A barley UDP-glucosyltransferase inactivates nivalenol and provides Fusarium Head Blight resistance in transgenic wheat

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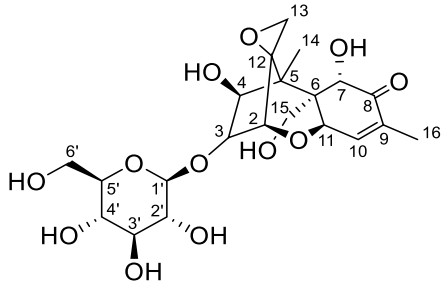
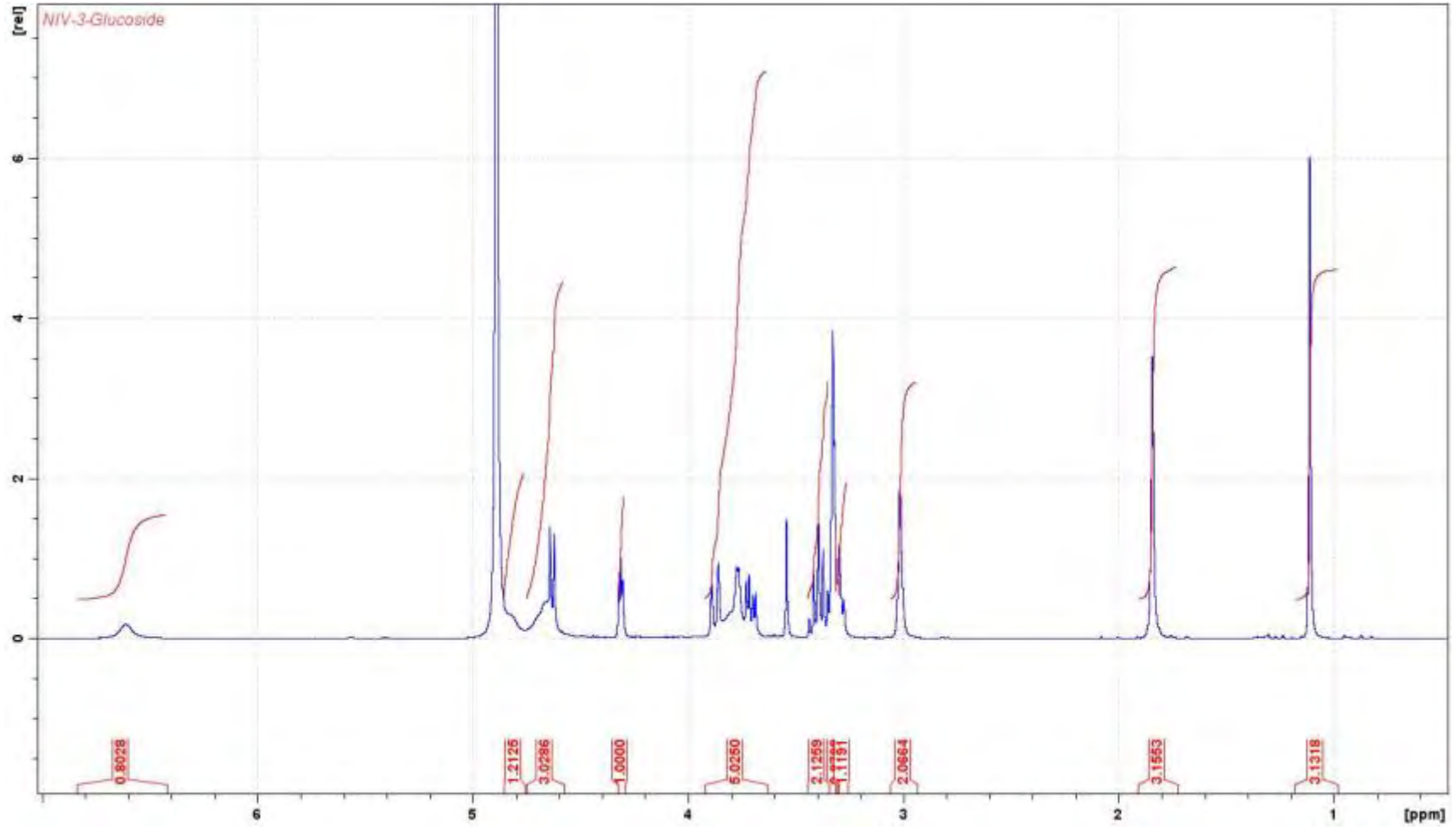
Supplementary File

Fig. S1. NMR data of NIV-3-*O*-β-D-glucoside. ^{1}H, ^{13}C and correlated spectra revealing NIV3G as the only product of NIV detoxification by OsUGT79 and HvUGT13248. All chemical shifts are displayed on the x-axis and are given in ppm relative to tetramethylsilane. The calibration was done using residual solvent signals methanol-*d4*). Multiplicities are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet) and b (broad signal).

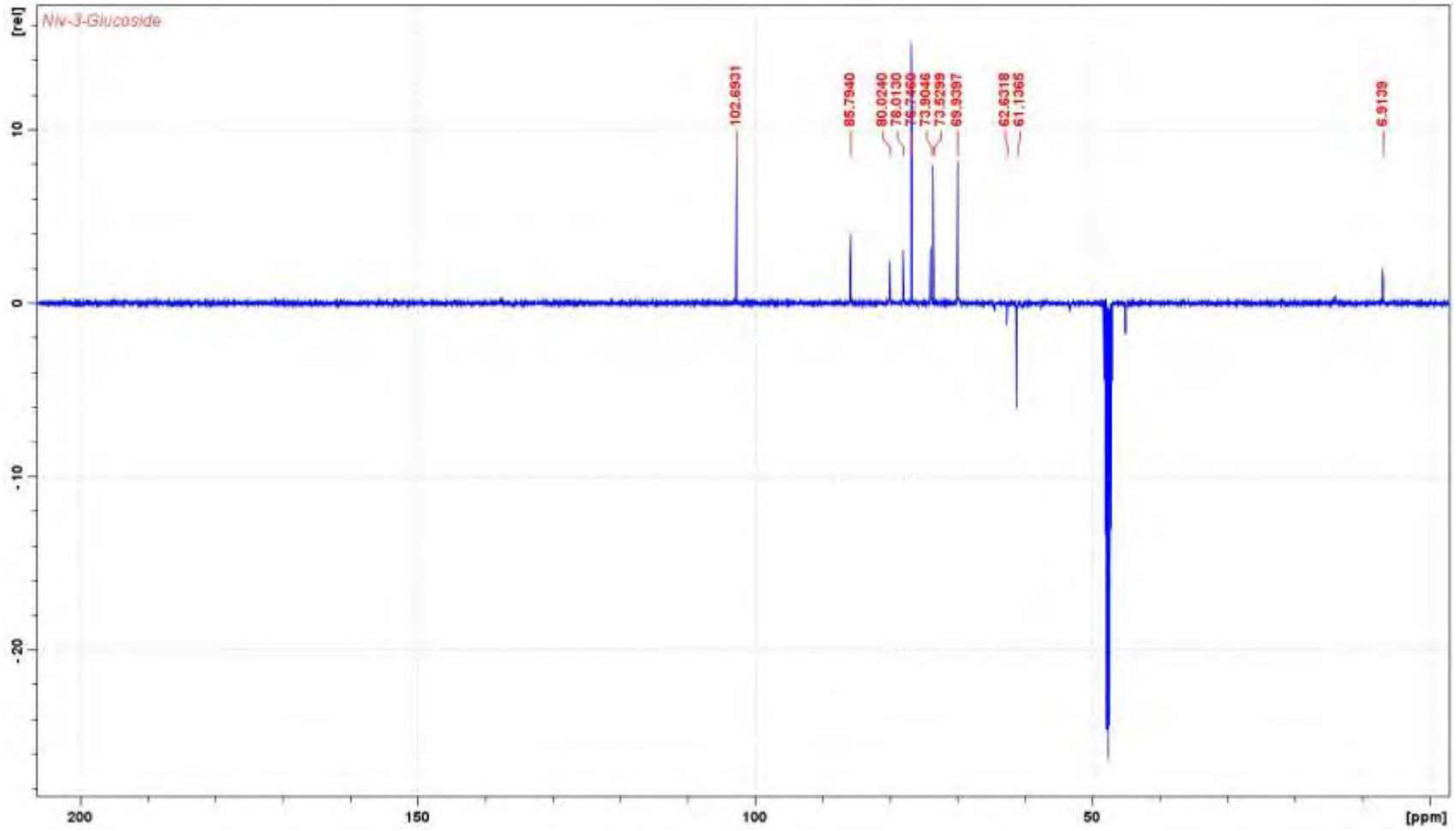


a) ^{1}H spectrum of NIV-3-β-D-glucoside



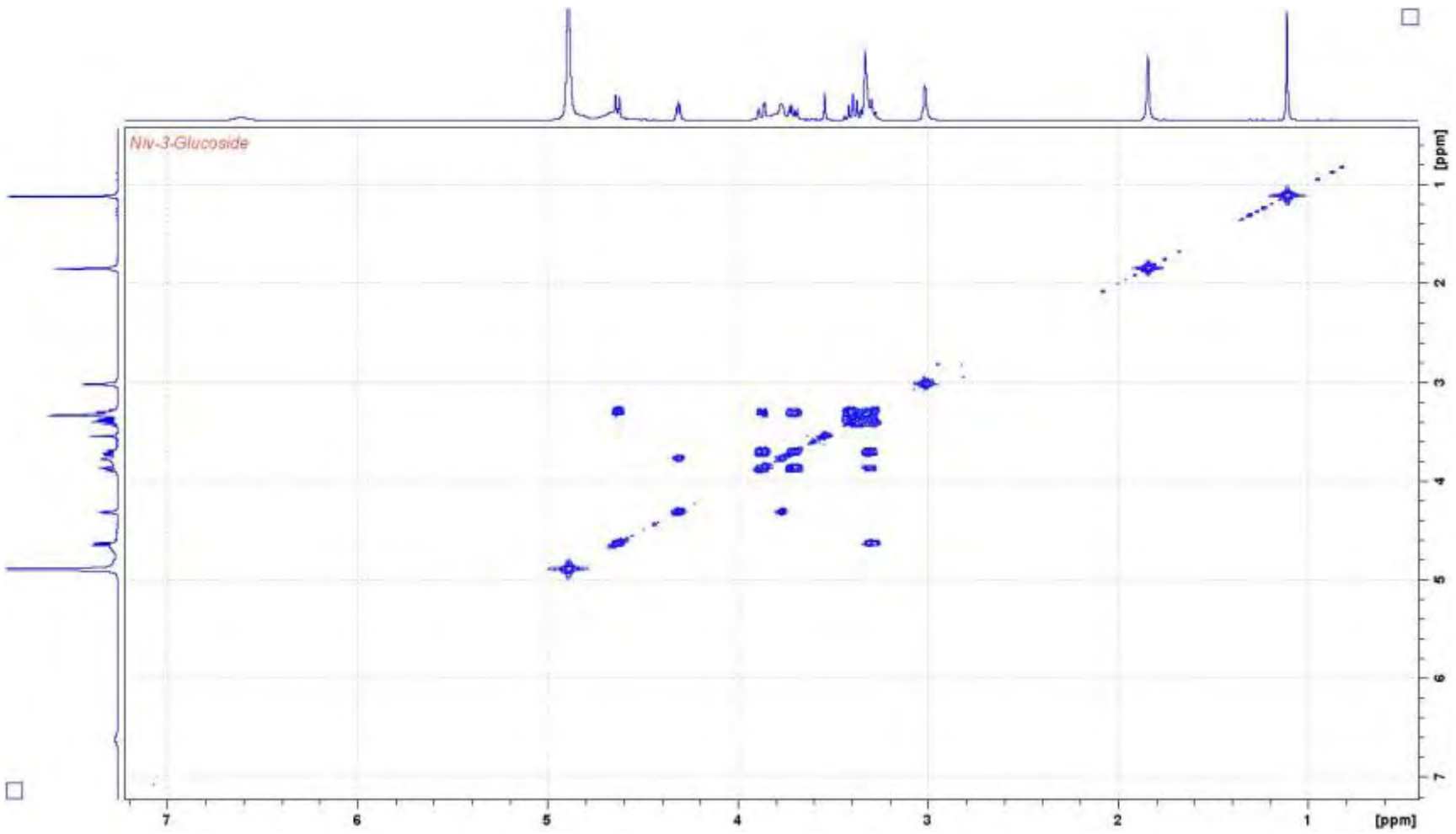
^{1}H NMR (400 MHz, methanol-d_4) δ 6.61 (b, 1H), 4.82 (b, 1H), 4.69 (b, 1H), 4.66 (b, 1H), 4.63 (d, *J*=7.6 Hz, 1H), 4.31 (dd, *J*=4.5, 3.7 Hz, 1H), 3.88 (dd, *J*=12.0, 2.1 Hz, 1H), 3.77 (d, *J*=4.1 Hz, 1H), 3.71 (dd, *J*=12.1, 5.0 Hz, 1H), 3.70 – 3.90 (m, 2H), 3.40 (t, *J*=7.9 Hz, 1H), 3.37 (t, *J*=8.5 Hz, 1H), 3.31 (b, 1H), 3.29 (b, 1H), 3.02 (b, 2H), 1.84 (s, 3H), 1.11 (s, 3H)

b) ^{13}C spectrum of NIV-3-β-D-glucoside

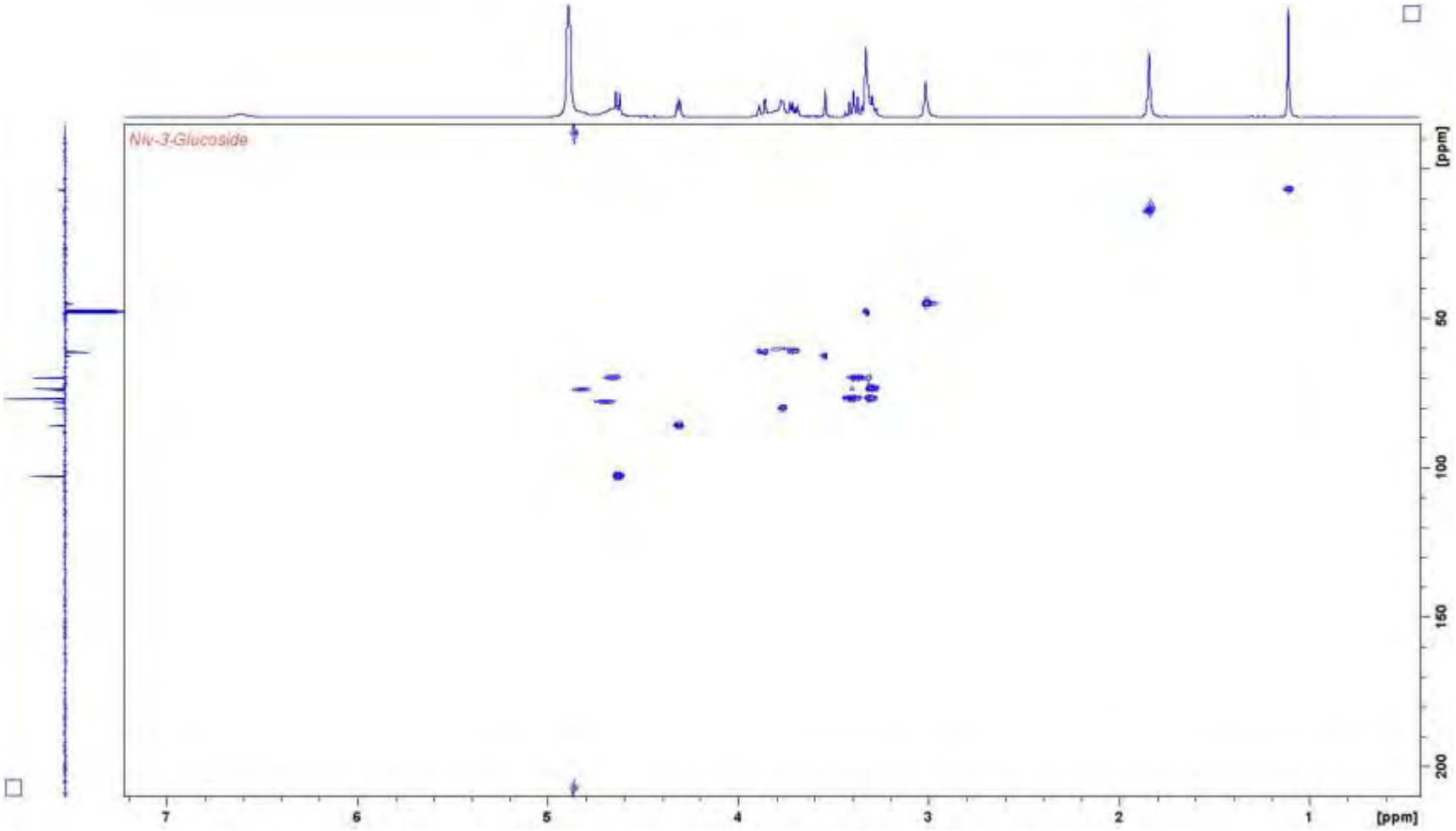


^{13}C NMR (100 MHz, methanol-d_4) δ 200.1 (s, 1C), 137.5 (d, 1C), 136.1 (s, 1C), 102.7 (d, 1C), 85.8 (d, 1C), 80.0 (d, 1C), 78.0 (d, 1C), 76.7 (d, 2C), 74.0 (d, 1C), 73.5 (d, 1C), 69.9 (d, 1C), 69.6 (d, 1C), 64.5 (s, 1C), 61.1 (t, 1C), 60.0 (t, 1C), 53.3 (s, 1C), 48.8 (s, 1C), 45.1 (t, 1C), 13.8 (q, 1C), 6.9 (q, 1C)

c) H-H-COSY spectrum of NIV-3-β-D-glucoside



d) HSQC spectrum of NIV-3-β-D-glucoside with ^{1}H on the x-axis and ^{13}C on the y-axis



e) HMBC spectrum of NIV-3-β-D-glucoside with ^{1}H on the x-axis and ^{13}C on the y-axis. The arrows indicate the connection between position 3 of DON and position 1' of glucose - red arrow = 85.8/4.63 ppm; blue arrow = 102.7/4.31 ppm.

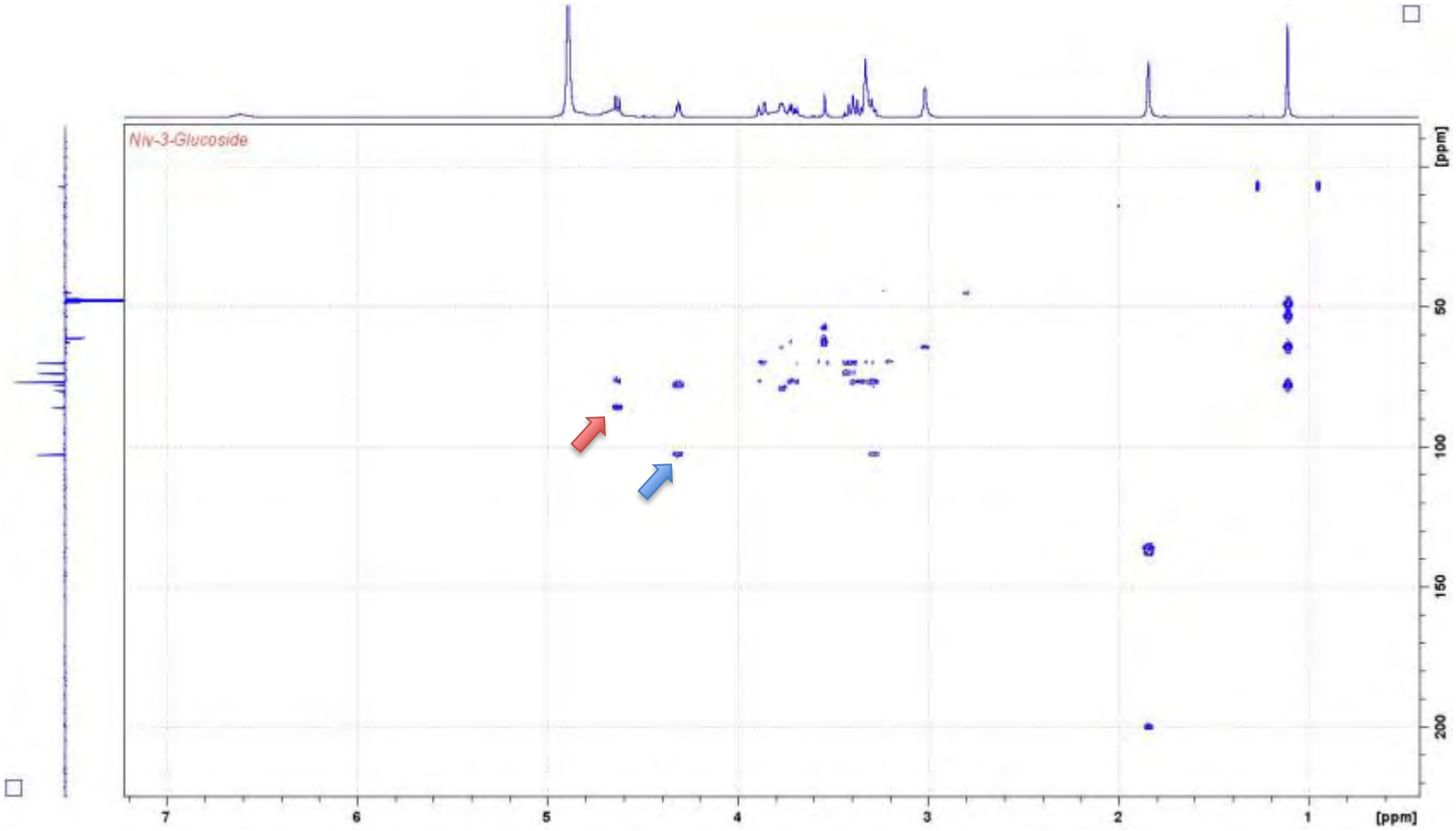

Niv-3-Glucoside
7
6
5
4
3
2
1
[ppm]
50
100
150
200
[ppm]

Fig. S2. NIV-3-glucoside is less inhibitory than NIV for rabbit reticulocyte ribosomes.

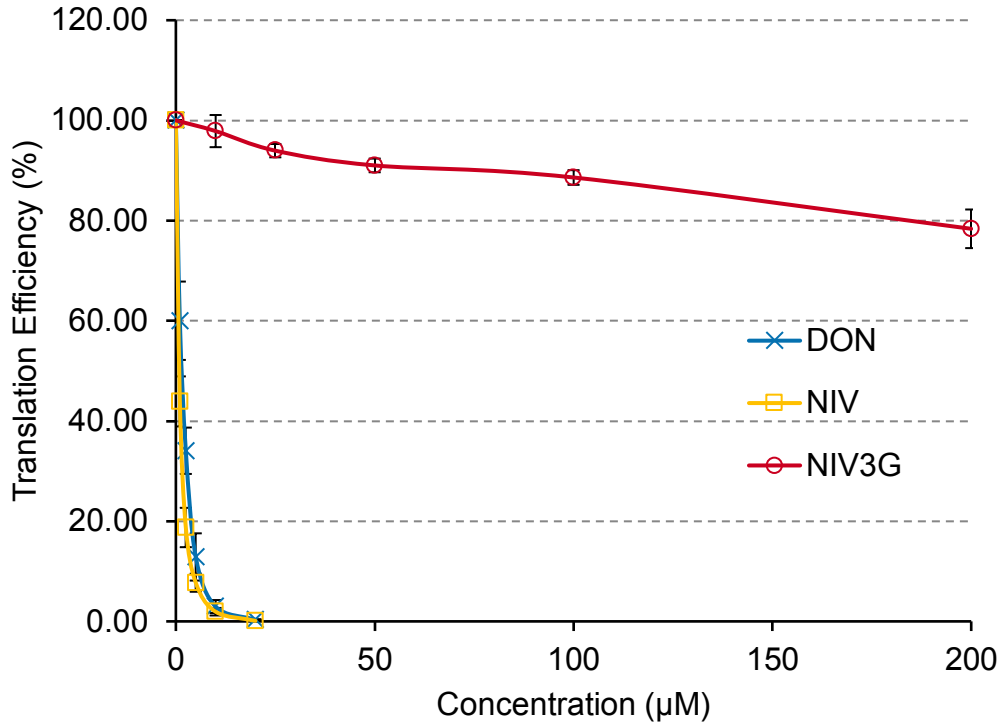


Fig. S3. Root growth of Col-0 wildtype and transgenic *A. thaliana* expressing *HvUGT13248* on half strength MS medium containing 0 mg L^{-1} NIV at 7 days and 100 mg L^{-1} NIV at 14 days after germination. Scale bars = 2 cm.

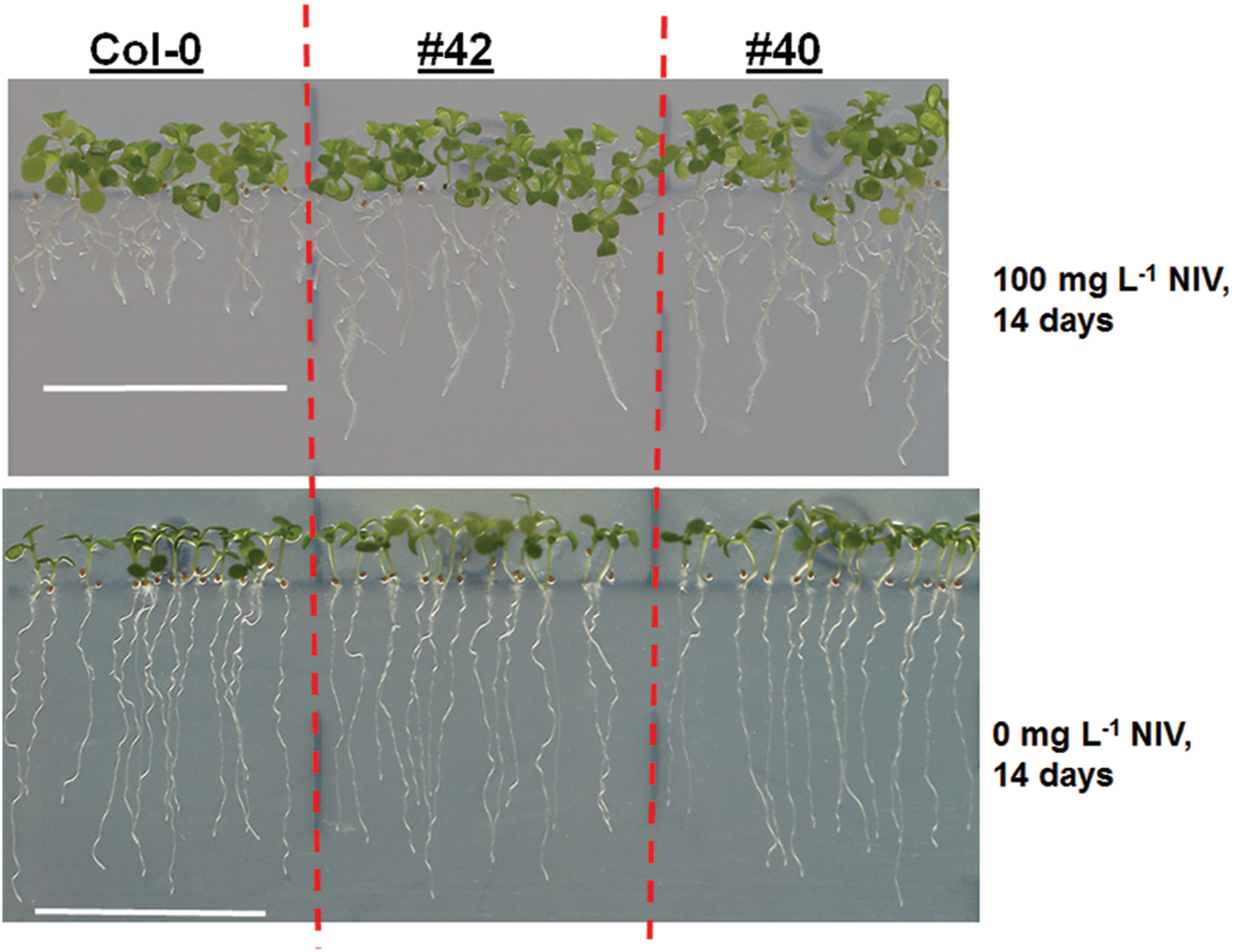


Table S1. Summary of transgenic wheat expressing *HvUGT13248* in greenhouse point-inoculation tests with NIV-producing *F. graminearum* strain.

	2013 Spring			2013 Fall			2014 Fall		
Genotype[a]	**No.[b]**	**Sev.[c] (%)**	**Red.[d] (%)**	**No.**	**Sev. (%)**	**Red. (%)**	**No.**	**Sev. (%)**	**Red. (%)**
#8	31	8.2 ± 0.8***	75.4	27	20.2 ± 4.0***	71.1	16	9.4 ± 0.9***	83.8
#15	32	6.8 ± 0.1***	79.5	27	7.3 ± 0.1***	89.6	14	9.6 ± 1.1***	83.4
#19	20	6.7 ± 0.2***	79.9	18	7.9 ± 0.5***	88.6	14	15.1 ± 2.6***	73.9
#37	31	6.9 ± 0.3***	79.3	27	6.8 ± 0.1***	90.3	16	13.2 ± 1.9***	77.1
Bobwhite	22	33.2 ± 6.6	NA[e]	25	69.8 ± 5.0	NA	16	57.7 ± 4.4	NA
Wheaton	23	58.6 ± 7.3	NA	20	82.9 ± 6.4	NA	15	86.9 ± 5.2	NA
Sumai 3	22	8.0 ± 1.3	NA	18	7.0 ± 1.6	NA	18	6.5 ± 0.6	NA

[a]Events #8, #15, #19 and #37 were transgenic lines, and 'Bobwhite' was the non-transformed control. 'Sumai 3' was the resistant check, and 'Wheaton' was the susceptible check.

Values provided are the means ± standard error.

*, ** and *** indicate significance at the 0.05, 0.01, and 0.001 levels compared with the non-transformed 'Bobwhite' control (Student's t test).

[b]No.: number of plants examined in the disease screen

[c]Sev.: FHB severity shown as the percentage of symptomatic spikelets in the inoculated spikes

[d]Red.: percent disease reduction rate as compared to the corresponding non-transformed 'Bobwhite' control.

[e]NA: not applicable.

Table S2. *HvUGT13248* converts NIV to NIV3G faster in transgenic wheat than in non-transformed 'Bobwhite'. Numbers shown are mean value ± standard error. *, ** and *** indicate significance at the 0.05, 0.01, and 0.001 levels compared with the non-transformed 'Bobwhite' control (Student's t test).

	Non-transformed 'Bobwhite'				***HvUGT13248*-#19 transgenic wheat**			
Time[a] (h)	**NIV (nmol)**	**NIV3G (nmol)**	**NIV3G/ NIV[b]**	**NIV+NIV3G[c] (nmol)**	**NIV (nmol)**	**NIV3G (nmol)**	**NIV3G/ NIV**	**NIV+NIV3G (nmol)**
0	106.69 ± 7.17	0	0	106.69 ± 7.17	83.57 ± 5.82*	0	0	83.57 ± 5.82*
2	87.89 ± 3.40	0.44 ± 0.11	0.01 ± 0	88.39 ± 3.39	75.06 ± 3.13*	2.52 ± 0.22***	0.03 ± 0***	77.58 ± 3.32*
6	120.45 ± 6.18	5.02 ± 0.44	0.04 ± 0	125.47 ± 6.20	85.96 ± 5.85**	19.20 ± 2.11***	0.22 ± 0.02***	105.16 ± 7.20
12	66.92 ± 5.05	16.37 ± 0.77	0.26 ± 0.02	83.29 ± 5.22	70.35 ± 7.90	23.41 ± 1.90**	0.36 ± 0.05	93.75 ± 7.91
24	59.55 ± 8.95	24.24 ± 1.57	0.45 ± 0.04	83.79 ± 9.64	49.13 ± 4.13	52.28 ± 2.97***	1.11 ± 0.09***	101.41 ± 5.84
36	35.04 ± 2.55	47.53 ± 1.60	1.40 ± 0.09	82.56 ± 3.26	33.44 ± 3.33	48.19 ± 3.44	1.59 ± 0.20	81.63 ± 2.60
48	27.64 ± 4.62	53.62 ± 2.76	2.20 ± 0.24	81.26 ± 6.64	19.84 ± 2.29	55.47 ± 2.19	3.10 ± 0.36	75.31 ± 2.33
72	18.76 ± 2.22	51.34 ± 1.72	2.99 ± 0.32	70.10 ± 2.83	20.52 ± 2.42	63.18 ± 2.49**	3.31 ± 0.29	74.40 ± 10.09*
96	15.58 ± 1.92	56.89 ± 3.61	3.91 ± 0.33	64.42 ± 9.23	10.45 ± 1.41*	52.71 ± 3.13	6.24 ± 1.21	63.16 ± 3.31
336	5.41 ± 0.89	61.75 ± 2.43	13.40 ± 1.87	59.70 ± 7.87	10.35 ± 1.83*	64.12 ± 4.25	7.72 ± 1.30*	66.20 ± 9.47

[a]Time after NIV treatment when samples were collected.

[b]Ratio of NIV3G over NIV at each time point in each genotype.

[c]Sum of NIV3G and NIV at each time point in each genotype.