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1 **Transcriptional analysis of wheat seedlings inoculated with *Fusarium culmorum* under continual**
2 **exposure to disease defence inductors**
3
4 **Short title: Gene expression in wheat exposed to disease defence inductors**
5
6 Zuzana Antalová[1,2], Dominik Bleša[1,3], Petr Martinek [1], Pavel Matušinsky [1,4*]
7 **1** Agrotest Fyto, Ltd, Havlíčkova 2787/121, 767 01 Kroměříž, Czech Republic,
8 **2** Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural
9 Research, Šlechtitelů 31, CZ Olomouc, Czech Republic,
10 **3** Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 61137
11 Brno, Czech Republic,
12 **4** Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 27, 783 71
13 Olomouc, Czech Republic
14
15 *Corresponding author e-mail: matusinsky@vukrom.cz
16
17 **Abstract**
18 A facultative parasite of cereals, *Fusarium culmorum* is a soil-, air- and seed-borne fungus causing foot
19 and root rot, fusarium seedling blight, and especially Fusarium head blight, a spike disease leading to
20 decreased yield and mycotoxin contamination of grain. In the present study, we tested changes in
21 expression of wheat genes (*B2H2*, *ICS*, *PAL*, and *PR2*) involved in defence against diseases. We first
22 compared expression of the analysed genes in seedlings of non-inoculated and artificially inoculated
23 wheat (variety Bohemia). The second part of the experiment compared expression of these genes in
24 seedlings grown under various treatment conditions. These treatments were chosen to determine the
25 effects of prochloraz, sodium bicarbonate, ergosterol, aescin and potassium iodide on expression of the
26 analysed defence genes. In addition to the inoculated and non-inoculated cultivar Bohemia, we examined
27 two other varieties of wheat with contrasting resistance to *Fusarium* sp. infection. These were the blue
28 aleurone layer variety Scorpion that is susceptible to *Fusarium* sp. infection and variety V2-49-17 with
29 yellow endosperm and partial resistance to *Fusarium* sp. infection. In this manner, we were able to
30 compare potential effects of inductors upon defence gene expression among three varieties with different
31 susceptibility to infection but also between inoculated and non-inoculated seedlings of a single variety.
32 The lowest infection levels were detected in the sodium bicarbonate treatment. Sodium bicarbonate had
33 not only negative influence on *Fusarium* growth but also positively affected expression of plant defence
34 genes. Expression of the four marker genes shown to be important in plant defence was significantly
35 affected by the treatments. The greatest upregulation in comparison to the water control was identified

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36 under all treatments for the *B2H2* gene. Only expression of *PAL* under the ergosterol and prochloraz
37 treatments were not statistically significant.
38

39 **Keywords** Fusarium head blight, Fusarium seedling blight, aescin, ergosterol, sodium bicarbonate,
40 chitinase, qPCR, potassium iodide
41

42 Introduction

43 *Fusarium culmorum* is a ubiquitous soil-borne fungus with a highly competitive saprophytic capability.
44 As a facultative parasite, it can cause foot and root rot (1). *Fusarium culmorum* is also seed-borne and
45 causes fusarium seedling blight when infected seed is used in sowing. Seedling blight can cause extensive
46 damage to growing seedlings (2) that can lead to reduced plant establishment, number of heads per square
47 meter, and also grain yield (3). Fusarium head blight (FHB) is one of the most severe diseases responsible
48 for decrease in grain yield and quality. Furthermore, presence of mycotoxins produced by this fungus
49 (deoxynivalenol, nivalenol, zearalenone, and many others) can harm human and animal health. FHB in
50 wheat is mainly caused by *Fusarium graminearum, F. culmorum*, and *F. poae*. *Fusarium culmorum*
51 infection is dominant in colder regions, such as north, east, and central Europe (1). The major reservoirs
52 of *Fusarium* sp. inoculum are crop residues on the soil surface. The fungus can survive on a wide range of
53 living plant species (wheat, corn, barley, soybean, and rice) (see Bai and Shaner for a review (4)).

54 There are several means to fight this disease: use of fungicides, cultural practices, resistant cultivars,
55 and biological agents (5). Although seed treatment is used to control soil-borne infection caused by
56 *Fusarium* spp. (6), there is no definite way to defeat this complex of Fusarium diseases. Efficacy of
57 fungicide treatments against FHB is only 15–30% (7). Fully resistant cultivars are not available to date,
58 but some cultivars have useable levels of partial resistance that limit yield loss and mycotoxins
59 accumulation (8). FHB resistance has a quantitative nature and identification of responsible genes is
60 difficult. Even though numerous quantitative trait loci have been described to date (see Duba et al. for a
61 review (9)), just a few such genes have been definitively identified, sequenced, and their causal mutations
62 determined. Kage et al. (10) identified an FHB resistance gene on chromosome 2DL as the *TaACT* gene
63 encoding agmatine coumaroyl transferase. They suggest that several single nucleotide polymorphisms
64 (SNPs) and two inversions may be important for gene function. The second identified gene, *Fhb1*, confers
65 resistance in variety Sumai 3. It is pore-forming toxin-like (*PFT*) gene (11). Further, a number of
66 pathogenicity and virulence factors have been characterized (11, 12, 13).

67 The expression of defence-related genes also can be important in the plant's reaction against
68 pathogens. *PR-1*, *PR-2* (glucanases), *PR-3* (chitinase), *PR-4* (thaumatin-like proteins), *PR-5*, and
69 peroxidase have been shown to be induced in both resistant and susceptible cultivars after point
70 inoculation (14). These proteins were detected as early as 6 to 12 h after inoculation and peaked after 36

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to 48 h. Earlier and greater expression of PR-4 and PR-5 transcripts were observed, however, in resistant cultivar Sumai 3 than in susceptible cultivar Wheaton (14). Larger amounts of β-1,3-glucanase and chitinase enzymes also have been detected in resistant cultivar Sumai (15). The overexpression of defence response genes in wheat could enhance FHB resistance in both greenhouse and field conditions.

A large number of organic and inorganic compounds have previously been described as affecting plant defence mechanisms (16, 17). For example, such plant or fungal-derived compounds as monoterpenes or ergosterol can induce plant defence (18, 19).

Our study is focused upon comparing expression of different genes in non-inoculated and inoculated varieties and under various treatment conditions. The first part of the experiment compares expression of the genes *β-1,3-glucanase (PR2)*, *chitinase (B2H2), phenylalanine ammonia-lyase (PAL),* and *isochorismate synthase* (*ICS)* (the last two being genes from the salicylate pathway) in healthy seedlings versus seedlings of *Triticum aestivum* var. Bohemia artificially inoculated with *F. culmorum*. The second part of the experiment focused on how expression of these genes is influenced by different treatment solutions (based upon prochloraz, aescin, ergosterol, sodium bicarbonate and potassium iodide) within which seedlings were grown. How these treatments influence expression of the aforementioned genes is compared with a water control in inoculated and healthy seedlings of Bohemia as well as in the moderately *Fusarium*-resistant yellow endosperm variety V2-49-17 and the susceptible blue aleurone layer variety Scorpion. The originality of this research consists in using artificially infected seeds during anthesis of mother plants and continuous exposure of seedlings to potential inductors.

Materials and methods

Plant material

Inoculated and non-inoculated (healthy) groups of wheat seeds (var. Bohemia) were used for the first part of the experiment. Inoculated and healthy seeds were acquired from plants grown under field conditions during the 2017 season. Inoculated seeds were collected from plants that had been sprayed during mid-anthesis phase by *F. culmorum* (tribe KM16902) macroconidia at concentration 5×10^5 conidia ml^{-1}. A previous study had shown a high level of virulence and strong production of deoxynivalenol (DON) by this tribe (20). The seeds from inoculated and healthy variants were collected and stored at room temperature and low humidity. The plant material further consisted of two bread wheat cultivars differing in grain colour and in susceptibility to *F. culmorum* infection (V2-49-17 – yellow kernels, medium resistant to infection; Scorpion – blue aleurone layer, highly susceptible to infection).

Confirmation of *F. culmorum* presence in inoculated seeds

The presence of *F. culmorum* in seeds was confirmed in two ways, using quantitative (real-time) polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA). DNA from 50 mg

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106 of inoculated and healthy seeds was isolated using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany)
107 according to the manufacturer's instructions. The DNA concentration was measured by Qubit (Thermo
108 Fisher Scientific, Waltham, MA, USA), and the DNA was diluted to 10 ng μl^{-1}. Primers and conditions
109 for the qPCR reaction were as described by Moradi et al. (21). The qPCR solution consisted of 1× SYBR
110 Master Mix (Top-Bio, Prague, Czech Republic), 10 ng DNA, and water to volume 15 µl. The qPCR
111 analyses were performed using the CFX96TM Real-Time PCR Detection System (Bio-Rad, Hercules,
112 CA, USA). The gDNA isolated from mycelium of *F. culmorum* tribe KM16902 with known
113 concentration was used for inspecting the amount of *F. culmorum* DNA in inoculated seeds. The second
114 way of confirmation was to measure mycotoxin content by ELISA in both inoculated and non-inoculated
115 seeds. For the ELISA method, R-Biopharm AG kits (Darmstadt, Germany) were used. A combination of
116 RIDASCREEN®DON and RIDACREEN®FAST DON kits was used to determine DON. The limit of
117 quantification for DON was 20 µg kg^{-1}.
118
119 **Growth chamber test under controlled conditions**
120 Growth chamber test of 50 kernels of all three cultivars (Bohemia – inoculated and healthy; Scorpion, and
121 V2-49-17) were laid with 1 cm separation distance into two layers of filtrate paper and rolled up. The
122 rolls were immersed into the treatment solutions. Four repetitions (200 kernels in total) were made for
123 each combination of cultivar and treatment solution. The treatment solutions consisted of 25 µg ml^{-1}
124 solution of ergosterol, 25 µg ml^{-1} solution of aescin, 1 µg ml^{-1} solution of prochloraz, 1% solution of
125 potassium iodide and 0.1 M solution of sodium bicarbonate. The sodium bicarbonate solution was boiled
126 at 120°C for half an hour. During boiling, the sodium bicarbonate gradually decomposed to sodium
127 carbonate, water and carbon dioxide. This reaction led to alkalization of pH. Distilled water was used as a
128 control solution.
129 Seedlings were cultivated under controlled conditions (20°C/18°C, 12/12 h of light/dark) until the
130 two-leaves growth stage. At this stage, the whole leaves were collected from three plants representing
131 three biological replicates. The leaves showed no signs of *F. culmorum* infection. Symptoms of *F.*
132 *culmorum* infection were visible only on the lower parts of plants and around the seeds (**Fig 1**). The
133 leaves were immediately frozen in liquid nitrogen and preserved at −80°C until RNA isolation. The
134 numbers of infected seeds and seeds with no sign of infection were counted and the results were
135 statistically analysed by ANOVA in conjunction with Tukey's post hoc test ($P < 0.05$). (Statistica 12
136 software).
137
138 **Fig 1. Inoculated and non-inoculated seedlings of cv. Bohemia under different treatment**
139 **conditions.** (A) non-inoculated seedlings in water, (B) inoculated seedlings in water, (C) inoculated
140 seedlings in prochloraz, (D) inoculated seedlings in potassium iodide, (E) inoculated seedlings in sodium

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141 bicarbonate. Pink–white coloration around kernels and dark discoloration of coleoptiles and stem bases
142 are symptoms of *F. culmorum* infection.
143
144 **RNA isolation and qPCR**
145 Leaves were homogenized in a TissueLyser II (Qiagen) for 2 minutes at 27 Hz. Caution was taken during
146 homogenization to avoid sample melting. The homogenized samples were immediately placed into liquid
147 nitrogen. The RNA was isolated using the RNeasy Plant Mini Kit (Qiagen) while following the
148 manufacturer's instructions. DNA was removed during the RNA purification using the RNase-Free
149 DNase Set (Qiagen). The isolated RNA was stored at −80°C. cDNA was synthesized using the
150 Transcription High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) according to
151 the manufacturer's instructions with 1 µg of total RNA and anchored-oligo (dT) primers. The
152 concentration of cDNA was measured with Qubit (Thermo Fisher Scientific) and cDNA was diluted to
153 concentration 10 ng μl^{-1}. The expression analysis of the chosen plant defence genes (chitinase [*B2H2*], β-
154 1,3-glucanase [*PR2*], isochorismate synthase [*ICS*], and phenylalanine ammonia-lyase [PAL]) was
155 performed using the CFX96TM Real-Time PCR Detection System (Bio-Rad). The qPCR mix consisted
156 of 1× SYBR Green (Top Bio), 0.2 µM forward and reverse primers (**Table 1**), 15 ng cDNA, and water to
157 final volume 15 µl. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
158 according to Travella et al. (2006) (22) and Sun et al. (2014) (23). The control sample consisted of equal
159 amounts of cDNA from all three replicates of healthy, untreated Bohemia seedlings diluted to 10 ng μl^{-1}.
160 The primers specificity and presence of primer dimers were verified by melting analysis. The data were
161 analysed using the $2^{-\Delta\Delta Cq}$ method with CFX Manager 3.0 software (Bio-Rad, USA). Three biological as
162 well as three technical replicates were run.
163
164 **Table 1. Primer pairs used in the study.** Names, sequences of forward and reverse primers, publication
165 sources of primer pairs, and gene functions are listed.

Gene name	Forward primer	Reverse primer	Publication	Function
B2H2	TCTATCGAAACGCCATTGTTACA	AGAGGCCGTTCGCATAGTCA	(43)	chitinase
PR2	CCGCACAAGACACCTCAAGATA	CGATGCCCTTGGTTTGGTAGA	(44)	β-1,3-glucanase
PAL	TTGATGAAGCCGAAGCAGGACC	ATGGGGGTGCCTTGGAAGTTGC	(45)	salicylate pathway
ICS	AGAAATGAGGACGACGAGTTTGAC	CCAAGTAGTGCTGATCTAATCCCAA	(45)	salicylate pathway
GAPDH	TTAGACTTGCGAAGCCAGCA	AAATGCCCTTGAGGTTTCCC	(23)	reference gene

166
167 **Results**
168

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169 **Determination of *Fusarium* infection level in growth chamber test**
170 Inoculated and healthy seeds of wheat cv. Bohemia were treated with different solutions: water,
171 prochloraz, aescin, ergosterol, sodium bicarbonate and potassium iodide. Inoculated seeds contained high
172 levels of *F. culmorum* DNA (confirmed by qPCR, data not shown), the mean of three replications being
173 5,048 μg kg^{-1} of DON (analysed by ELISA, data not shown). No *F. culmorum* DNA was detected in the
174 non-inoculated seeds, and their DON content (if any) was under the detection limit. The plants were
175 visually inspected at the two-leaves stage for presence of *Fusarium* infection. The number of infected
176 seeds under every treatment was compared to that for the control (treated only with water). The presence
177 of *Fusarium* infection was detectable by pink–white mycelia growing around kernels and dark
178 discoloration of the coleoptile and stem (**Fig 1**).
179 In the variant without inoculation there were no infected seeds. On the contrary, the inoculated seeds
180 that had been submerged in water showed high level of infection (**Fig 1**). This level of infection was
181 decreased under every treatment except for that of potassium iodide. The level of infection in the
182 potassium iodide treatment group was even increased in comparison to the water-treated inoculated seeds.
183 In evaluating the 200 seeds from each combination, significant differences were detected between
184 individual groups. The results suggest that the lowest level of infection in inoculated seeds was detected
185 in the sodium bicarbonate treatment, followed (in order from lowest to highest) by prochloraz, ergosterol,
186 aescin, water, and potassium iodide (**Fig 2**). Thus, the treatment with 0.1M sodium bicarbonate was more
187 potent in suppressing fungal growth than was the treatment with 1 μg ml^{-1} prochloraz.
188
189 **Fig 2. Percentage of *F. culmorum*-infected plants in different treatments with cultivar Bohemia.**
190 Two hundred inoculated seeds were evaluated for each treatment solution (water [control], ergosterol,
191 aescin, sodium bicarbonate, prochloraz and potassium iodide). Error bars indicate 95% confidence
192 intervals around the mean.
193
194 **Expression levels of four genes involved in plant–pathogen interaction in wheat seedlings**
195 The expression of four important plant defence genes (*B2H2*, *ICS*, *PAL*, and *PR2*) against *Fusarium*
196 infection was detected. These genes were analysed in three technical and biological replications of each
197 studied variety under each treatment. The fold differences (FDs) in their expression levels were first
198 compared among the reference (water control) and other experimental conditions (ergosterol, aescin,
199 sodium bicarbonate, prochloraz, and potassium iodide) of all studied varieties (**Fig 3**). In this manner, the
200 efficiency of different treatments in enhancing expression of plant defence genes with and without
201 pathogen infection was examined. The treatments did not influence expression of the genes in an even
202 manner, as expression was stronger in some genes and weaker in others. Only insignificant changes were

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203 detected in inoculated Bohemia under the ergosterol treatment (*ICS, PAL*) as well as in Scorpion under
204 the aescin treatment (*PAL*).
205
206 **Fig 3. Heat map summarizing expression levels of four chosen genes in leaves of healthy and**
207 **inoculated plants of wheat variety Bohemia and healthy V2-49-17 and Scorpion varieties.** The
208 expression of four genes (*B2H2*, *ICS*, *PAL*, and *PR2*) were detected in (A) healthy and (B) inoculated
209 Bohemia, (C) V2-49-17 variety with yellow endosperm, and (D) cv. Scorpion with blue aleurone layer in
210 different treatment conditions (water, ergosterol, aescin, sodium bicarbonate, prochloraz, potassium
211 iodide). Colour range from light to dark represents increasing expression. This scale was determined for
212 every gene individually. Significant differences between water control and experimental treatments are
213 indicated by asterisks ($P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***); $P < 0.0001$ (****). The statistical
214 analyses were carried out separately within each gene and treatment. FD indicates fold change difference
215 between expression levels of experimental treatment vs. water control.
216
217 *B2H2* expression was upregulated by almost all treatments in all studied varieties (**Fig 3**). The highest
218 expression level of this gene was achieved under the sodium bicarbonate (V2-49-17) treatment followed
219 by the prochloraz (V2-49-17) and potassium iodide (healthy Bohemia) treatments. It was downregulated
220 just under the ergosterol (healthy Bohemia and Scorpion), aescin (Scorpion), and prochloraz (Scorpion)
221 treatments. This gene achieved the largest FD (up to 7,370; potassium iodide) in comparison to the other
222 genes. The FD was lowest in inoculated Bohemia. The FD of *B2H2* was in some cases near to 1,000 in
223 comparison to the water control. This high FD was not achieved solely in the inoculated Bohemia and the
224 Scorpion variety. In inoculated Bohemia, for example, the FD ranged from 0.95 under the sodium to 7.45
225 under the iodide treatment. In healthy Bohemia, the expression ranged from −0.99 to 7,370 FD in
226 comparison to the water control. A similarly large increase of expression in comparison to the water
227 control as in healthy Bohemia was identified also in V2-49-17. The V2-49-17 variety showed significant
228 increase of *B2H2* expression in comparison to healthy Bohemia while the expression of all other genes
229 was lower.
230 The *ICS* gene manifested the smallest fold increase under almost all analysed treatments. Its
231 expression was often downregulated under some experimental treatments in some analysed varieties (**Fig**
232 **3**). The largest fold difference was detected in the V2-49-17 variety under the prochloraz treatment, the
233 smallest in inoculated Bohemia under the prochloraz treatment. Under the iodide treatment, all FDs were
234 in negative values for all analysed varieties.
235 FDs for *PAL* expression were increased under almost every treatment. These increases were not to
236 such large extent as seen for *B2H2*. The largest FDs were achieved under prochloraz (82.05 FD) and
237 sodium bicarbonate (57.79 FD) treatments in the V2-49-17 variety. Downregulation of *PAL* expression in

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238 comparison to the water control was identified under the ergosterol treatment in healthy Bohemia, under
239 potassium iodide treatment in V2-49-17, and under potassium iodide and aescin treatments in the
240 Scorpion variety (**Fig 3**). The lowest FD was detected under iodide in the yellow variety.
241 The FDs for *PR2* expression in comparison to the water control were elevated under almost all
242 treatments and in all analysed varieties except for the ergosterol treatment in healthy Bohemia. The
243 largest FD was seen in the V2-49-17 variety under all treatments except for prochloraz and iodide, in
244 which cases the FDs were greater in the Scorpion variety. The largest FD for *PR2* was observed under the
245 iodide treatment (from 20.75 to 49.85 FD). The lowest FD in almost all cases was found in healthy
246 Bohemia (**Fig 3**).
247 We further compared the expression of all four genes between the inoculated and healthy Bohemia
248 under all treatments. The strongest *B2H2* expression in inoculated Bohemia was identified under
249 potassium iodide treatment and the weakest under the control. The strongest *B2H2* expression in healthy
250 Bohemia was identified under potassium iodide treatment followed by that for sodium bicarbonate (**Fig**
251 **4**). The expression of *B2H2* was increased in inoculated Bohemia under all treatments.
252
253 **Fig 4. Expression profiles of *B2H2*, *ICS*, *PAL*, and *PR2* in leaves of inoculated and healthy**
254 **plants of wheat variety Bohemia under different treatment conditions (A, B, C, D).** Expression levels
255 were relative to healthy cv. Bohemia seeds and were normalized with the wheat reference gene *GAPDH*.
256 Expression levels shown are mean values and standard deviation for three repetitions. Statistically
257 significant differences between healthy and inoculated cv. Bohemia plants are indicated by asterisks
258 above every treatment ($P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***); $P < 0.0001$ (****).
259
260 Expression of *ICS* was significantly downregulated in inoculated Bohemia under almost all
261 treatments, the exception being the ergosterol treatment, in which case the difference was not statistically
262 significant (**Fig 4**). The strongest *ICS* expression was detected under the prochloraz treatment in healthy
263 Bohemia. The weakest was under the iodide and ergosterol treatments.
264 Comparison of healthy versus inoculated Bohemia showed increase of *PAL* expression in inoculated
265 Bohemia under all treatments. The strongest expression of *PAL* in healthy Bohemia was identified under
266 the sodium bicarbonate treatment. The greatest expression in inoculated Bohemia was identified under the
267 prochloraz and sodium bicarbonate treatments (**Fig 4**).
268 *PR2* expression was elevated in all treatments other than the aescin treatment in inoculated Bohemia.
269 The difference between healthy and inoculated Bohemia under the aescin and prochloraz treatments was
270 not statistically significant. The expression of *PR2* in inoculated Bohemia was elevated under the iodide
271 (in comparison to healthy Bohemia), and ergosterol treatments with high significance. Small increases
272 were detected under the water and sodium bicarbonate treatments (**Fig 4**).

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273

274 **Discussion**

275 In current study was tested the expression of chosen marker genes of wheat seedlings after various
276 treatments by potential plant defence inductors. The effect of plant defence inductors was previously
277 widely studied (18, 19) and their effect on defence genes expression was taken to the account. Effect of
278 chitinase genes in increasing plant resistance to fungal diseases has been observed in previous studies (see
279 Fahmy et al. for a review (24)). Transgenic wheat with barley chitinase II was shown to be resistant
280 against powdery mildew, leaf rust pathogens, and *F. graminearum* (25, 26, 27). Chitinase from wheat,
281 barley, and maize kernels has been shown to inhibit hyphal elongation of the fungi (28). In the present
282 study, expression of the chitinase gene in the V2-49-17 variety was greater than was its expression in
283 healthy Bohemia under the control variant (water) as well as under the experimental treatments. This
284 higher value of chitinase expression can be connected to partial resistance of the yellow variety to
285 *Fusarium* sp. infection. Higher chitinase levels in resistant cultivars already have been detected in
286 previous studies (29, 30). The largest fold increase for *B2H2* expression in healthy Bohemia was detected
287 under the potassium iodide treatment. This 7,000 FD could be explained by the negative effect of this
288 treatment on seedlings growth. This retardation of growth was connected to increase of *F. culmorum*
289 infection in potassium iodide-treated varieties regardless of variety or presence of *Fusarium* infection.
290 However, more than 1,000 FD (1,363) in comparison to the water control was detected just in V2-49-17.
291 The growth retardation of seedlings under iodide treatment in our experiment is substantiated by the
292 previous study of Brenchley (31), who reported a negative effect of high concentration of potassium
293 iodide on germination of barley seeds and even of low concentration on the survival of barley seedlings.
294 Iodine at concentration 10 ppm was observed to be toxic to barley. Nevertheless, a concentration 0.5–1
295 ppm had a positive effect on barley plants (32). The iodine is most toxic in its iodide form (33). This is
296 the form we used in our experiments at 10,000 ppm concentration. The observed toxicity of potassium
297 iodide for wheat seedlings is thus understandable. The strong expression of *B2H2* is just a result of this
298 treatment and does not reflect an effect of potassium iodide's increasing resistance in the plant. The
299 potassium iodide showed inhibitory effect against *F. culmorum* growth *in vitro* (data not shown). This
300 inhibitory effect was more potent than the effects of sodium bicarbonate and prochloraz. Significant
301 increase of *B2H2* expression could be seen also in the sodium bicarbonate treatment. This increase
302 exceeded those under the prochloraz treatments. It can be seen across all analysed varieties with the
303 exception of the inoculated Bohemia. Our results imply that sodium bicarbonate is significantly effective
304 in enhancing expression of defence genes. Contrary to potassium iodide, sodium bicarbonate has no
305 negative effect on wheat seedlings' growth. Comparison of all treatments in inoculated wheat showed that
306 the lowest numbers of seedlings with detectable *Fusarium* infection at the three-leaves stage were under
307 the prochloraz and sodium bicarbonate treatments. This suggests that sodium bicarbonate has potential for

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308 increasing plant resistance. The addition of sodium bicarbonate to experimental treatments was
309 conditioned by its alkaline pH. This was in accordance with studies showing inhibition of *TRI* genes
310 expression in *Fusarium* by alkaline pH (34, 35, 36). *TRI* genes expression is important for synthesis of
311 DON, which is known to be a virulent factor aiding in the establishment and propagation of *Fusarium*
312 infection within the spikes (37, 38). In preliminary experiments, the sodium bicarbonate showed potential
313 for inhibiting *Fusarium* growth *in vitro*. This inhibition was comparable to inhibition induced by
314 prochloraz (data not shown). Indeed, the sodium bicarbonate showed great potential also *in planta*.
315 Sodium bicarbonate had not only a negative influence on *Fusarium* growth but also a positive effect on
316 expression of plant defence genes. The sodium bicarbonate has been shown to be potent in inhibiting
317 growth also of other *Fusarium* species (39, 40).

318 We analysed the SA pathway's function in plant defence by examining expression of the two genes
319 *ICS* and *PAL*, both of whose biosynthetic pathways are known to be involved in SA production within
320 *Arabidopsis* (41). Hao et al. (41) had previously detected that suppression of the *ICS* gene compromised
321 plant resistance to *F. graminearum* but that similar suppression of *PAL* genes had no significant effect.
322 Those authors also found that *F. graminearum*-inoculated plants with stronger expression of *ICS* were
323 comparable to wild-type control plants (41) and that plants with *ICS* suppressed did not accumulate SA
324 during pathogen infection and were more susceptible to *Fusarium*. In the present study, the suppression of
325 growth and higher rate of *Fusarium* infection connected to lower *ICS* expression were detectable
326 predominantly under the potassium iodide treatment, where *ICS* had negative FD in comparison to the
327 water treatment in every analysed variety. Similarly, *ICS* was downregulated under all treatments in the
328 inoculated wheat cv. Bohemia. On the other hand, *ICS* expression was upregulated in all other treatments
329 except for a few exceptions in Scorpion and inoculated Bohemia. It was upregulated in V2-49-17 under
330 all treatments other than that of potassium iodide, which can be connected to this variety's partial
331 *Fusarium* resistance. Upregulation of *ICS* in inoculated Bohemia in comparison to the water control was
332 detected only under sodium bicarbonate and aescin treatments, which can indicate effects of these
333 treatments on expression of defence genes. Hao et al. (41) have suggested that *ICS* plays a unique role in
334 SA biosynthesis in barley, which, in turn, confers a basal resistance to *F. graminearum* by modulating the
335 accumulation of H_2O_2, O_2, and reactive oxygen-associated enzymatic activities. In the present study, the
336 greatest increase in *PAL* expression was detected in the V2-49-17 and Scorpion varieties. We found no
337 correlation between higher *PAL* expression and the level of resistance to *F. culmorum*, because V2-49-17
338 is partially resistant but Scorpion is susceptible. In healthy versus inoculated Bohemia, *PAL* expression
339 was generally increased under most treatments and especially under the potassium iodide treatments.
340 Wildermuth et al. had detected increased *PAL* and decreased *ICS* expression after *F. graminearum*
341 infection (42). They also found a difference in timing whereby earlier increase of *PAL* expression was

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342 detected in a partially resistant variety (Wangshuibai). They found no difference, however, between
343 resistant and susceptible varieties in the timing of decrease in *ICS* expression.
344

345 **Conclusion**

346 We conclude that sodium bicarbonate has the greatest potential for increasing plant resistance without
347 having a negative effect on plant growth. According to our findings, the sodium bicarbonate had not only
348 a negative influence on *Fusarium* growth but also a positive effect on upregulating the expression of plant
349 defence genes.
350

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354
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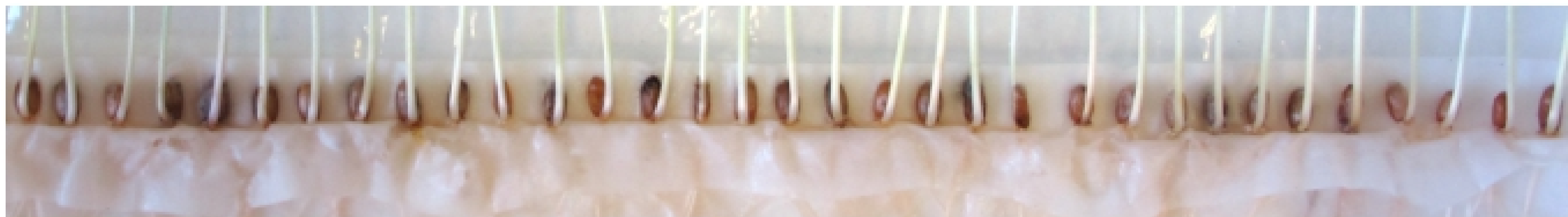
A



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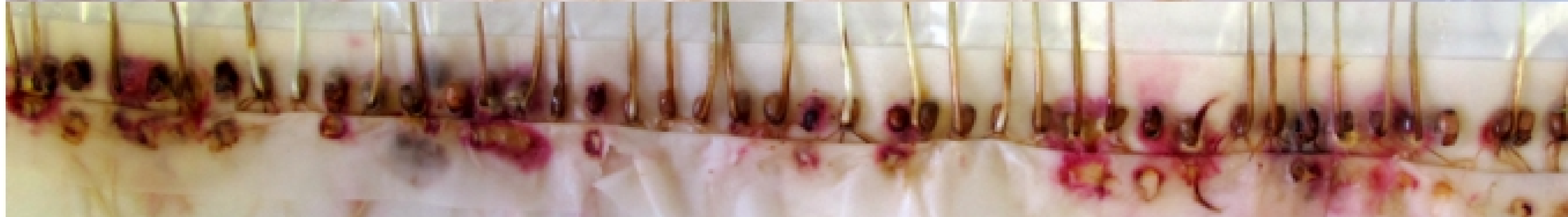

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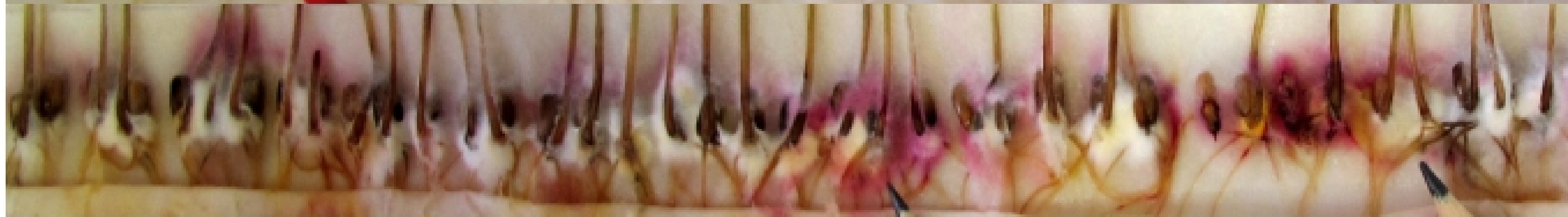

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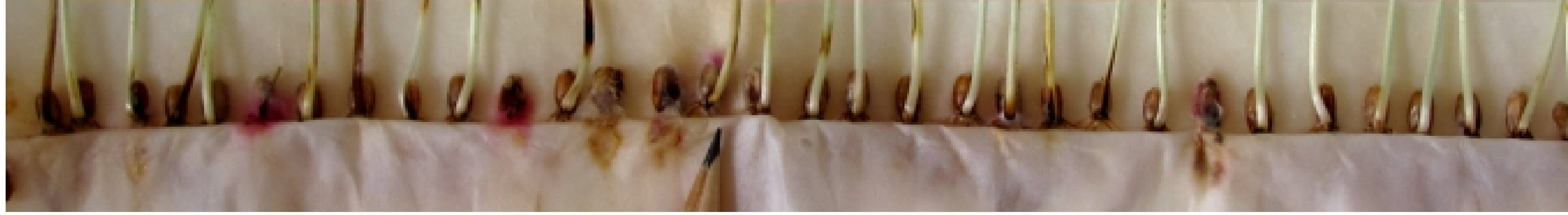

Figure 1

Figure 2

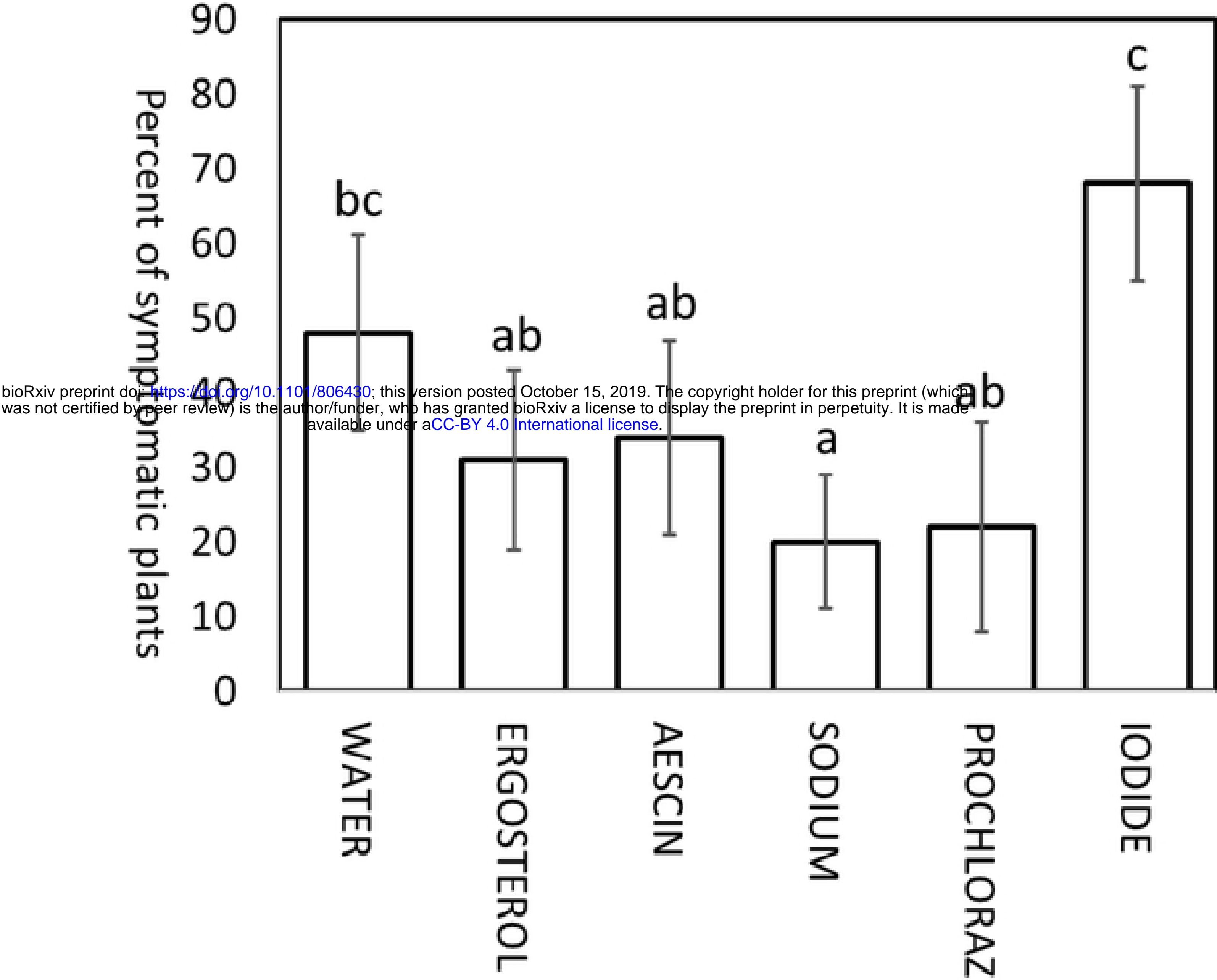

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Figure 2

Figure 3

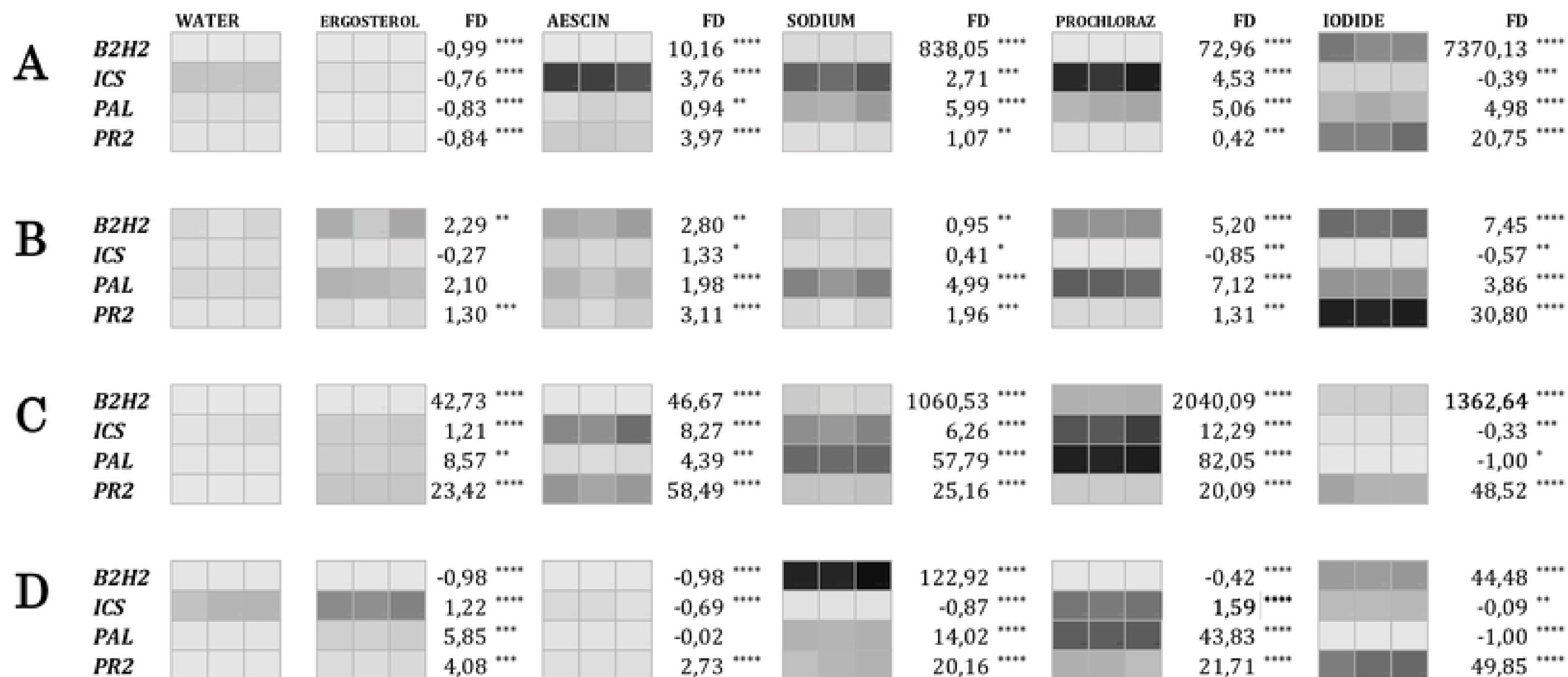

Figure 3

Figure 4

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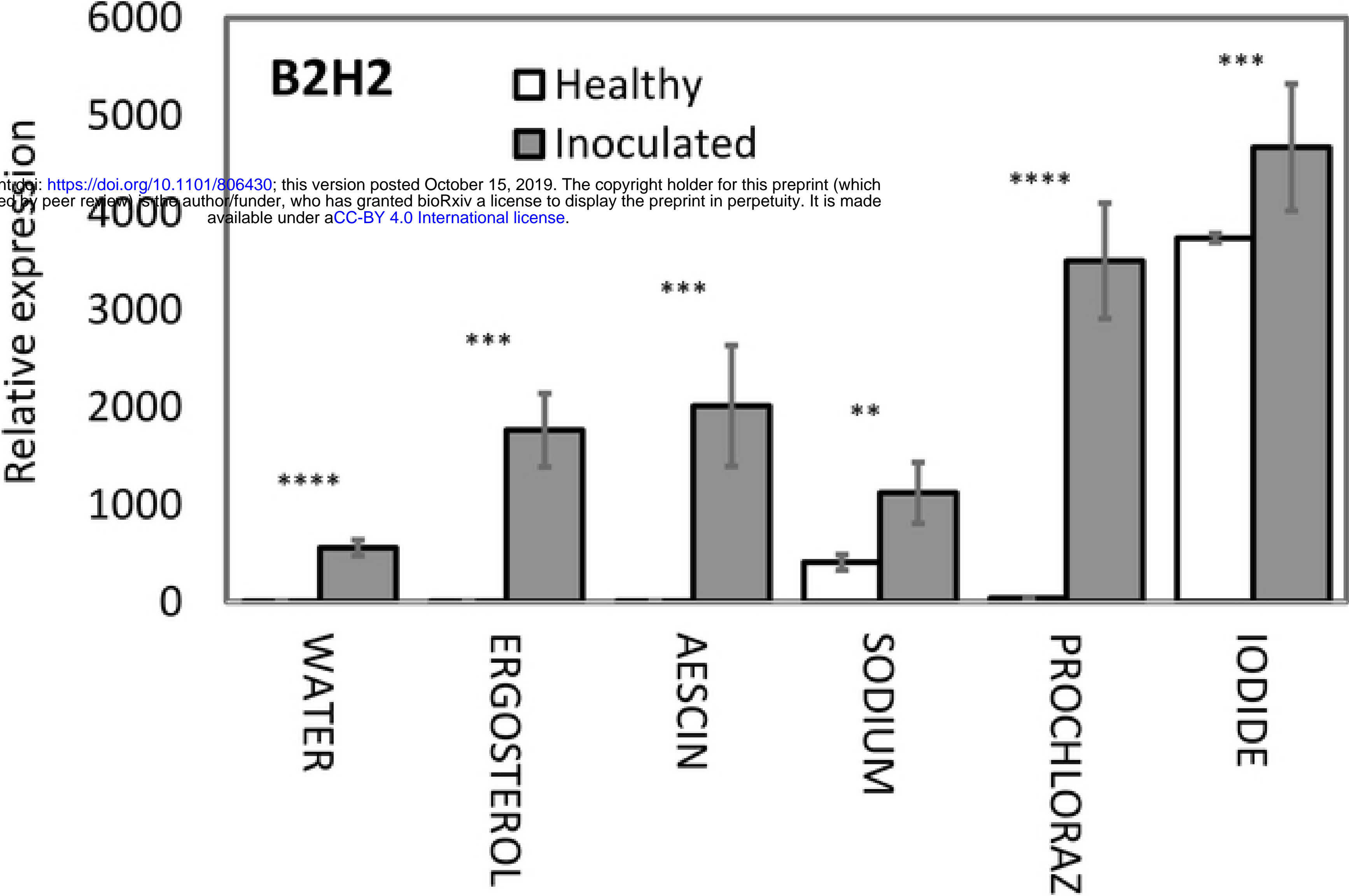

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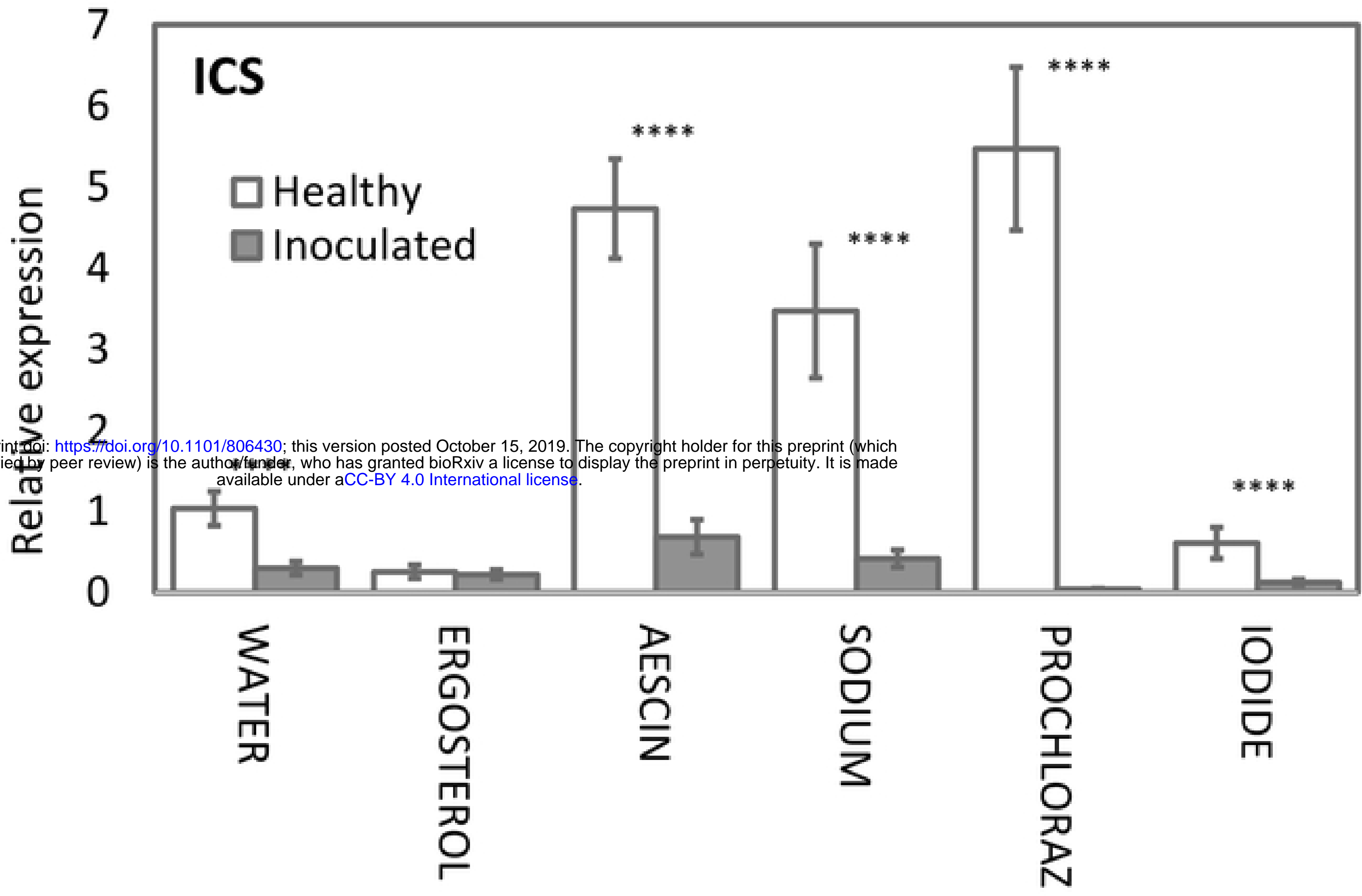

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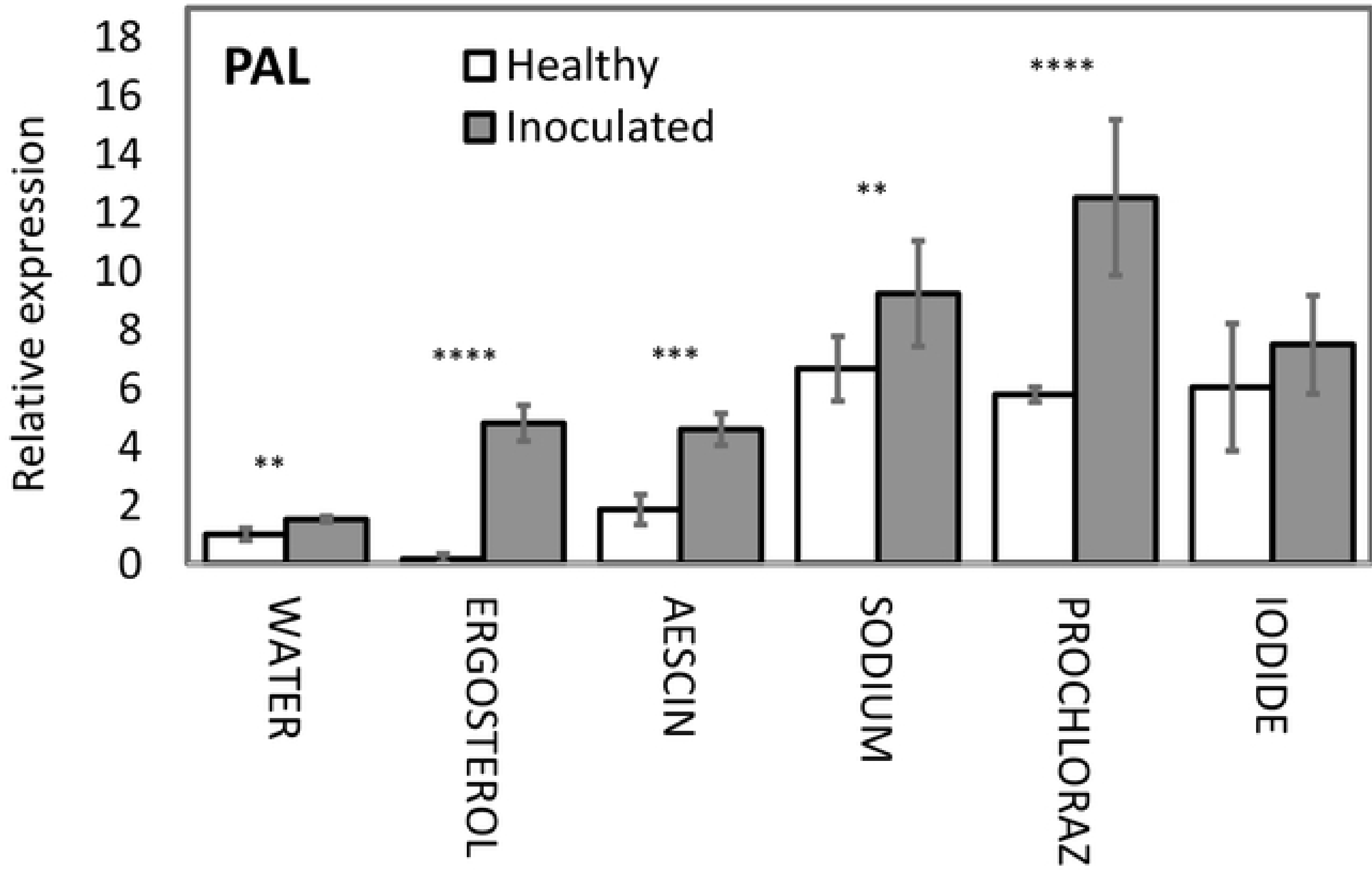

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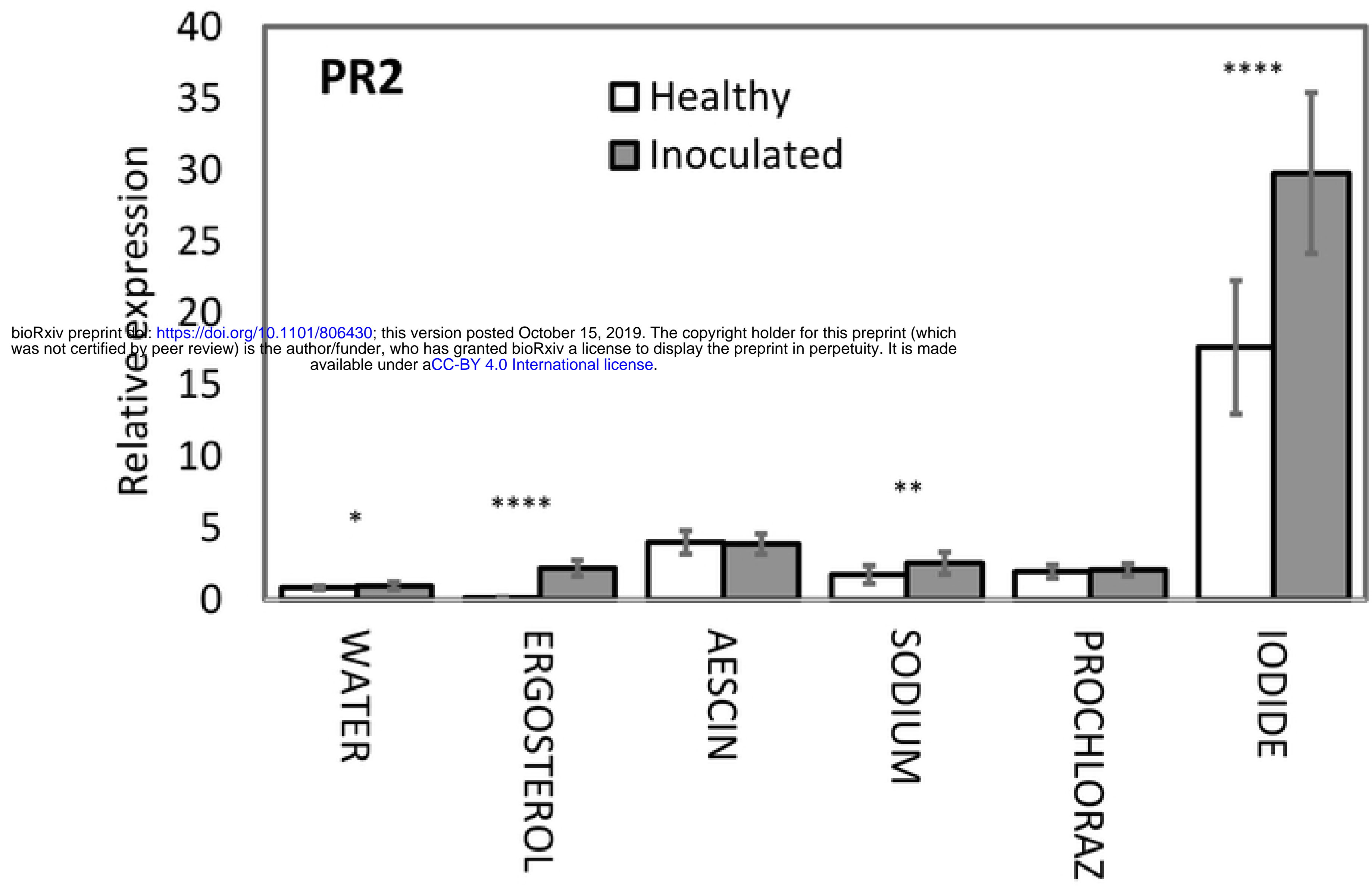

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