ± 3.4	21.0 ± 4.1	19.0 ± 3.9	23.0 ± 4.2	19.0 ± 3.9	14.0 ± 3.5
0.25	10.0 ± 3.0	20.0 ± 4.0	20.0 ± 4.0	22.0 ± 4.2	16.0 ± 3.7	14.0 ± 3.5
0.50	9.0 ± 2.9	17.0 ± 3.8	18.0 ± 3.9	20.0 ± 4.0	13.0 ± 3.4	12.0 ±3.3
1.00	7.0 ± 2.6	14.0 ± 3.5	16.0 ± 3.7	14.0 ± 3.5	12.0 ± 3.3[1a]	10.0 ± 3.0
SA_1 sprayed once with 0.05 mM SA						
0	10.0 ± 3.0	23.0 ± 4.2	18.0 ± 3.9	25.0 ± 4.4	19.0 ± 3.9	16.0 ± 3.7
0.05	8.0 ± 2.7/G_1	18.0 ± 3.9	17.0 ± 3.8	20.0 ± 4.0	16.0 ± 3.7	16.0 ± 3.7
0.25	10.0 ± 3.0	18.0 ± 3.9/G_1	17.0 ± 3.8	19.0 ± 3.9	12.0 ± 3.3[1]	12.0 ± 3.3
0.50	9.0 ± 2.9	20.0 ± 4.0	16.0 ± 3.7	22.0 ± 4.2	15.0 ± 3.6/G_1	9.0 ± 2.9
1.00	7.0 ± 2.6	18.0 ± 3.9	15.0 ± 3.6/G_a	15.0 ± 3.6	12.0 ± 3.3[1]	9.0 ± 2.9
SA_1 sprayed twice with 0.05 mM SA						
0	13.0 ± 3.4	19.0 ± 3.9	23.0 ± 4.2	26.0 ± 4.4	19.0 ± 3.9/G_1	16.0 ± 3.7
0.05	11.0 ± 3.1	18.0 ± 3.9	23.0 ± 4.2	22.0 ± 4.2	18.0 ± 3.9/G_1	10.0 ± 3.0
0.25	10.0 ± 3.0	19.0 ± 3.9	20.0 ± 4.0	21.0 ± 4.1	16.0 ± 3.7	11.0 ± 3.1
0.50	8.0 ± 2.7	17.0 ± 3.8	19.0 ± 3.9	17.0 ± 3.8	14.0 ± 3.5/G_1	10.0 ± 3.0
1.00	7.0 ± 2.6	16.0 ± 3.7	15.0 ± 3.6	11.0 ± 3.1[1b]	10.0 ± 3.0[1]	7.0 ± 2.6[1a]
SA_1 sprayed three times with 0.05 mM SA						
0	9.0 ± 2.9	20.0 ± 4.0	17.0 ± 3.8	15.0 ± 3.6	13.0 ± 3.4	13.0 ± 3.4
0.05	11.0 ± 3.1	17.0 ± 3.8	18.0 ± 3.9	14.0 ± 3.5	14.0 ± 3.5	11.0 ± 3.1
0.25	10.0 ± 3.0	15.0 ± 3.6/G_1	19.0 ± 3.9	16.0 ± 3.7	13.0 ± 3.4	11.0 ± 3.1
0.50	8.0 ± 2.7	16.0 ± 3.7	15.0 ± 3.6/G_a	13.0 ± 3.4[1]	12.0 ± 3.3[1]	10.0 ±3.0
1.00	6.0 ± 2.4	14.0 ± 3.5	12.0 ± 3.3[1]	12.0 ± 3.3[1]	8.0 ± 2.7[2]	6.0 ± 2.4[2]

1,a – P < 0.05; 2, b – P < 0.01; 3,c – P < 0.001; 1,2,3 – compared with germinating grains from plants in SA_1 absolutely untreated (unsoaked and unsprayed) with SA (**0**); a, b, c – compared with germinating grains from plants in SA_1 sprayed with 0.05 mM SA, but unsoaked in SA (0); **L** – length of roots and **G** – germination capacity, where $L_{1,2,3}$, $L_{a,b,c}$, $G_{1,2,3}$, $G_{a,b,c}$ are respective mean of P in comparison 1,2,3 or a,b,c; $L_{1,2,3}$, $L_{a,b,c}$, or $G_{1,2,3}$, $G_{a,b,c}$ in italic – where meaning is decreased; A3 – *WT*/ initial cv. 'Auksiniai 3'.

mutations for immunodeficiency to fungal infection [1, 3], what has been also confirmed in the present work with six *tw* type mutants of different history of origin: from the barley initial cultivar 'Auksiniai II' (Table 1) or from the initial cultivar 'Auksiniai 3' (Table 2). In the present investigation, germinating grains of *tw* mutants arisen from cv. 'Auksiniai 3' were even more susceptible to micromycetes: the frequency of moldy germinating grains (without any treatment) among *tw* mutants arisen from cv. 'Auksiniai II' was only 1.55–1.65 times higher than among grains of the initial cv. 'Auksiniai II' (Table 1), while moldy grain frequency among *tw* mutants from the initial cv. 'Auksiniai 3' was higher 2.2–2.4 times (Table 2).

Unexpectedly, the frequency of moldy germinating grains among the *branched ear* – be_1 and be_2 barley mutants was high. The frequency of moldy grains among these mutants was 1.7–2.2 times higher than in 'Auksiniai 3' (Table 2). Mutants of *be* type were chosen in this experiment only for comparison as ear developmental mutants. They have a branched ear. In field conditions they did not show a higher susceptibility to the fungal diseases tested – ergot (*Claviceps purpurea*), smut (*Ustilago nuda*), powdery mildew (*Blumeria graminis* sp. *hordei*), leaf rust (*Puccinia hordei*). The only exception was a higher susceptibility of both *be* mutants to net blotch (*Drechslera teres*) [8]. Absence of clearly expressed differences between *WT* and *be* type mutants in field conditions was the main reason why those mutants were not examined for moldy germinating grain frequency. The effect of SA on the seed quality of both *be* mutants tested has also been fixed (Table 2).

Table 3. **Root length in SA_2 of germinating grains of barely cv. 'Auksiniai 3' and mutants arisen from it and in SA_1 exposed to salicylic acid (SA)**

Grain treatment in SA_1/ SA mM	Root length, cm					
	A3	tw_7	tw_8	tw_{11}	be_1	be_2
SA_1 not sprayed with SA						
0	4.7 ± 0.2	4.5 ± 0.3	4.1 ± 0.2	4.6 ± 0.2	5.0 ± 0.3	4.7 ± 0.3
0.05	4.8 ± 0.3	4.8 ± 0.2	3.6 ± 0.4	4.8 ± 0.2	5.2 ± 0.2	4.3 ± 0.3
0.25	4.8 ± 0.2	4.6 ± 0.3	4.1 ± 0.2	4.4 ± 0.3	5.7 ± 0.3	4.2 ± 0.2
0.50	6.1 ± 0.3^{3c}	4.4 ± 0.3	5.1 ± 0.3^{2b}	5.4 ± 0.3^{1a}	6.0 ± 0.2^{2b}	5.6 ± 0.3
1.00	5.1 ± 0.2	4.9 ± 0.3	5.3 ± 0.3^{2b}	5.0 ± 0.3	5.4 ± 0.3	5.0 ± 0.3
SA_1 sprayed once with 0.05 mM SA						
0	7.0 ± 0.2^3	6.4 ± 0.3^3	6.2 ± 0.2^3	5.4 ± 0.2^2	6.5 ± 0.2^3	6.3 ± 0.2^3
0.5	6.4 ± 0.2^3	5.9 ± 0.3^3	7.0 ± 0.3^{3a}	5.5 ± 0.4^1	6.1 ± 0.3^2	6.9 ± 0.3^3
0.25	7.2 ± 0.2^3	4.8 ± 0.1^c	5.6 ± 0.2^{3a}	6.3 ± 0.2^3	7.5 ± 0.2^{3b}	6.3 ± 0.2^3
0.50	6.7 ± 0.2	6.1 ± 0.2^3	5.3 ± 0.2^{3c}	6.0 ± 0.2^3	7.1 ± 0.3^3	6.9 ± 0.3^3
1.00	7.2 ± 0.3^3	5.5 ± 0.2^{2b}	5.2 ± 0.3^{2b}	5.9 ± 0.3^3	7.2 ± 0.3^3	6.9 ± 0.2^3
SA_1 sprayed twice with 0.05 mM SA						
0	6.0 ± 0.3^3	5.6 ± 0.3^2	5.8 ± 0.3^3	5.3 ± 0.3^1	5.6 ± 0.2	5.6 ± 0.2
0.05	5.9 ± 0.2^3	6.1 ± 0.2^3	4.8 ± 0.2^{2b}	4.5 ± 0.2^b	5.3 ± 0.3	6.0 ± 0.2^3
0.25	6.0 ± 0.2^3	5.3 ± 0.3	5.0 ± 0.2^{2a}	4.8 ± 0.1	5.1 ± 0.2^a	5.9 ± 0.3^2
0.50	5.6 ± 0.2^2	5.4 ± 0.3^1	5.4 ± 0.2^3	5.3 ± 0.2^1	5.8 ± 0.3	5.7 ± 0.2
1.00	5.8 ± 0.2^2	5.0 ± 0.2	5.0 ± 0.2^{2a}	5.1 ± 0.2	6.1 ± 0.3^2	5.8 ± 0.3^2
SA_1 sprayed three times with 0.05 mM SA						
0	7.5 ± 0.3^3	5.7 ± 0.2^3	5.7 ± 0.3^3	6.8 ± 0.3^3	7.1 ± 0.3^3	7.0 ± 0.2^3
0.05	6.2 ± 0.3^{2b}	6.7 ± 0.3^3	6.0 ± 0.3^3	5.7 ± 0.4^{1a}	6.1 ± 0.3^{2a}	6.4 ± 0.4^3
0.25	6.5 ± 0.5	6.9 ± 0.2^3	6.2 ± 0.5^3	6.1 ± 0.5^2	6.1 ± 0.4^{1a}	6.0 ± 0.4^{2a}
0.50	5.5 ± 0.3	6.4 ± 0.3^3	5.9 ± 0.3^3	6.7 ± 0.3^3	7.0 ± 0.4^3	6.9 ± 0.4^3
1.00	7.3 ± 0.3^3	4.8 ± 0.5	6.0 ± 0.3^3	6.5 ± 0.3^3	6.6 ± 0.4^2	7.0 ± 0.4^3

1,a – $P < 0.05$; 2, b – $P < 0.01$; 3, c – $P < 0.001$; 1,2,3 – compared with germinating grains from plants in SA_1 absolutely untreated (unsoaked and unsprayed) with SA (**0**); a, b, c – compared with germinating grains from plants in SA_1 sprayed with 0.05 mM SA, but unsoaked in SA (0); *1, 2, 3,* or *a, b, c* – if root length is decreased; A3 – *WT* /initial cv. 'Auksiniai 3'

The unreal idea that SA treatment in one plant generation could increase immunoresistance of seed material in the next generation gave unexpected results. Even seed treatment with SA alone without plant spraying with SA (the conditions most remote in time from the next seed-harvest) gave a positive result if the seed material in SA_1 had been treated with the highest 1.0 mM SA concentration. The effect of SA on seed quality in SA_2 was even more evident when SA_1 plants were also sprayed three times with 0.05 mM SA. This effect was present in all barley mutants tested in the present work, but differences among separate mutants were also evident. So, most responsive to SA-spraying were two mutants – tw_{11} and be_1 (Table 2). In SA_2, for both mutants the effect of SA was very clear. For mutants tw_1, be_1 and be_2 the frequency of moldy germinating grains was decreased to statistically significant values if a 3-time spraying with 0.05 mM SA was applied (Table 2).

It is also evident that the effect of SA treatment on seed material quality can be discovered only on mutants of *tw* or *be* type, which are characterized by an increased frequency of moldy germinating grains. It is an advantage of such mutants, which offers a new field of their application.

As to the effect of 3-time SA spraying in SA_1 and in SA_2, it was not so clear in SA_1. Increased immunoresistance was observed only for *Puccinia hordei* (in the group of *tw*, tw_1, tw_2 mutants) and for *Drechslera teres* (among *tw*, tw_2 and be_2 plants) [7].

Besides the frequency of moldy germinating grains, also germination capacity and root length were analysed. In Tables 1 and 2 only statistically significant differences are shown. For the mutants arisen from cv. 'Auksiniai II', the consequences of SA

treatment in SA_1 on the characteristics of germinating grains in SA_2 were irregular.

However, an unexpected effect was discovered for SA action in SA_2 on the root length of germinating grains (Table 3). After spraying the plants with 0.05 mM SA in SA_1, roots in SA_2 were significantly longer. However, like susceptibility to *Puccinia hordei* or *Drechslera teres* in field conditions of SA_1 [7], in SA_2 the effect was not a specificity of the mutants but a common peculiarity of the basic *WT* genotype (initial cv. 'Auksiniai 3') from which all those mutants arose.

Thus, many characteristics of induced mutants can be determined not only by the features of a mutant itself, but also by the initial genotype from which the mutant arose. Therefore, for investigation purposes it is correct to use only mutants of common history.

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MIEÞIØ *TW* TIPO MUTANTØ SËKLINËS MEDÞIAGOS KOKYBË PAGAL JAUTRUMÀ MIKROMICETAMS PO TËVINËS KARTOS POVEIKIO SALICILO RÛGÐTIMI

Santrauka

Mieþiø *tweaky spike* (*tw*) mutantams bûdingas padidëjæs dygstanèiø grûdø pelijimas. Ðiame darbe minëtas reiðkinys nustatytas ir kitai mieþiø mutantø grupei – *branched ear* (*be*). SA poveikis labai pagerina sëklinës medþiagos kokybæ: pastebimai sumaþëja supelijusiø dygstanèiø grûdø. Ðià salicilo rûgðties ypatybæ galima aptikti tik su tokiais mutantais kaip *tw* arba *be*. Jø panaudojimas atveria naujas galimybes tiriant augalø indukuotà imunitetà.