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1 **A Plant Nutrient- and Microbial Protein-Based Resistance Inducer Elicits Wheat**
2 **Cultivar-Dependent Resistance Against *Zymoseptoria tritici***

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18

19 **Abstract**

20 The induction of plant defense mechanisms by resistance inducers is an attractive and
21 innovative alternative to reduce the use of fungicides on wheat against *Zymoseptoria tritici*, the
22 responsible agent of septoria tritici blotch (STB). Under controlled conditions, we investigated
23 the resistance induction in three wheat cultivars with different susceptible levels to STB as a
24 response to a treatment with NECTAR Céréales® (a sulfur, manganese sulfate and protein-
25 based resistance inducer). While no direct anti-germinative effect of NECTAR Céréales® was
26 observed *in planta*, more than 50 % reduction on both symptoms and sporulation were recorded
27 on the three tested cultivars. However, an impact of the wheat genotype on this resistance
28 induced by NECTAR Céréales® was highlighted, which concerns the host penetration, cell
29 colonization and the production of cell-wall degrading enzymes by the fungus. Moreover, in
30 the most susceptible cultivar Alixan, NECTAR Céréales® upregulated *POX2*, *PAL*, *PR1* and
31 *GLUC* gene expressions in both non-infectious and infectious contexts and *CHIT2* in non-
32 infectious context only. In contrast, defense responses induced in Altigo, the most resistant
33 cultivar, seem to be more specifically mediated by the phenylpropanoid pathway in non-
34 infectious as well as infectious contexts, since *PAL* and *CHS* were most specifically upregulated
35 in this cultivar. In Premio, the moderate resistant cultivar, NECTAR Céréales® elicits mainly
36 the octadecanoid pathway, *via* *LOX* and *AOS* induction in noninfectious context. We concluded
37 that NECTAR Céréales® protects wheat against *Z. tritici* by stimulating the cultivar-dependent
38 plant defense mechanisms.

39 **Key words:** *Zymoseptoria tritici*, wheat, manganese, induced resistance, defense mechanisms.

Introduction.

40 Winter soft wheat (*Triticum aestivum* L.) is a major economically important crop in France and
41 Septoria Tritici Blotch (STB) is currently one of the most damaging foliar wheat diseases,
42 causing yield losses reaching up to 40% (Eyal, 1999; Palmer and Skinner, 2002). The infectious
43 process of the hemibiotrophic fungus *Zymoseptoria tritici*, responsible for STB, is characterized
44 by an approximately 2 to 3 weeks-long symptomless period. Later on, the fungus switches to a
45 necrotrophic phase, associated with an increase of fungal biomass, the formation of pycnidia in
46 colonized substomatal cavities, necrosis (Kema et al., 1996; Selim et al., 2011, 2014; Siah et al.
47 2010a; Ors et al. 2017) and the production of fungal cell-wall degrading enzymes (CWDE)
48 (Douaiher et al., 2007a, 2007b; Siah et al., 2010a).

49 At the field level, STB is mainly controlled by the extensive use of fungicides. An alternative
50 to chemical control is breeding for new and, if possible, fully resistant wheat cultivars. The
51 wheat resistance against *Z. tritici* is controlled by (i) gene-for-gene relationships leading to
52 incompatible interactions (Brading et al., 2002) and/or by (ii) non-specific and partial resistance
53 expressed during compatible interactions (Chartrain et al., 2004a). While *Z. tritici* is able to
54 overcome specific resistance controlled by one of the 18 known *stb* wheat genes (Goodwin,
55 2007; Goodwin and Thompson, 2011; Simón et al., 2012), partial resistance, controlled by
56 several quantitative trait loci (QTL), is more durable at the field level (Orton et al., 2011).

57 Plant resistance can be considered as the result of an effective defense against the pathogen
58 attack. Defense responses are triggered by the recognition of molecules derived from pathogens.
59 The first level of defense is based on the perception of pathogen-associated molecular pattern
60 (PAMP) or microbe-associated molecular pattern (MAMP), and is called (PAMP)-triggered
61 immunity (PTI) (Nürnberger and Scheel, 2001; Deller et al., 2011; Henry et al., 2012; Mengiste,
62 2012). To overcome PTI, effectors have emerged in pathogens, that are released within the host
63 during infectious process to suppress PTI (Li et al., 2013), which results in a compatible
64 interaction associated with partial resistance, depending on the degree of effectiveness of
65 triggered defense pathways. In turn, plant receptors can recognize such pathogen effectors,
66 resulting in a second type of immunity called effector triggered immunity (ETI), leading to an
67 incompatible interaction (Jones and Dangl, 2006; Deller et al., 2011) and total resistance.

68 Wheat defenses against *Z. tritici* have been studied during compatible and incompatible
69 interactions, revealing an early induction of peroxidase (POX activity and H₂O₂ accumulation
70 in wheat cells, associated with wheat resistance to *Z. tritici* (Shetty et al., 2003, 2007). Different
71 defense-related genes, such as genes encoding PR-proteins, were described as early and highly
72 up-regulated during the biotrophic phase of an incompatible interaction, while a slight induction
73 only or a repression were observed during a compatible interaction (Adhikari et al., 2007; Ray
74 et al., 2003; Yang et al., 2013a, 2013b). However, by comparing defense related genes
75 expression in eight combinations of cultivars and isolates leading either to compatible or
76 incompatible interactions, no significant difference was observed in the responses during the
77 symptomless phase of colonization, suggesting that induction of defense gene expression is not
78 specific of incompatible interactions (Orton et al., 2017). Moreover, a comparison between
79 cultivars with different relative levels of susceptibility against *Z. tritici* during compatible
80 interactions highlighted a constitutive expression of defense associated genes at a higher level
81 in the less susceptible studied cultivars, in non-infectious context (Ors et al., 2017).
82 Surprisingly, during the symptomless phase of fungal development, many defense genes were
83 induced in the most susceptible cultivar, while no induction or only slight inductions were
84 observed in the less susceptible cultivar (Ors et al., 2017). These results underline the
85 complexity of the mechanisms involved in specific or partial resistances of wheat against *Z.*
86 *tritici*. The induction of plant defense mechanisms by resistance inducers is an attractive and
87 innovative alternative to reduce the use of fungicides because induced resistance is a natural
88 and potentially effective, persistent and durable strategy (Walters et al., 2005, 2008; Samain et

90 al., 2019). So far, a number of resistance inducers have been developed, based on either natural
91 or synthetic compounds, that could be used in crop protection at the field (Vavrina et al., 2004;
92 Walters et al., 2008; Thakur and Sohal, 2013; Tayeh et al., 2014). Induced resistance could be
93 related to a direct elicitation of defense responses as well as to a sensitization by a priming
94 agent, which results in the expression of earlier and/or stronger basal defense mechanism upon
95 a pathogen attack (Conrath et al., 2006; Walters et al., 2005).

96 The only resistance inducer currently registered so far on wheat against STB in France is
97 Vacciplant[®] (Goëmar, France) which contains laminarin ([https://ephy.anses.fr/ppp/iodus-2-
98 cereales/](https://ephy.anses.fr/ppp/iodus-2-cereales/)). NECTAR Céréales[®] is currently commercialized as a complete foliar fertilizer
99 based on manganese and sulfur enriched with an active complex (www.agronutrition.com).
100 NECTAR Céréales[®] is investigated in this work for its protective efficacy against STB and for
101 its wheat defense activation properties. We compared the resistance induced by NECTAR
102 Céréales[®] against a given *Z. tritici* strain in three distinct wheat cultivars characterized by
103 different partial resistance levels in the field. On the one hand, the impact of the treatment on
104 the infectious process and the pathogenicity of the fungus were measured by cytological and
105 biochemical approaches during both biotrophic and necrotrophic phases of *Z. tritici*. On the
106 other hand, various defense pathways were investigated at biochemical and molecular levels
107 during the early period following treatment with NECTAR Céréales[®] in both infectious and
108 non-infectious contexts. The influence of the wheat genotype on the induced resistance has been
109 discussed.

110 **Experimental procedures**

111 **Fungal inoculum preparation**

112 *Zymoseptoria tritici* strain T01193 (GeneBank accession number KX356102), isolated from
113 Northern France (Lorgies location), is moderately resistant to Sterol 14 α -demethylase inhibitor
114 (DMI) fungicides, characterized by three SNP mutations (M-281-V, A-379-G, I-381-V) in the
115 *cyp51* gene encoding for Sterol 14 α -demethylase (Selim, 2016). Fungal material (*in vitro*
116 cirrhous) of this strain stored at - 80 °C was activated by transfer to fresh potato dextrose agar
117 (PDA) medium (39 g.L⁻¹; Sigma). After seven days incubation at 18 °C, with a 12-h
118 photoperiod, spore suspensions were prepared by washing the cultures using sterile distilled
119 water. Then, the concentration of spores was adjusted to desired concentration using a Malassez
120 cell.

121 **Direct antifungal assays**

122 Antifungal activity of NECTAR Céréales[®] against *Z. tritici* (on both fungal growth and spore
123 germination) was assessed *in vitro* using PDA medium amended with different concentrations
124 of the product (1.2, 3.7, 11.1, 33.3 and 100 mL.L⁻¹), using the spotting and spore germination
125 methods described by Siah et al. (2010b). The product was added to the medium at 30 °C after
126 autoclaving. The effect on fungal growth was recorded by measuring the colony perpendicular
127 diameters after 10 days incubation at 21 °C in the dark, while the effect on spore germination
128 was evaluated after one day incubation by determining the percentage of germinated spores
129 calculated from 100 spores using light microscope (Nikon, Eclipse80i) for each plate (Siah et
130 al., 2010). Three different plates were used as replicates for each condition, including controls,
131 within both spotting and spore germination bioassays.

132 **Plant growth and protection tests**

133 Alixan, Premio and Altigo wheat cultivars were used in this study. These cultivars exhibited
134 various levels of partial resistance against STB under field conditions in 2009 and were noted
135 as 4, 5.5, and 7, respectively, on a scale from 1 (highly susceptible) to 9 (highly resistant).
136 Wheat grains were germinated in Petri dishes and wheat plantlets were grown in the greenhouse
137 as described by Ors et al. (2017). After 3 weeks (when third leaves from the base of plants were
138 fully expanded), plants of each pot were sprayed with 2.5 mL/plant of a solution containing
139 NECTAR Céréales[®] at 8.73 μ L/mL of distilled water. This dose of NECTAR Céréales[®]

140 (previously named FSOV7) was identified by Ors et al. (2012, 2013) as the most effective dose
141 to protect wheat against *Z. tritici*. Control plants were treated with distilled water. Plants were
142 inoculated 48 h after treatment (hat) as mentioned in Selim et al. (2014). Briefly, 21-day-old
143 plants were infected by spraying a 2 mL *Z. tritici* inoculum (2×10^6 spores/mL) in 10 mM
144 MgSO_4 amended with 0.1% polyoxyethylene-sorbitan monolaurate surfactant (Tween 20,
145 Sigma). Controls were sprayed with 2 mL/plant of the same solution. Five repetitions were
146 carried out for each condition. Plants in pot were covered with transparent polyethylene bag for
147 three days and the disease was scored at 21 days after inoculation (dai) by noting the percentage
148 of the third leaf's area covered by lesions (chlorosis and necrosis) and by noting their pycnidial
149 density on a scale from 0 (absence of pycnidia) to 5 (high density of pycnidia) as described by
150 Ors et al. (2017).

151 **Histological analyses**

152 Two centimeters leaf segments were harvested from the third expanded wheat leaf at different
153 time points (1, 5, and 21 dai) and observed microscopically. Spore germination and leaf
154 penetration by *Z. tritici* was performed using Fluorescence Brightener 28 (Calcofluor, Sigma)
155 at wavelength 365 nm according to Siah et al. (2010a). Mesophyll colonization was investigated
156 using Trypan blue according to Ors et al. (2017). The percentage of germinated spores (GS)
157 was recorded at 1 dai. Total germinated spores with penetrated germ tube (PS) and within PS,
158 penetration through stomata (PSS) were accounted at 5 dai. At 21 dai, total colonized
159 substomatal cavities (CS) among 150 randomly chosen cavities were recorded and, within CS,
160 percentages of colonized substomatal cavities bearing pycnidia (CSP) were estimated.

161 **Protein extraction and enzymatic assays**

162 **Cell wall degrading enzymes assay.** Protease, β -1,4-endoglucanase and β -1,4-endoxylanase
163 activities were measured according Ors et al. (2017) and total protein concentrations were
164 determined at 595 nm using the method Bradford et al. (1976). The bovine serum albumin
165 (Sigma A7906) was used as standard.

166 **Plant enzymes assays.** Peroxydase (POX) activity was measured according to Mitchell et al.
167 (1994) modified by Randoux et al. (2006) and phenylalanine ammonia lyase (PAL) activity
168 according to Randoux et al. (2010) at 0, 12, 24, 48, 54, 60, 72 and 120 hat (hours after
169 treatment).

170 **RNA extraction and gene expression quantification by quantitative real-time RT-PCR**

171 The third expanded wheat leaves were sampled at 0, 12, 24, 48, 51, 54, 60, 72, 120 and 168
172 hours after treatment (hat). Total RNA extraction and real time RT-PCR was performed
173 according to Ors et al. (2017) to quantify the expression pattern of 9 genes: *PRI*, *CHIT2*, *gluc*,
174 *LOX*, *AOS*, *PAL*, *CHS*, *POX2* and *OXO*. The *TUB6* and *GAPDH* genes were used as
175 endogenous reference for each treatment. The primers used in this study were published
176 previously in Ors et al. (2017).

177 **Statistical analysis.**

178 All experiments were carried out using at least three technical repetitions and two biological
179 replicates. Data represent continuous variables and were analyzed using ANOVA (Tukey's test
180 at $P \leq 0.05$) assuming a normal distribution. Variances were stabilized by logarithmic
181 transformation of data if necessary, as described by Ors et al. (2017). All data were analyzed
182 by Xlstat software (Addinsoft, Paris, France).

183 **Results**

184 **Protection efficacy conferred by NECTAR Céréales® on Alixan, Premio and Altigo 185 inoculated with *Z. tritici***

186 In order to evaluate the protection efficacy of NECTAR Céréales® against *Z. tritici*, the
187 percentage of leaf area with symptoms and their pycnidial density were assessed on the third
188 fully expanded wheat leaves from the three cultivars at 21 dai (Figure 1 and supplementary
189 data).

190 In non-treated inoculated (nTI) control, the percentage of leaf area with symptoms (Figure 1A
 191 and supplementary data) recorded in Alixan and Premio was not significantly different (41.7 %
 192 and 42.6%, respectively), but was significantly lower in Altigo (27.7 %). In plants treated with
 193 NECTAR Céréales®, disease severity (10.8, 10.1 and 2.9 % in Alixan, Premio and Altigo,
 194 respectively) was significantly lower than in their nTI controls, corresponding to reductions by
 195 74.1, 76.3 and 89.5 % on Alixan, Premio and Altigo, respectively.

196 In nTI controls, the pycnidial density (Figure 1B and supplementary data) recorded in Alixan
 197 was significantly higher (2.9) than in Premio (1.1) and Altigo (1.1). In plants treated with
 198 NECTAR Céréales®, a significant reduction of pycnidial density was observed in Alixan,
 199 Premio and Altigo (55.3, 66.3 and 91.3 % reductions respectively).

200 The figure 2 shows the third wheat leaves harvested at 21 dai from Alixan, Premio and Altigo
 201 non-treated and non-inoculated (nTnI), non-treated inoculated with *Z. tritici* (nTI) and treated
 202 with NECTAR Céréales® before inoculation (NECTAR Céréales®I). In each cultivar, nTI
 203 leaves presented chlorosis and necrosis related to the disease development, compared with nTnI
 204 leaves. Symptoms were drastically reduced in NECTAR Céréales®I leaves. However, the
 205 NECTAR Céréales® treated non-inoculated leaves, especially in Altigo cultivar, displayed a
 206 lighter green color than nTnI controls, and chlorosis at the top (data not shown).

207 **Alteration of the *Z. tritici* infectious process by NECTAR Céréales® treatment**

208 In order to evaluate the impact of NECTAR Céréales® on the development of *Z. tritici* in the
 209 three cultivars, five cytological parameters were assessed at different time points after infection
 210 (Figure 3 and supplementary data).

211 At 1 dai, more than 60 % of fungal spores were germinated on the nTI controls of the three
 212 cultivars (Figure 3A). No significant effect of NECTAR Céréales® treatment on spore
 213 germination was observed on the three cultivars (Figure 3A). This result found *in planta* agrees
 214 with the *in vitro* antifungal assays, which showed no significant antifungal effect of NECTAR
 215 Céréales® on both the growth and spore germination of *Z. tritici* when tested on PDA medium
 216 (data not shown).

217 At 5 dai, the percentage of germinated spores with penetrated germ tube (PS) recorded in
 218 Alixan, Premio and Altigo nTI controls were not significantly different (between 39.3 and 55.9
 219 %) (Figure 3B). Alixan plants treated with NECTAR Céréales® presented a percentage of PS
 220 significantly lower than that of nTI control (33.3 % compared to 53% in nTI) corresponding to
 221 a reduction by 37.2 % (Figure 3B). No significant difference was detected for the percentages
 222 of penetrations through stomata (PSS) between the three nTI cultivars (between 24.2 and 28.2
 223 %) and between NECTAR Céréales®-treated plants and their nTI control (between 15.6 and
 224 26.2 %) (Figure 3C).

225 Concerning the percentage of total colonized substomatal cavities (CS) recorded at 21dai, no
 226 significant difference in Alixan, Premio and Altigo nTI controls was found (between 72.0 and
 227 79.2 %). In Premio and Altigo treated with NECTAR Céréales® the percentage of CS (31.4
 228 and 27.8 %, respectively) was significantly lower than in their nTI controls, corresponding to
 229 reductions by 56.4 and 61.9 %, respectively, but not in Alixan (Figure 3D). Likewise, in the
 230 case of CS bearing pycnidia (CSP), only Premio treated with NECTAR Céréales® presented a
 231 percentage significantly lower (3.8 %) than in nTI controls, corresponding to a reduction by
 232 84.1 % (Figure 3E).

233 **Cell-wall degrading enzyme activities**

234 The fungal β -1,4-endoxylanase, β -1,4-endoglucanase and protease activities were measured in
 235 leaves from Alixan, Premio and Altigo (Table 1, 2 and 3 and supplementary data). At 1, 5 and
 236 11 dai, corresponding to the biotrophic phase, all of these enzymes' activities were at weak
 237 levels and not significantly different from those of nTnI controls.

238 Later on, the β -1,4-endoxylanase activity levels recorded in Alixan and Premio nTI controls at
 239 17 dai (0.08 and 0.04 U.mg⁻¹ total proteins) were significantly higher than in their nTnI controls

240 (0.03 and 0.01 U.mg⁻¹ total proteins, respectively), unlike in Altigo (between 0.02 and 0.03
 241 U.mg⁻¹ total proteins for both nTI and nTnI) (Table 1). At this time point, this activity, recorded
 242 in Alixan and Premio treated with NECTAR Céréales® and inoculated (0.03 and 0.02 U.mg⁻¹
 243 total proteins, respectively), was significantly lower than in their nTI controls, without
 244 significant differences with their nTnI controls. At 21 dai, the β-1,4-endoxylanase activity was
 245 significantly higher in the three nTI cultivars (0.34, 0.08 and 0.05 U.mg⁻¹ total proteins in
 246 Alixan, Premio and Altigo, respectively) than in their nTnI controls, and was significantly
 247 higher in Alixan than in Premio and Altigo. After treatment, this activity was significantly
 248 lower only in Alixan treated with NECTAR Céréales® (0.10 U.mg⁻¹ total proteins) when
 249 compared to its nTI control.

250 The β-1,4-endoglucanase activity (Table 2) was significantly higher in Alixan and Premio nTI
 251 (0.21 and 0.14 U.mg⁻¹ total proteins respectively) than in their nTnI controls at 17 dai (0.10 and
 252 0.06 U.mg⁻¹ total proteins respectively). At this time point, the β-1,4-endoglucanase level
 253 recorded in Alixan nTI (0.21 U.mg⁻¹ total proteins) was significantly higher than in Altigo (0.10
 254 U.mg⁻¹ total proteins) but not Premio. At the same time point, this activity recorded in Alixan
 255 and Premio treated with NECTAR Céréales® (0.12 and 0.09 U.mg⁻¹ total proteins, respectively)
 256 was significantly lower than in their nTI controls. At 21 dai, the β-1,4-endoglucanase activity
 257 was significantly higher in Alixan, Premio and Altigo nTI controls (0.62, 0.18 and 0.15 U.mg⁻¹
 258 total proteins, respectively) than in their nTnI controls. The maximal value was recorded in
 259 nTI Alixan, and was significantly higher than in nTI Premio and Altigo plants. The activity
 260 amounts obtained in NECTAR Céréales®-treated leaves were significantly lower than in the
 261 corresponding nTI control in Alixan cultivar only (0.26 compared to 0.62 U.mg⁻¹ total proteins).
 262 The protease activity (Table 3) was significantly higher in Alixan nTI (0.85 mU.mg⁻¹ total
 263 proteins) than in its nTnI control (0.09 mU.mg⁻¹ total proteins) at 17 dai. At this time point, the
 264 protease activity recorded in Alixan nTI was significantly higher than in Premio nTI plants
 265 (0.07 mU.mg⁻¹ total proteins) and in Altigo nTI plants (0.15 mU.mg⁻¹ total proteins). Only the
 266 value recorded in Alixan treated with NECTAR Céréales® (0.22 mU.mg⁻¹ total proteins) was
 267 significantly lower than that of its nTI control. At 21 dai, this activity reached 2.66 mU.mg⁻¹
 268 total proteins in Alixan nTI plants and was significantly higher than in nTI Premio (0.51
 269 mU.mg⁻¹ total proteins) and Altigo (0.72 mU.mg⁻¹ total proteins) plants. Only Premio treated
 270 with NECTAR Céréales® presented an activity significantly reduced (0.07 mU.mg⁻¹ total
 271 proteins) compared to its nTI control.

272 ***Induction of POX and PAL activities by NECTAR Céréales® in Alixan, Premio and Altigo.***

273 Total peroxidase (POX) and phenylalanine ammonia lyase (PAL) activities were measured in
 274 Alixan, Premio and Altigo at 0, 24, 48 and 72 hat with NECTAR Céréales® in both infectious
 275 and non-infectious contexts (Figure 4 and 5, and supplementary data).

276 POX activity in plants treated with NECTAR Céréales® was significantly higher than in nTnI
 277 control in Alixan (Figure 4A) at both 48 and 72 hat (8.0 and 14.8 nKat.mg⁻¹ proteins,
 278 respectively), and in Premio (Figure 4B) and Altigo (Figure 4C) at 72 hat (10.0 and 13.3
 279 nKat.mg⁻¹ proteins, respectively). In infectious context, the POX activity in NECTAR
 280 Céréales®-treated and inoculated (NECTAR Céréales®I) Altigo plants recorded at 72 hat (10.1
 281 nKat.mg⁻¹ proteins) was significantly lower than in NECTAR Céréales®-treated plants, and
 282 higher than in nTI plants, whereas in Alixan and Premio, this activity presented no significant
 283 difference with that measured in NECTAR Céréales®-treated ones.

284 PAL activity recorded in non-infectious context in Alixan treated with NECTAR Céréales®
 285 (3.6 pKat.mg⁻¹ proteins) was significantly higher than in nTnI, nTI and NECTAR Céréales®I
 286 plants at 72 hat (0.8, 1.5 and 0.7 pKat.mg⁻¹ proteins, respectively). The value obtained in
 287 NECTAR Céréales®I plants (0.7 pKat.mg⁻¹ proteins) was significantly lower than that obtained
 288 in NECTAR Céréales® and nTI plants (3.6 and 1.5 pKat.mg⁻¹ proteins, respectively) (Figure
 289 5A). At the same time course in Premio in non-infectious context, NECTAR Céréales®-treated

290 plants presented a PAL activity (2.1 pKat.mg^{-1} proteins) significantly higher than nTnI controls
 291 (Figure 5B). In infectious context, the activity recorded in Premio NECTAR Céréales®I plants
 292 (1.3 pKat.mg^{-1} proteins) was significantly higher than in nTnI and nTI conditions, but
 293 significantly lower than in NECTAR Céréales® plants. In Altigo, the PAL activity recorded in
 294 NECTAR Céréales® plants 48 and 72 hat (2.1 and 1.1 pKat.mg^{-1} proteins respectively) was
 295 significantly higher than in the nTnI control (1.3 and 0.9 pKat.mg^{-1} proteins, respectively)
 296 (Figure 5C). In infectious context, the PAL activity recorded at 72 hat in NECTAR Céréales®I
 297 plants (4.6 pKat.mg^{-1} proteins) was significantly higher than in NECTAR Céréales® plants,
 298 nTnI and nTI controls.

299 ***Induction by NECTAR Céréales® of defense-related gene expression in Alixan, Premio and***
 300 ***Altigo in non-infectious context***

301 We established transcript profiles of a set of nine distinct defense genes associated with four
 302 types of wheat defenses: ROS metabolism (*POX2*, *OXO*) (Figure 6 and supplementary data),
 303 octadecanoid (*LOX*, *AOS*) (Figure 7 and supplementary data), phenylpropanoid (*PAL*, *CHS*)
 304 (Figure 8 and supplementary data) pathways and PR protein biosynthesis (*PRI*, *CHIT2*, *GLUC*)
 305 (Figure 9 and supplementary data). Gene expression profiles in Alixan, Premio and Altigo
 306 plants were followed at 12, 24, 48 51, 54, 60, 72, 120 and 168 hat with NECTAR Céréales®
 307 and were compared to their respective (nTnI) controls (Figure 6, 7, 8 and 9) for which
 308 expression was considered to be at 1. For a meaningful biological approach, significantly
 309 differentially expressed transcripts were reported according to a strict selection criterion;
 310 namely, a ≥ 2.0 -fold (upregulation) or a < 0.5 -fold (downregulation) change threshold.

311 Concerning ROS metabolism, the *POX2* gene was induced in the Alixan cultivar all over the
 312 time course except at 24, 60 and 168 hat (Figure 6). This induction ranged from 3.2- at 120 hat
 313 to 17.8-fold at 54 hat compared to the nTnI control. On the other hand, the *POX2* expression
 314 was induced at 12, 24, 48, 51 and 60 hat in Altigo (ranging from 3.1- at 51 hat to 17.3 –fold at
 315 24 hat) and at 24, 51, 54 and 60 hat in Premio (from 4.6- at 51 hat to 12.5-fold at 60 hat).
 316 Moreover, the strongest inductions were observed in Altigo at 24 hat and later in Alixan at 54
 317 hat. The *OXO* gene expression was induced at 60 hat (2.7-fold) in Premio, and at 72 hat (4.1-
 318 and 6.8-fold, respectively) in Alixan and Altigo.

319 Concerning the jasmonate pathway, Premio showed an early induction of the *LOX* gene
 320 expression level at 12, 24, 48 and 54 hat, ranging from 3.6- at 54 hat to 28.8-fold at 48 hat
 321 compared to the nTnI control (Figure 7). In Altigo, this gene expression was induced at 24 hat
 322 (10.1-fold). We could notice that the transcription level of this gene exhibited statistically
 323 significant but very small down regulations at 60, 72 and 120 hat at least in Premio and Altigo.
 324 As for *LOX* gene, Premio showed an early significant induction of the *AOS* gene expression at
 325 12, 24 and 48 hat (77.2-, 5.1- and 8.7-fold, respectively) and in Altigo at 24 and 48 hat (5.6-
 326 and 11.5-fold respectively), whereas in Alixan, this gene was induced later at 54 and 120 hat
 327 (6.5- and 2.0-fold). Again, minor down regulations of this gene were measured at 72 and 168
 328 hat in Alixan and Premio.

329 Concerning the salicylic acid (SA) pathway, an early induction of the *PAL*, the gene marker of
 330 the phenylpropanoid pathway, was recorded in the Altigo cultivar at 12, 24, 51 and 72 hat,
 331 ranging from 2.3- at 12 hat to 35.0- fold at 72 hat compared to the nTnI control (Figure 8). This
 332 gene expression was induced at 48, 51, 54 and 120 hat (ranging from 2.7- at 51 hat to 14.0-fold
 333 at 54 hat) in Alixan and at 60 hat only in Premio (2.3-fold). Moreover, the up-regulation of *PAL*
 334 expression level recorded in Altigo at 24 and 72 hat were significantly higher than in Alixan
 335 and Premio. Concerning the *CHS* gene, Altigo showed an induction of its expression level at
 336 54 and 72 hat (22.1- and 15.0-fold, respectively). Moreover, the up-regulation of *CHS*
 337 expression level recorded at 54 and 72 hat in Altigo were significantly higher than in Alixan
 338 and Premio.

339 Concerning the PR protein biosynthesis, Altigo showed only an early but very small induction
 340 of the *PRI*, the SA pathway marker of the systemic acquired resistance, at 24 hat (2.3-fold)
 341 (figure 9). In Alixan, the *PRI* gene expression level was induced at 48 and 51 hat (6.2- and
 342 18.1-fold, respectively), and this level was significantly higher than in Premio and Altigo.
 343 Moreover, this gene expression was down regulated at 72 hat in Alixan and Premio (0.3- and
 344 0.2-fold, respectively). On the other hand, the *CHIT2* gene expression was induced in Altigo at
 345 12 hat and 72 hat (4.6- and 5.9-fold, respectively), whereas in Alixan, it was only induced at 72
 346 hat (9.1-fold). No induction was observed in Premio. The expression of the *GLUC* gene was
 347 induced in Premio at 12, 24 and 54 (5.1-, 3.0- and 2.5-fold, respectively). In Alixan, this gene
 348 induction was recorded at 24, 48 and 54 hat (4.3-, 9.8- and 2.7-fold, respectively) and at 60 and
 349 72 hat in Altigo (2.1- and 5.2-fold respectively). Moreover, this gene expression presented
 350 minor down-regulations at 60 and 120 hat in Alixan and Premio.

351 ***Induction of defense-related gene expression by NECTAR Céréales® in Alixan, Premio and*** 352 ***Altigo in infectious context***

353 The transcription profiles of the same set of gene were also established in plants treated with
 354 NECTAR Céréales® in infectious context. Gene expression profiles were followed at 6, 12 and
 355 24 hai and were compared with nTnI and nTI controls, and plants treated with NECTAR
 356 Céréales® (Figure 10 and supplementary data). Only genes that were differently regulated in
 357 infectious context are displayed.

358 The *POX2* gene expression level recorded in Alixan at 6 hai (68-fold compared to the nTnI
 359 control) was significantly higher than in nTI controls (17.8-fold) and NECTAR Céréales® -
 360 treated plants (17.3-fold), whereas no significant increase was recorded in Premio and Altigo.

361 The up-regulation of the *PAL* gene expression level recorded in Alixan NECTAR Céréales®I
 362 at 6 hai (26.7-fold compared with nTnI control) was significantly higher than in nTI controls
 363 (5.6-fold) and NECTAR Céréales® treated plants (14-fold). The expression level of this gene
 364 recorded at 24 hai in Altigo NECTAR Céréales®I plants (84.6-fold compared with nTnI
 365 control) was significantly higher than in nTI controls (1.3-fold) and NECTAR Céréales®-
 366 treated plants (35.0-fold). In Premio, NECTAR Céréales®I plants presented no significant
 367 induction of this gene.

368 In Alixan, the up-regulation of the *PRI* gene expression level recorded in NECTAR Céréales®I
 369 plants at 12 hai (7.4-fold compared with nTnI control) was significantly higher than in nTI
 370 controls (0.4-fold) and NECTAR Céréales® treated plants (1.4-fold). No significant induction
 371 of this gene was observed in Premio and Altigo. Concerning the *GLUC* gene, its expression
 372 was significantly higher at 6 and 12 hai in Alixan (7.4- and 2.6-fold compared with nTnI control,
 373 respectively) than in nTI (0.9- and 0.5-fold respectively) and in NECTAR Céréales®-treated
 374 plants (2.7- and 0.4-fold, respectively). This gene expression level recorded at 12 and 24 hai in
 375 Altigo NECTAR Céréales®I (5.9- and 32.5-fold compared with nTnI control, respectively) was
 376 significantly higher than in nTI (2.6- and 0.9-fold, respectively) and in NECTAR Céréales®-
 377 treated plants (2.1- and 5.2-fold, respectively). In Premio NECTAR Céréales®I plants, the
 378 *GLUC* gene expression level was not significantly different from nTI and NECTAR Céréales®
 379 treated plants.

380

381 **Discussion**

382 The present study aims to evaluate the elicitor potential of a sulfur, manganese sulfate and
 383 microbial protein-based commercial product NECTAR Céréales® against STB, taking into
 384 account the wheat genotype response. During the first part of our project, we determined the
 385 efficient dose, timing and number of applications of NECTAR Céréales® (previously named
 386 FSOV7) to protect wheat against STB (Ors et al., 2012, 2013). Significant reductions >70% of
 387 disease symptoms and >50% of pathogen sporulation on the three studied cultivars were
 388 obtained when leaves were pre-treated 48h before inoculation with 30 mL of NECTAR

389 Céréales® in the dose of 8.73 µL/mL. While the efficient dose of this product didn't show any
390 direct effect on spore germination and mycelium growth, the upregulation of at least one wheat
391 defense gene marker, such as *POX2*, *PAL*, *LOX*, *AOS*, *OXO*, *CHIT2* or *GLUC* genes was
392 recorded in the three studied cultivars after leaf pre-treatment. The involvement of these genes
393 in the resistance of wheat to *Z. tritici* was suggested previously in numerous studies (Adhikari
394 et al., 2007; Shetty et al., 2009; Diani et al., 2009; Yang et al., 2013b; Samain et al., 2017; Ors
395 et al., 2017).

396 Many microbial proteins have been identified as inducers of plant resistance to pathogen attack.
397 The pebC1 protein from *Botrytis cinerea* reduced grey mould in tomato (Zhang et al., 2010,
398 2014b), an elicitor and a 75 kDa protein elicitor purified from *Phytophthora palmivora* induced
399 resistance in *Hevea brasiliensis* against this pathogen (Dutsadee and Nunta, 2008), the SsCut
400 cutinase from *Sclerotinia sclerotiorum* induced defense mechanisms in various plant species
401 (Zhang et al., 2014a) and the Hrip1 protein from *Alternaria tenuissima* elicited defense
402 mechanisms in tobacco (Kulye et al., 2012). However, minerals and ions are also able to induce
403 defenses in plants against pathogens. Low concentrations of CuSO₄ elicited the production of
404 phytoalexins in grapevine leaves and protected them against *B. cinerea* and *Plasmopara viticola*
405 (Aziz et al., 2006). Moreover, high leaf manganese concentrations induced protection against
406 *Uncinula necator*, responsible for powdery mildew in grapevine, and the synthesis of proteins
407 related to pathogen resistance (Yao et al., 2012).

408 Nevertheless, a light chlorosis was observed on wheat leaves treated with NECTAR Céréales®.
409 In their study, Fecht-Christoffers et al. (2003) described the same symptoms in leaf cowpea
410 caused by the toxic effect of an excessive manganese supply. The authors reported an increase
411 of the POX activity and an accumulation of proteins showing homologies to PR-proteins such
412 as glucanase and chitinase. As discussed below, treatment with NECTAR Céréales® induces
413 similar accumulations in the wheat cultivars Alixan, Premio and Altigo. It could be suggested
414 that manganese contained in NECTAR Céréales® is at least partially responsible for such
415 induction and possibly involved in the slight toxicity observed during the induced resistance.
416 Abiotic stresses, such as an excessive Mn supply, could induce plant defense mechanisms.
417 Mithöfer et al. (2004) hypothesized that heavy metals as well as biotic stress lead to an
418 accumulation of the reactive oxygen species (ROS) involved in the oxidation of unsaturated
419 fatty acids which initiate the octadecanoid pathway. The authors suggested that the formation
420 of oxylipins (through LOX and AOS activities) could elicit plant secondary metabolism and
421 accumulation of defense compounds as induced by a biotic stress. Taken together, these results
422 suggest that the protection efficacy conferred by NECTAR Céréales® on wheat against *Z. tritici*
423 results from the induction of resistance mechanisms that could be attributed to one or several
424 of its components.

425 Whatever the cultivar, wheat defense responses were activated during the first 72 hours after
426 treatment, even if many differences concerning timing and intensity were recorded between
427 cultivars. More specifically, the RT-qPCR analysis showed an important upregulation of *POX2*
428 gene as a response to NECTAR Céréales® pre-treatment in the three wheat cultivars, in a non-
429 infectious context. At the biochemical level, our results confirmed the elicitation of POX
430 activity in the three cultivars 72 hours after treatment. The *pox* gene encodes for the peroxidase
431 enzyme plays an important role of ROS pathway by catalyzing genes implicated in oxidative
432 radicals. In addition, peroxidases are implicating in the lignin production and in the plant cell
433 wall reinforcement (Pechanova et al., 2010). This mode of action represent the first physical
434 defense barrier face to pathogen attack (Underwood et al., 2012). In addition to lignification,
435 oxidative radicals with other relate defense proteins and polysaccharides have restricted
436 strongly cell wall invasion by fungal pathogens including *Verticillium* spp. and *Melampsora*
437 spp. (Pechanova et al., 2012; Floerl et al., 2012). Also, the overexpression of the hydrogen
438 peroxide (H₂O₂)-producing enzyme oxalate oxidase (*OXO*) as a response to NECTAR

439 Céréales® pre-treatment indicates the implication of the H₂O₂ in the induced resistance against
440 STB. However, H₂O₂ has shown direct antagonistic effect against phytopathogenic
441 microorganisms (Peng et al., 1992). It has been previously reported the correlation between
442 *OXO* gene and the resistance to *Septoria musiva* (Liang et al., 2001). The production of ROS
443 was reported as an earlier response during the biotrophic phase of *Z. tritici* starting in the
444 infection point and moves to the entire leaf tissue during the necrotrophic phase (Yang et al.,
445 2015). Contrarily to the necrotrophs which often enhance ROS production to stimulate the
446 program cell death and facilitate their nutrients uptake (Govrin et al., 2000), this mode of action
447 could be efficient to stop the infection process of *Z. tritici* which reacts as a biotrophe during
448 the first nine days of its contact with wheat plants (Selim et al., 2014).

449 However, our results showed variations between cultivars at the level of the elicitation timing
450 and upregulation intensities of other tested genes. Such as variations were interpreted
451 previously by the impact of the plant genotype (Bruce, 2014; Walters et al., 2005, 2011; Samain
452 et al., 2019). In contrast to Alixan and Premio, the most resistant cultivar Altigo treated with
453 NECTAR Céréales® presented an earlier and a significant upregulation of the *PAL* and the
454 *CHS* genes at 12 hat, in a non-infectious context. The overexpression of *PAL* reached the
455 maximum at 72 hat and was associated with a high induction level of *CHS* and with transient
456 but significant inductions of *chit*, *GLUC* and *OXO* genes. The *PAL* and *CHS* genes encode for
457 the phenylalanine ammonia-lyase and chalcone synthase enzymes, respectively, that are known
458 as gene markers of the phytoalexins and phenylpropanoid pathway. Indeed, *PAL* catalyzes the
459 phenylalanine as the first step of serial reactions where many other enzymes such as *CHS* are
460 implicated. These reactions result to the biosynthesis of large varieties of compounds with
461 antimicrobial and antioxidants activities, flavonoids, phytoalexins and lignin (Lamb et al.,
462 1997). In addition, the upregulation of *chit* and *GLUC* genes code for chitinase and glucanase
463 enzymes could be efficient in the control of *Z. tritici* by their known role in the digesting of the
464 chitin and β-glucans, respectively, of the fungal cell wall (Samain et al., 2017). Moreover, after
465 inoculation with *Z. tritici*, the same defense pathways are still stimulated in Altigo treated with
466 NECTAR Céréales®, since an overexpression of *PAL* and *GLUC* genes was observed during
467 the first 24 hours after infection in comparison with plants treated with NECTAR Céréales®
468 only or inoculated with *Z. tritici* only. Phenylpropanoid pathway and glucanase seem
469 definitively involved in Altigo's defense response induced by NECTAR Céréales® against *Z.*
470 *tritici*.

471 In the moderately resistant wheat cultivar Premio, any of the tested genes were up-regulated in
472 infectious context after treatment. Defenses mechanisms were mainly induced just after
473 treatment, suggesting an eliciting effect rather than a priming effect of NECTAR Céréales® in
474 this cultivar. In the non-infectious context, *LOX* and *AOS* gene expressions were strongly up-
475 regulated at the same times 12, 24 and 48 hat indicating the stimulation of jasmonic acid (JA)
476 and ethylene (ET) signaling pathways. *LOX* and *AOS* enzymes are implicated in the
477 biosynthesis of the oxylipins (Gottwald et al., 2012) which are involving in pathogen
478 recognition (Wasternack, 2007) and in different plant defense mechanisms such as proteinase
479 inhibitor induction, secondary metabolites accumulation and eliciting of other defenses as PR
480 genes (Blée, 2002; Mithöfer et al., 2004; Robert-Seilaniantz et al., 2007). Moreover, some of
481 them, such as thaumatin-like proteins have fungicide activities inhibiting spore germination and
482 mycelium development (Prost, 2005).

483 In the most susceptible cultivar Alixan treated with NECTAR Céréales®, in non-infectious
484 context, a significant up-regulation of different gene expression (such as *POX2*, *OXO*, *PAL*,
485 *PR1*, *CHIT2* and *GLUC*) as well as an increase of *POX* and *PAL* activities were recorded and
486 sometimes earlier (e.g. *pox* at 12 hat) and stronger (e.g. *PR1* 18.1 fold at 51 hat) than in Premio
487 and Altigo. Moreover, in infectious context, a priming effect of NECTAR Céréales® in Alixan
488 was noticed through the induction of an overexpression of *pox*, *PR1*, *PAL* and *GLUC* at 6 and

489 12 hours after infection. The association of overexpression of *PR1*, *PR2* (β -1,3-glucanase) and
490 *pox*, which have been identified previously as types of PR protein (Van Loon et al., 1999), were
491 observed more in incompatible than in compatible interactions in several cereal pathosystems
492 (Boyd et al., 1997; Desmond et al., 2008) and specially in the wheat-*Z. tritici* pathosystem
493 (Adhikari et al., 2007; Ray et al., 2003; Samain et al., 2019). This suggests that *PR1* proteins
494 may play an important role in the resistance against *Z. tritici* alone or in concert with other
495 defense-related genes or pathways such as phenylpropanoid and ROS pathways. The absence
496 of *PR1* overexpression in the other two cultivars Premio and Altigo as a response to NECTAR
497 Céréales® pre-treatment contradicts the idea that *PR1* is the wheat protection marker against
498 STB, as previously proposed by Adhikari et al., 2007. The percentage of germinated spores
499 with penetrated germ tubes (PS) recorded at 5 dai was reduced in Alixan treated with NECTAR
500 Céréales® compared with non-treated plants suggests that in this cultivar, the elicitation of
501 *POX2*, *OXO*, *PR1*, *PAL*, *CHIT2*, and *GLUC* gene expressions as well as the increase of *POX2*,
502 *PR1*, *PAL*, and *GLUC* gene expressions in infectious context, affect the penetration of *Z. tritici*.
503 A high level of these gene expression seems to be required, since in this susceptible cultivar,
504 *POX2*, *CHIT2* and *PR1* are already induced after infection and without NECTAR Céréales®
505 pre-treatment (Ors et al., 2017).

506 The effect of NECTAR Céréales® on the pathogenicity of *Z. tritici* was investigated at the
507 cytological and biochemical levels. Results showed that CWDE activities increased in the
508 infectious context (nTI) on the three wheat cultivars according to the sporulation level of *Z.*
509 *tritici* and the highest levels were recorded in the most susceptible cultivar Alixan at 21 dai. It
510 has been previously reported that the CWDE activities are correlated with the pycnidial
511 formation (Douaiher et al., 2007a, 2007b; Siah et al., 2010a) and wheat susceptibility (Tian et
512 al., 2009). As a response to a pretreatment with NECTAR Céréales®, the CWDE activities
513 were reduced significantly in Alixan only (except protease activity which was also reduced in
514 Premio), reaching levels close to those of nTI Premio and Altigo cultivars. Surprisingly, the
515 pretreatment with NECTAR Céréales® affected the colonization of substomatal cavities in
516 Premio and Altigo but not in Alixan, where the reduction of CWDE activities was the most
517 important. According to microscopic observations, only direct penetration attempts were
518 reduced in Alixan. At the macroscopic level, leaf areas with symptoms and sporulation level
519 were both reduced in the three cultivars. However, the reduction of CWDE activities cannot be
520 considered as a strict marker of the induced resistance as a response to the elicitor pretreatment
521 in wheat against STB since a protection has been obtained without influencing these enzymatic
522 activities.

523 In conclusion, under controlled conditions, the NECTAR Céréales®, the manganese, sulfur-
524 and microbial protein-based product, induces a non-genotype wheat resistance conferring more
525 than 70% of protection against *Z. tritici*. Nevertheless, the mechanisms of this induced
526 resistance were genotype dependent and can be linked to an elicitor or both elicitor and priming
527 effects. However, these defense pathways could impact the penetration process and the first
528 days after infection in some cultivars or could interfere later with the fungus spreading in the
529 leaf tissues, leading to symptom and sporulation reduction. It seems that manganese contained
530 in NECTAR Céréales® induces an abiotic stress leading to plant defense responses and
531 chlorosis. Such an effect may be reduced at field level by applying a lower dose or on more
532 mature plant growth stages. In our next work, the interaction of NECTAR Cereals® with soil
533 microorganisms will be investigated. Indeed, if the treated plant engages a defense response, it
534 must be reflected in its exudations and could change the soil microbial populations. On the same
535 time, the efficiency of NECTAR Céréales® will be confirmed over high number of wheat
536 genotypes, at different growth stages, and using different *Z. tritici* strains. Moreover, field
537 experimentations will be conducted to determine its effective dose, timing and number of
538 applications, how long does it confer resistance (persistence), and its impact on grain yield.

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752

753 **Table 1.** Time course of β -1,4-endoxylanase activity ($\text{U}\cdot\text{mg}^{-1}$ total proteins) in Alixan, Premio
 754 and Altigo treated with NECTAR Céréales® 48h before inoculation with the *Z. tritici* strain
 755 T01193 (NECTAR Céréales®I). At each time point (1 to 21 days after inoculation (dai)),
 756 activity levels were compared with non-treated non-inoculated (nTnI) and non-treated
 757 inoculated (nTI) controls. Means tagged with the same letter at each time point are not
 758 significantly different using the Tukey test at $P \leq 0.05$.

		1dai	5dai	11dai	17dai	21dai
Alixan	nTnI	0.01 _b	0.01 _a	0.01 _a	0.03 _{bc}	0.02 _d
	nTI	0.02 _{ab}	0.01 _a	0.01 _a	0.08 _a	0.34 _a
	NECTAR Céréales®I	0.02 _{ab}	0.02 _a	0.02 _a	0.03 _{bc}	0.10 _b
Premio	nTnI	0.02 _{ab}	0.01 _a	0.01 _a	0.01 _d	0.01 _d
	nTI	0.01 _{ab}	0.01 _a	0.02 _a	0.04 _{ab}	0.08 _b
	NECTAR Céréales®I	0.02 _a	0.01 _a	0.01 _a	0.02 _{cd}	0.07 _b
Altigo	nTnI	0.02 _{ab}	0.01 _a	0.01 _a	0.02 _{cd}	0.02 _d
	nTI	0.02 _{ab}	0.01 _a	0.01 _a	0.03 _{bc}	0.05 _{bc}
	NECTAR Céréales®I	0.02 _{ab}	0.01 _a	0.02 _a	0.03 _{bc}	0.03 _{cd}

759

760

761 **Table 2.** Time course of β -1,4-endoglucanase activity ($\text{U}\cdot\text{mg}^{-1}$ total proteins) in Alixan, Premio
 762 and Altigo treated with NECTAR Céréales® 48h before inoculation with the *Z. tritici* strain
 763 T01193 (NECTAR Céréales®I). At each time point (1 to 21 days after inoculation (dai)),
 764 activity levels were compared with non-treated non-inoculated (nTnI) and non-treated
 765 inoculated (nTI) controls. Means tagged with the same letter at each time point are not
 766 significantly different using the Tukey test at $P \leq 0.05$.

		1dai	5dai	11dai	17dai	21dai
Alixan	nTnI	0.06 _{ab}	0.08 _a	0.08 _a	0.10 _{bc}	0.09 _{ef}
	nTI	0.06 _{ab}	0.08 _a	0.09 _a	0.21 _a	0.62 _a
	NECTAR Céréales®I	0.07 _{ab}	0.09 _a	0.1 _a	0.12 _{bc}	0.26 _b
Premio	nTnI	0.05 _b	0.07 _a	0.08 _a	0.06 _d	0.07 _f
	nTI	0.06 _{ab}	0.07 _a	0.09 _a	0.14 _{ab}	0.18 _{bcd}
	NECTAR Céréales®I	0.08 _a	0.07 _a	0.09 _a	0.09 _{cd}	0.24 _{bc}
Altigo	nTnI	0.05 _b	0.07 _a	0.08 _a	0.12 _{bc}	0.09 _{ef}
	nTI	0.05 _b	0.07 _a	0.07 _a	0.10 _{bc}	0.15 _{cd}
	NECTAR Céréales®I	0.06 _{ab}	0.08 _a	0.06 _a	0.10 _{bc}	0.12 _{de}

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768

769 **Table 3.** Time course of protease activity (mU.mg⁻¹ total proteins) in Alixan. Premio and Altigo
 770 treated with NECTAR Céréales® 48h before inoculation with *Z. tritici* strain T01193
 771 (NECTAR Céréales®I). At each time point (1 to 21 days after inoculation (dai)), activity levels
 772 were compared with non-treated non-inoculated (nTnI) and non-treated inoculated (nTI)
 773 controls. Means tagged with the same letter at each time point are not significantly different
 774 using the Tukey test at $P \leq 0.05$.

		1dai	5dai	11dai	17dai	21dai
Alixan	nTnI	0.05 _{ab}	0.13 _a	0.08 _a	0.09 _{bc}	0.04 _c
	nTI	0.07 _{ab}	0.08 _{ab}	0.06 _{ab}	0.85 _a	2.66 _a
	NECTAR Céréales®I	0.07 _{ab}	0.09 _{ab}	0.03 _{ab}	0.22 _b	1.12 _{ab}
Premio	nTnI	0.10 _a	0.13 _a	0.03 _{ab}	0.05 _c	0.05 _c
	nTI	0.05 _{ab}	0.06 _{ab}	0.05 _{ab}	0.07 _{bc}	0.51 _b
	NECTAR Céréales®I	0.02 _b	0.05 _{ab}	0.03 _b	0.09 _{bc}	0.07 _c
Altigo	nTnI	0.06 _{ab}	0.08 _{ab}	0.06 _{ab}	0.07 _{bc}	0.02 _c
	nTI	0.08 _a	0.04 _b	0.06 _{ab}	0.15 _{bc}	0.72 _{ab}
	NECTAR Céréales®I	0.03 _{ab}	0.07 _{ab}	0.05 _{ab}	0.12 _{bc}	0.57 _b

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776

777 **Figures legends:**

778 **Figure 1.** Disease level caused by the *Z. tritici* T01193 strain on Alixan, Premio and Altigo on
 779 non-treated inoculated control plants (nTI), and on plants treated with NECTAR Céréales® 48h
 780 before inoculation (NECTAR Céréales®I). Symptoms were evaluated by noting the percentage
 781 of the third leaf area covered by lesions (chlorosis and necrosis) (A), and the pycnidial density
 782 within symptoms was estimated on a scale from 0 (absence of pycnidia) to 5 (high density of
 783 pycnidia) (B), at 21 dai . Means tagged with the same letter are not significantly different using
 784 the Tukey test at $P \leq 0.05$.

785 **Figure 2.** Wheat leaves sampled from Alixan, Premio and Altigo in greenhouse conditions at
 786 21 dai. nTnI, non-treated non-inoculated; nTI, non-treated inoculated; NECTAR Céréales®I,
 787 treated with NECTAR Céréales® 48h before inoculation.

788 **Figure 3.** Microscopic observation of the infectious process of *Z. tritici* on Alixan, Premio and
 789 Altigo, treated with NECTAR Céréales® 48h before inoculation (NECTAR Céréales®I), or
 790 non-treated and inoculated (nTI). (A) Percentages of germinated spores (GS) at 1dai; (B)
 791 percentages of total germinated spores with penetrated germ tube (PS) and (C) penetrating
 792 spores through stomata (PSS) at 5dai; (D) percentages of colonized substomatal cavities (CS)
 793 and (E) percentages of pycnidia emerging from colonized substomatal cavities (CSP) at 21dai.
 794 Means tagged with the same letter are not significantly different using the Tukey test at $P \leq$
 795 0.05.

796 **Figure 4.** Time course of peroxidase (POX) activity in Alixan (A), Premio (B) and Altigo (C)
 797 treated with NECTAR Céréales® either non-inoculated (NECTAR Céréales®) or inoculated
 798 (NECTAR Céréales®I) with the *Z. tritici* strain T01193, compared to non-treated non-
 799 inoculated (nTnI) and non-treated inoculated (nTI) controls. Inoculation occurred 48h after
 800 treatment (arrow). Means tagged with the same letter are not significantly different for each
 801 time point using the Tukey test at $P \leq 0.05$.

802 **Figure 5.** Time course of phenylalanine ammonia lyase (PAL) activity in Alixan (A), Premio
 803 (B) and Altigo (C) treated with NECTAR Céréales® non-inoculated (NECTAR Céréales®)
 804 and inoculated (NECTAR Céréales®I) with the *Z. tritici* strain T01193, compared to non-
 805 treated non-inoculated (nTnI) and non-treated inoculated (nTI) controls. Inoculation occurred
 806 at 48h after treatment (arrow). Means tagged with the same letter are not significantly different
 807 for each time point using the Tukey test at $P \leq 0.05$.

808 **Figure 6.** Relative expressions of *POX2* and *OXO* wheat genes in Alixan, Premio and Altigo
 809 leaves treated with NECTAR Céréales® compared to their respective non-treated non-
 810 inoculated (nTnI) controls at 12, 24, 48, 51, 54, 60, 72, 120 and 168 hours after treatment (hat).
 811 Means tagged with the same letter are not significantly different for each time point using Tukey
 812 test at $P \leq 0.05$.

813 **Figure 7.** Relative expressions of *LOX* and *AOS* wheat genes in Alixan, Premio and Altigo
 814 leaves treated with NECTAR Céréales® compared to their respective non-treated non-
 815 inoculated (nTnI) controls at 12, 24, 48, 51, 54, 60, 72, 120 and 168 hours after treatment (hat).
 816 Means tagged with the same letter are not significantly different for each time point using Tukey
 817 test at $P \leq 0.05$.

818 **Figure 8.** Relative expressions of *PAL* and *CHS* wheat genes in Alixan, Premio and Altigo
 819 leaves treated with NECTAR Céréales® compared to their respective non-treated non-
 820 inoculated (nTnI) controls at 12, 24, 48, 51, 54, 60, 72, 120 and 168 hours after treatment (hat).
 821 Means tagged with the same letter are not significantly different for each time point using Tukey
 822 test at $P \leq 0.05$.

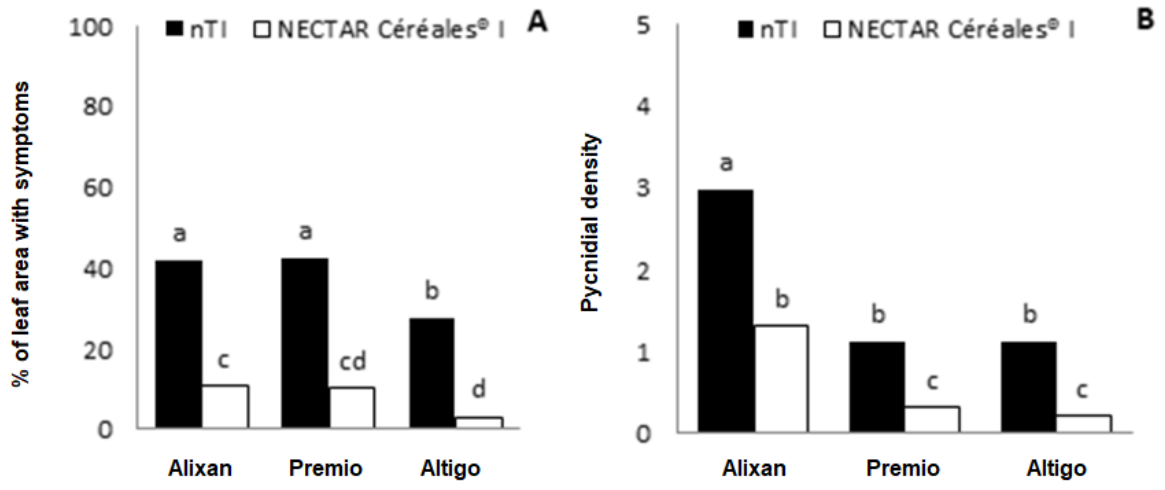
823 **Figure 9.** Relative expressions of *PRI*, *CHIT2* and *GLUC* wheat genes in Alixan, Premio and
 824 Altigo leaves treated with NECTAR Céréales® compared to their respective non-treated non-
 825 inoculated (nTnI) controls at 12, 24, 48, 51, 54, 60, 72, 120 and 168 hours after treatment (hat).

826 Means tagged with the same letter are not significantly different for each time point using Tukey
827 test at $P \leq 0.05$.

828 **Figure 10.** Relative expressions of *POX2*, *PAL*, *PR1* and *GLUC* wheat genes in Alixan, Premio
829 and Altigo leaves (i) treated with NECTAR Céréales® in infectious context (NECTAR
830 Céréales®I), (ii) non-treated inoculated (nTI) and (iii) treated non-inoculated (NECTAR
831 Céréales®) leaves at 6, 12 and 24 hours after inoculation (hai), compared to non-treated non-
832 inoculated (nTnI) respective controls. Means tagged with the asterisk are significantly higher
833 than in nTnI, nTI and NECTAR Céréales®-treated plants for each cultivar and for each time
834 point using Tukey test at $P \leq 0.05$.

835

836 Figure 1



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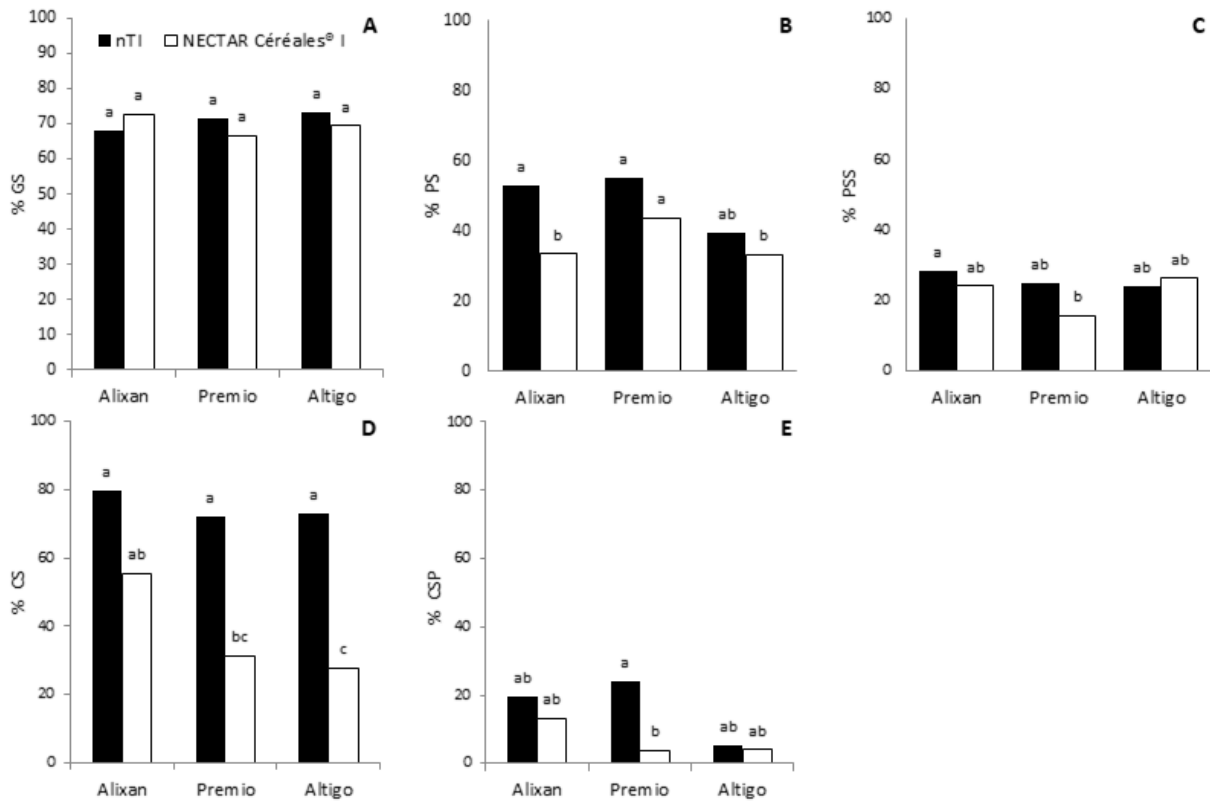
839 Figure 2



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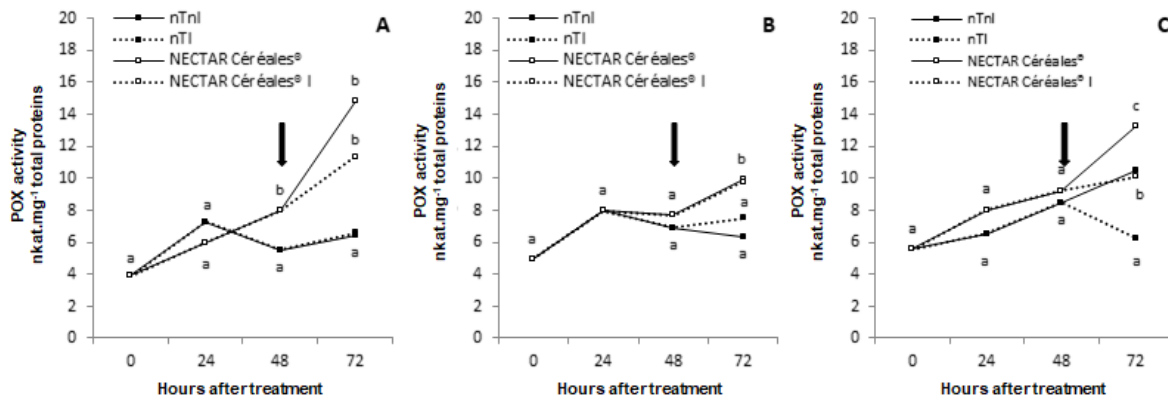
842 Figure 3



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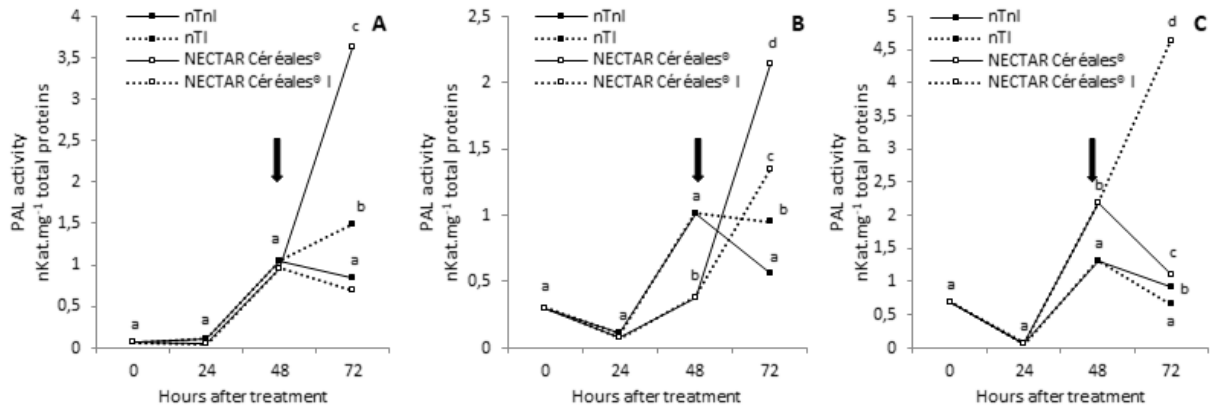
845 Figure 4



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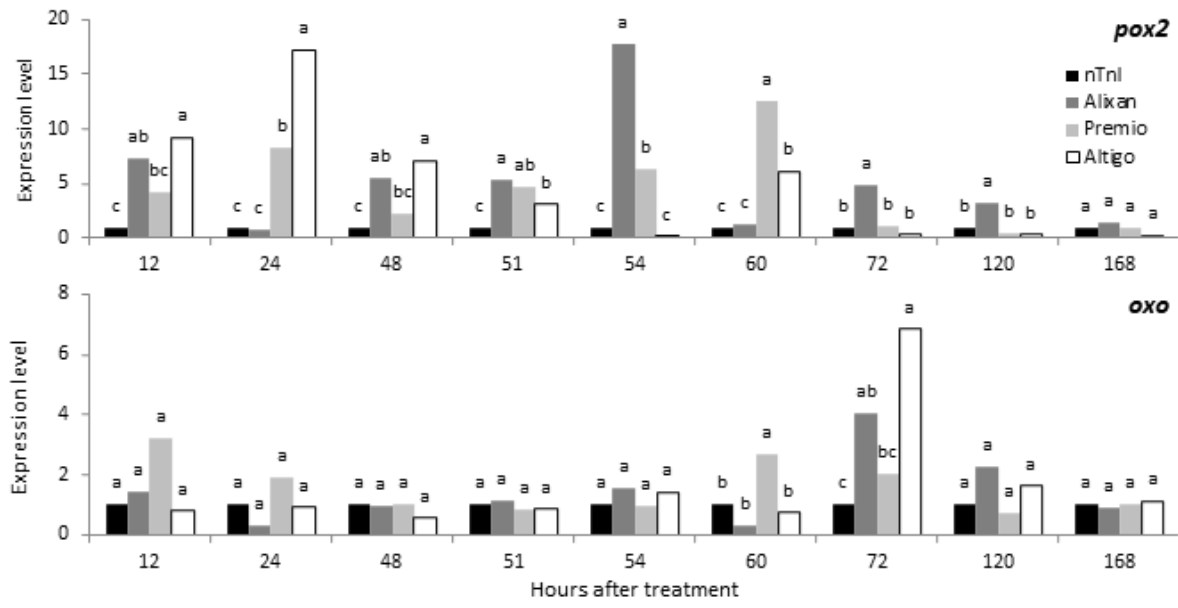
848 Figure 5



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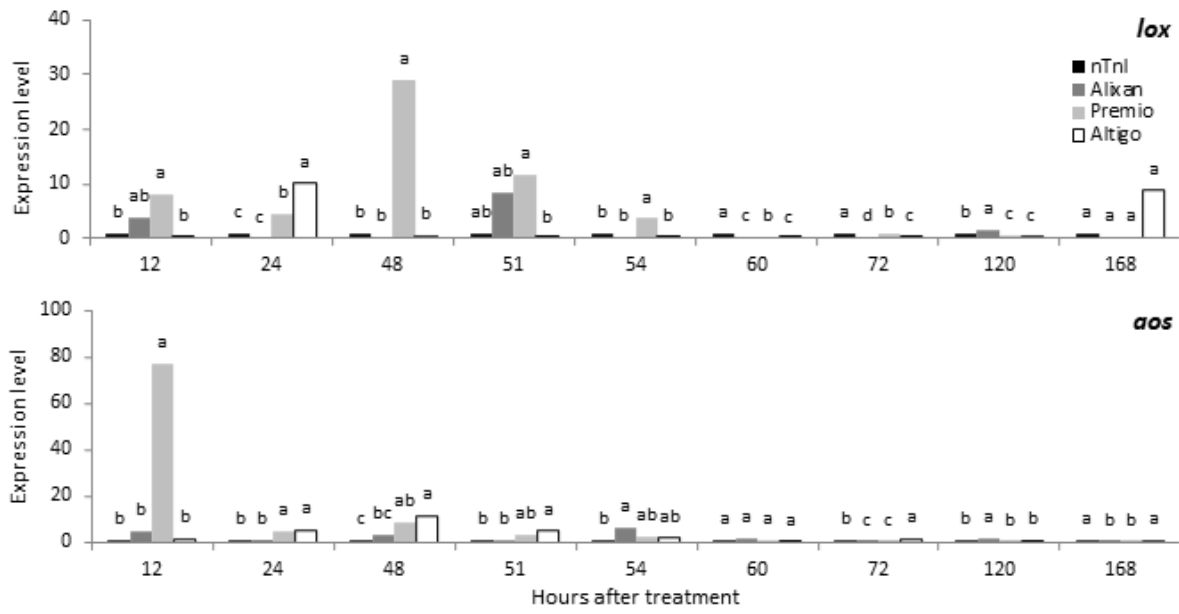
851 Figure 6



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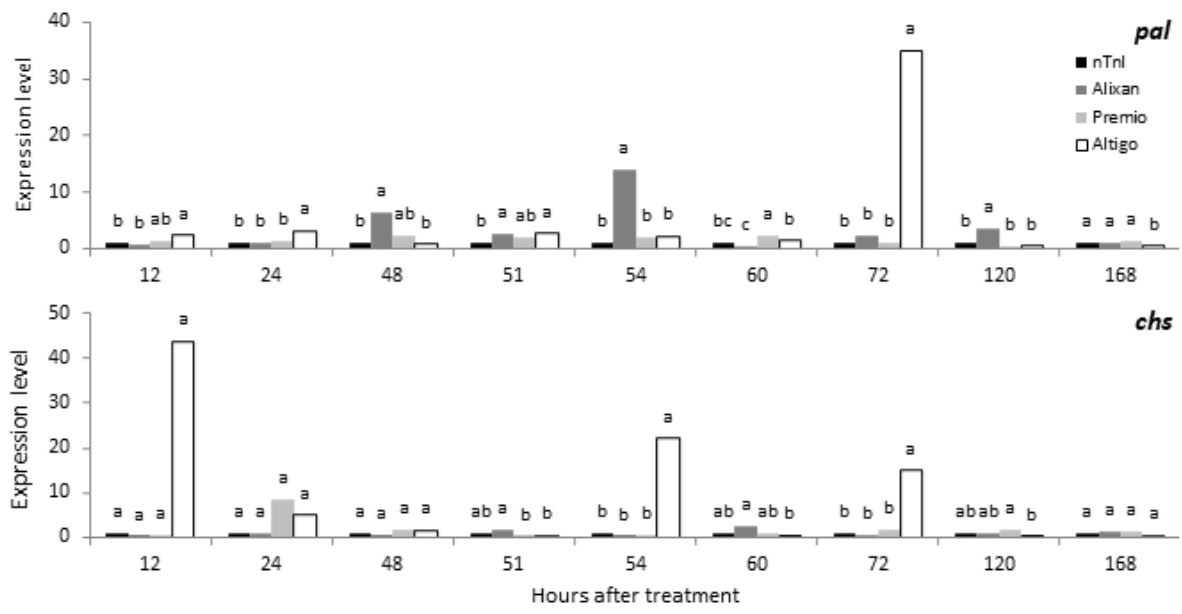
854 Figure 7



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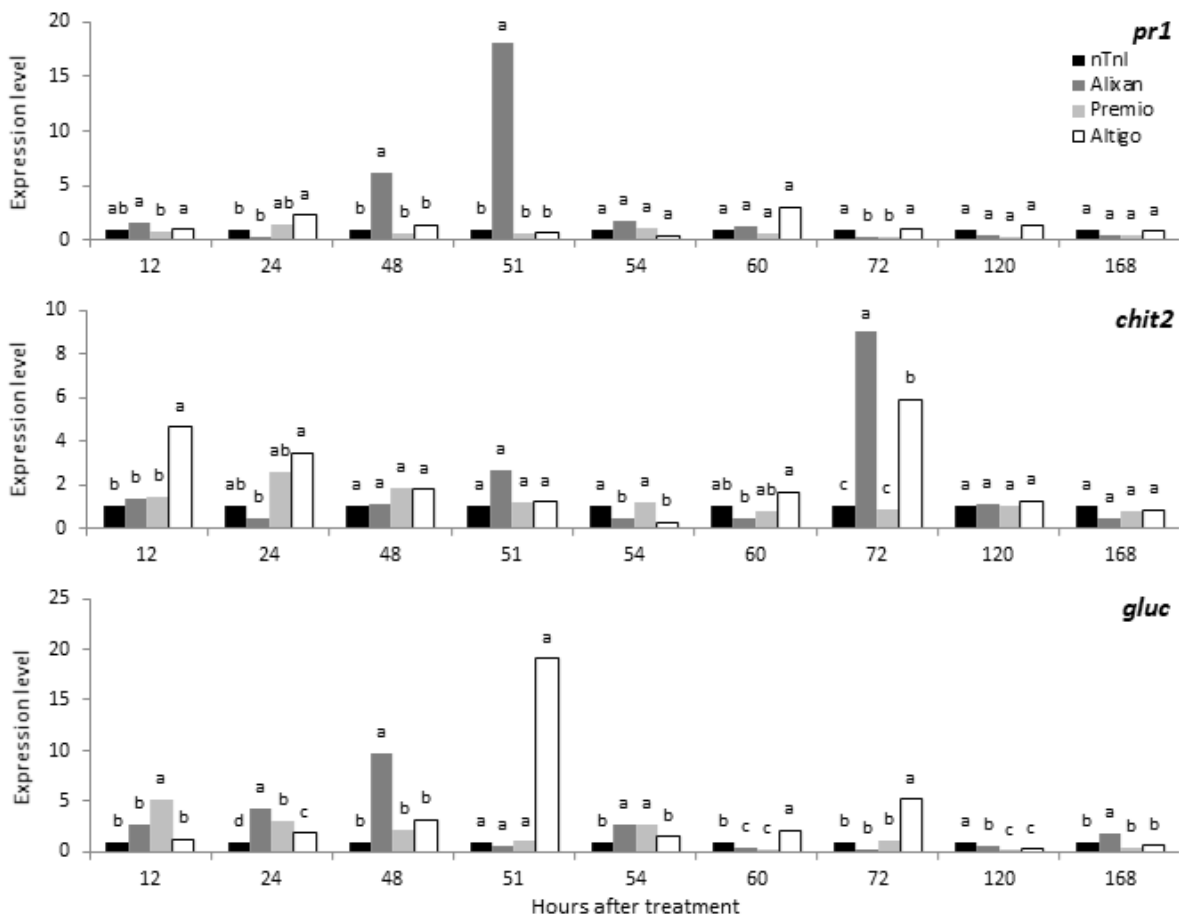
857 Figure 8



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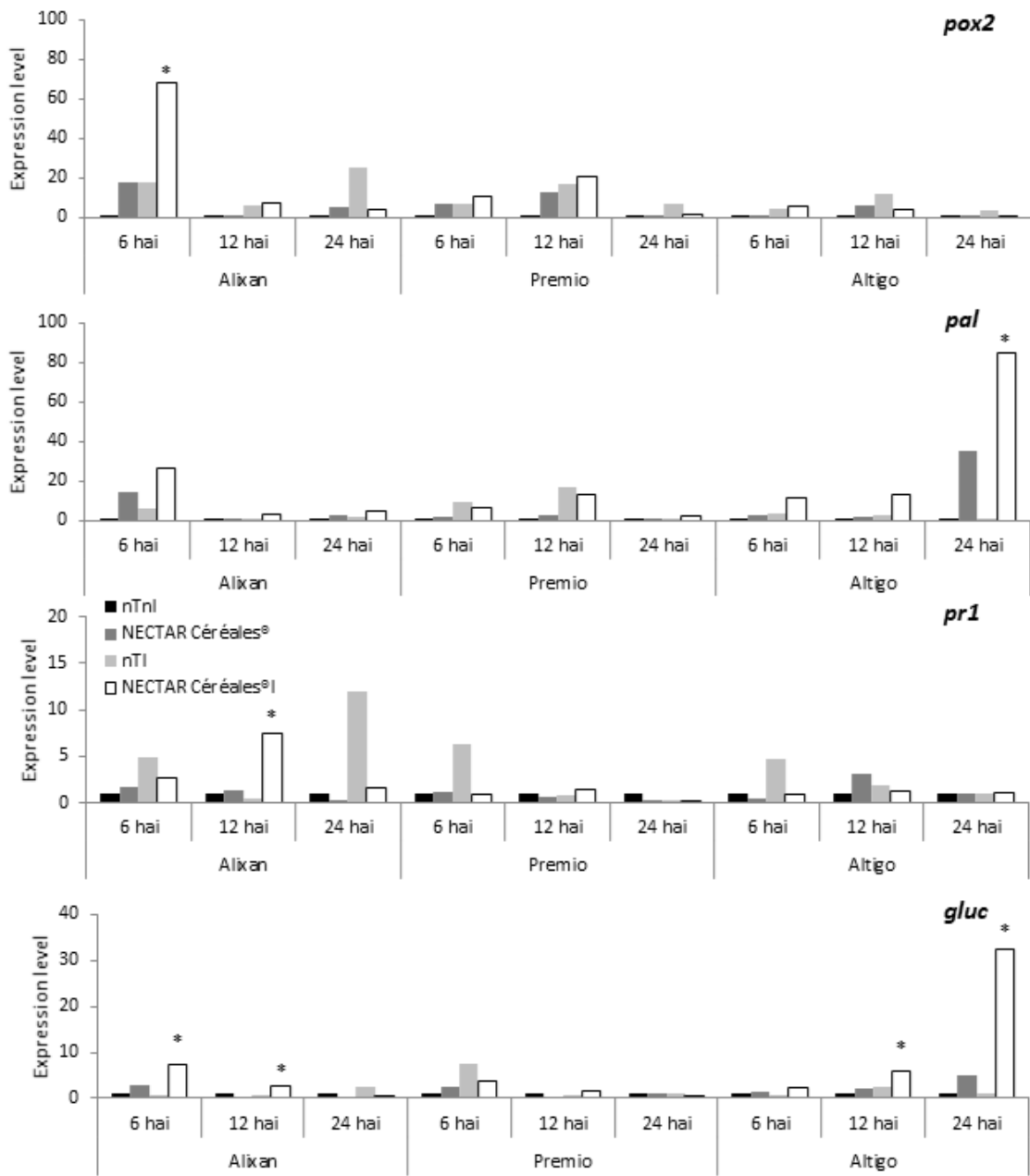
860 Figure 9



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863 Figure 10



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