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A Preliminary Approach in Mendelizing Barley FR-H1 and FR-H2 QTLs

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BACKGROUND

Considering **barley** (*Hordeum vulgare* L.) as a **model** specie for the ***Triticeae***, two locations were selected to assess the **winter survival rate (WSR)** and to evaluate the **frost resistance** of **Nure** (medium resistant winter type), **Tremois** (susceptible spring type) and **4 QTL-NILs**, carrying alternative alleles form Nure or Tremois varieties. The aim was to **mendelize** the effects of the ***FR-H1 (VRN-H1)*** and ***FR-H2 (CBF gene cluster)*** loci in **alternative backgrounds**. The QTL-NILs, and parents were also tested in a preliminary **single gene expression experiment** aimed at evaluating the immediate and early changes in **CBF's expression** in the first two days of **cold acclimation.**

MATERIALS and METHODS

- **Genotypes.** A marker-assisted backcross scheme was used to develop four QTL-Near Isogenic Lines (markers; *HvCBF3 – FR-H2* and *HvBM5 – FR-H1*). An initial indication of the genotypic status of the lines was obtained using 19 SSR markers scattered in the barley genome.
- **Field Trials.** Two locations were selected to assess the Winter Survival Rate (WSR): Reggio Emilia, Italy, temperate continental climate, and Wooster, Ohio, USA, humid continental climate.
- **Single Gene Expression Profiles.** Plants at third-leaf stage were sampled in the morning, afternoon, and night for three consecutive days (Figure 1). The relative gene expressions were calculated using the ΔΔCt method (Livak and Schmittgen, 2001) with Ct values normalized by the Ct values of house-keeping gene *HvCyclophilin* (Burton et al., 2004).

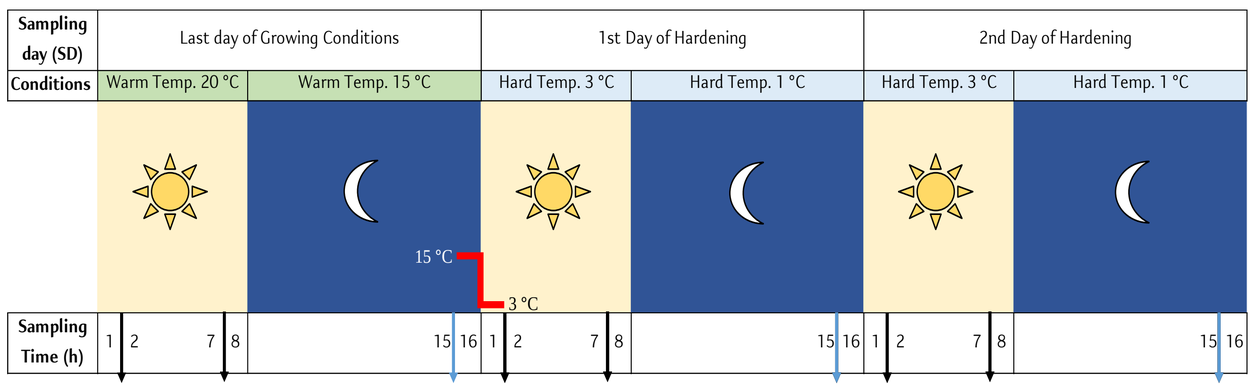

Figure 1. Outline of samplings during the single gene expression experiment under warm (20/15°C, 8/16 h day/night) and acclimation (3/1°C, 8/16 h day/night) conditions. Black arrows – sampling time during the day light. Blue arrows – sampling time during the night. Red lines - cold temperature application.

PHENOTYPING

- **Reggio Emilia, IT, Po valley area, temperate continental climate**. Due to **abnormal condition** in early stages and lacking freezing events during the trial **no significant difference** among genotypes was **observed.**
- **Ohio, US, humid continental climate.** ***FR-H1/VRN-H1*** **substitution** did **NOT modify** the frost **resistance** neither in winter nor spring background. **Nure** ***FR-H2/CBFs*** **increased** the frost **resistance** in the **Tremois/susceptible background** (Figure 2 and Table 1).

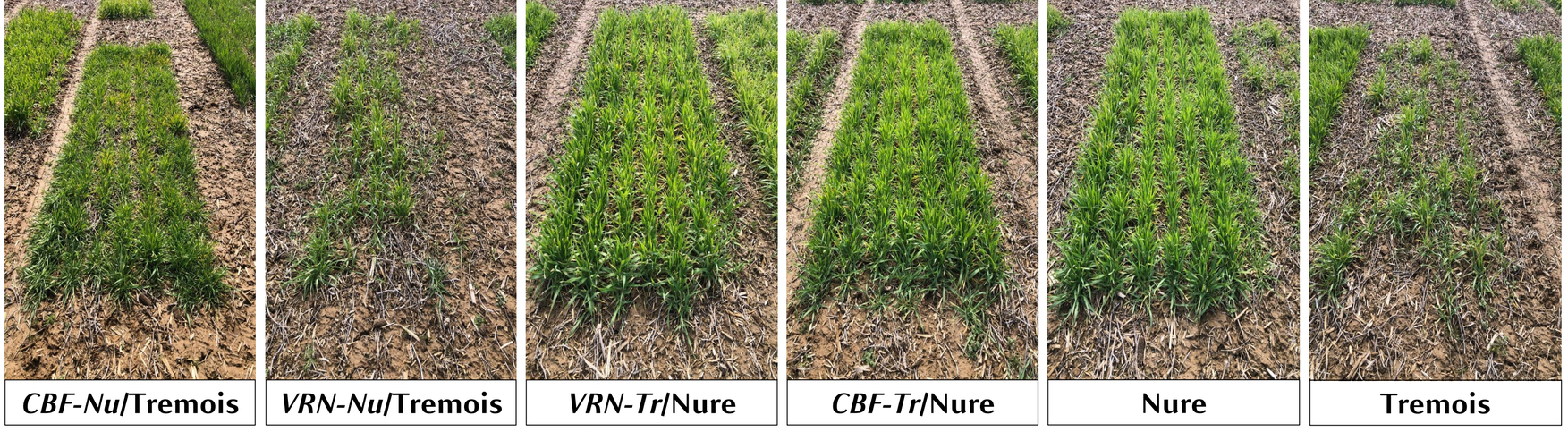

Figure 2. Pictures of the four QTL-NILs, Nure and Tremois at the end of the winter in Wooster (Ohio). Winter survival was measured at the end of the tillering phase (Zadoks growth stage Z29-Z30) 2-April 2024 by comparing the number of living tillers to winterkilled tillers in one-meter square sampling area for three reps.

Genotype	WSR (%)	RANK
CBF-Nu/Tremois	91.7	b
VRN-Nu/Tremois	36.7	c
VRN-Tr/Nure	100.0	a
CBF-Tr/Nure	100.0	a
Nure	100.0	a
Trèmois	35.0	c

Table 1. Statistical analysis of the WSR Wooster (Ohio). Kruskal-Wallis test for non-parametric multiple comparisons and ANOVA were used to evaluate the effects of genotype, treatment and their interaction. P-value Chisq: 0.00926478

GENE EXPRESSION

- **Rapid** and **strong temperature** decrease did **not determine** the **induction** for ***HvCBF2*** (Table 2).
- ***HvCBF4*** was **highly** expressed in **the frost-resistant** line ***CBF-Nu*/Tremois** (Table 3).
- ***HvCBF9*** **expression levels** were **higher** in those lines carrying the **winter allele** of ***FR-H2*** or harboring the **winter background** (Table 4).
- ***HVCBF14*** was **highly** expressed in the **winter resistant** genotype **Nure** carrying both resistant alleles (Table 5).

High expression
Low expression

HvCBF2	Last day of Growing Conditions 20/15 °C			1st Day of Hardening 3/1 °C			2nd Day of Hardening 3/1 °C		
	Morning	Afternoon	Night	Morning	Afternoon	Night	Morning	Afternoon	Night
Nure	1.59	79.96	8.96	9.25	39.91	32.30	22.13	21.31	2.85
Tremois	0.99	25.41	9.73	4.22	17.42	16.06	10.13	11.33	2.08
CBF-Nu/Tremois	3.32	71.83	6.98	8.26	44.49	3.99	2.45	9.83	0.65
VRN-Nu/Tremois	0.13	9.31	4.01	3.13	3.27	20.78	4.34	9.83	19.38
VRN-Tr/Nure	1.54	70.31	5.50	30.89	11.84	8.42	51.35	21.34	2.71
CBF-Tr/Nure	6.63	88.94	11.94	14.56	40.57	87.82	18.66	28.03	11.14

Table 2. Heatmap of the relative gene expression for the *HvCBF2*. Red arrow - cold temperature application.

HvCBF4	Last day of Growing Conditions 20/15 °C			1st Day of Hardening 3/1 °C			2nd Day of Hardening 3/1 °C		
	Morning	Afternoon	Night	Morning	Afternoon	Night	Morning	Afternoon	Night
Nure	3.68	217.66	2.83	24.35	350.10	681.70	595.24	108.23	56.11
Tremois	84.95	328.14	3.15	126.64	852.04	213.82	121.94	44.06	7.19
CBF-Nu/Tremois	665.54	1107.02	17.55	665.68	4461.00	656.64	242.08	74.16	14.17
VRN-Nu/Tremois	50.71	108.09	2.36	125.77	794.41	130.53	69.09	24.33	4.99
VRN-Tr/Nure	9.86	236.28	2.36	18.71	287.49	551.64	467.34	110.76	32.62
CBF-Tr/Nure	7.74	303.89	3.15	33.55	547.69	1126.72	461.15	164.05	26.21

Table 3. Heatmap of the relative gene expression for the *HvCBF4*. Red arrow - cold temperature application.

HvCBF9	Last day of Growing Conditions 20/15 °C			1st Day of Hardening 3/1 °C			2nd Day of Hardening 3/1 °C		
	Morning	Afternoon	Night	Morning	Afternoon	Night	Morning	Afternoon	Night
Nure	15.59	116.18	31.27	242.22	1452.19	1414.69	763.11	393.76	48.69
Tremois	13.82	3.93	3.87	162.32	1147.76	673.63	430.70	147.32	87.82
CBF-Nu/Tremois	9.61	152.49	7.06	354.75	1436.15	319.57	143.02	141.12	9.83
VRN-Nu/Tremois	3.88	142.18	5.84	140.11	912.72	318.53	163.93	181.42	20.31
VRN-Tr/Nure	6.92	6.68	10.60	240.23	1109.80	1373.88	882.38	71.54	53.08
CBF-Tr/Nure	6.81	12.11	9.74	378.37	1280.53	1421.45	621.83	191.59	31.03

Table 4. Heatmap of the relative gene expression for the *HvCBF9*. Red arrow - cold temperature application.

HvCBF14	Last day of Growing Conditions 20/15 °C			1st Day of Hardening 3/1 °C			2nd Day of Hardening 3/1 °C		
	Morning	Afternoon	Night	Morning	Afternoon	Night	Morning	Afternoon	Night
Nure	7.09	90.90	6.73	2292.66	1303.44	769.88	2348.46	299.04	50.23
Tremois	5.58	31.22	37.82	257.38	989.26	338.05	213.53	132.99	22.72
CBF-Nu/Tremois	3.48	74.34	5.72	344.21	1590.91	344.42	178.09	164.85	15.44
VRN-Nu/Tremois	3.188	46.877	6.781	247.596	1090.149	233.200	116.665	91.275	9.606
VRN-Tr/Nure	3.88	33.13	5.55	344.19	1615.74	568.10	372.87	193.80	37.31
CBF-Tr/Nure	35.12	37.43	8.34	433.18	1261.73	836.79	321.22	269.89	37.67

Table 5. Heatmap of the relative gene expression for the *HvCBF14*. Red arrow - cold temperature application.

SUMMARY AND PROSPECTS

Our study showed that **the winter allele** of ***FR-H2*** (*CBF* gene cluster) putatively appears to have a **greater effect** compared to ***FR-H1*** (*VRN-H1*) in **conferring resistance** in the **susceptible genotype**. On the other hand, in the **winter background** seems to be **other factors** that might be **involved** in the frost **resistance**. The recently identified ***FR-H3*** locus (Muñoz-Amatriaín et al., 2020) will be investigated in our plant material to reveal its allelic state.

The **expression** of the ***CBF*** genes showed **interesting pattern** after the **cold exposure** highlighting **higher levels in the resistant genotypes**. However, further **investigations** are required to analyze the **molecular response** of the **ICE-CBF-COR** and ***VRN-H1*** activity during the **cold acclimation** and **vernalization**.

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