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Effect of Powdery Mildew on Glutamate Dehydrogenase in Wheat Grain

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Abstract: Glutamate dehydrogenase (GDH) is a key enzyme in nitrogen (N) metabolism of wheat under stress conditions. There are two views about the reaction catalyzed by GDH in plants, namely aminating and deaminating activity, so the specific function of GDH remains to be clarified. In order to clarify the specific role of GDH in wheat grain and further study the changes of *GDH* expression and activity under wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*, *Bgt*) stress, field experiments were carried out with susceptible cultivar Xi'nong 979 and resistant cultivar Zhengmai 103 under three treatments. The three treatments were no inoculation (CK), inoculated once with *Bgt* (MP) and inoculated nine times with *Bgt* (HP). Results showed that GDH aminating activity was higher than that of deaminating activity in Zhengmai 103 in CK (natural conditions). In the same way, under powdery mildew conditions, there was a similar trend of higher aminating activity than deaminating activity of GDH in Zhengmai 103 at 21 and 28 days after anthesis (DAA) and Xi'nong 979 at 10 – 30 DAA in MP and HP. The results indicated that GDH mainly assimilated ammonia in grains under both natural and powdery mildew stress conditions. Meanwhile, under mild powdery mildew conditions, the *GDH* expression and GDH aminating activity increased with the increase of disease index (DI) at 21 and 28 DAA in Zhengmai 103 and at 10 – 15 DAA in Xi'nong 979. Under severe powdery mildew conditions, *GDH* expression and GDH aminating activity of Xi'nong 979 increased with the increase of DI during the period of 20 – 30 DAA. Therefore, both mild and severe powdery mildew stress could induce *GDH* expression and aminating activity. It showed that the main function of GDH in wheat grain was to assimilate ammonia, and powdery mildew could induce *GDH* expression and aminating activity.

Keywords: Wheat, Powdery Mildew, Glutamate Dehydrogenase, Enzyme Activity

1. Introduction

Glutamate dehydrogenase (GDH, EC l. 4.1.2) is a widely-expressed mitochondrial enzyme that catalyzes a reversible amination/deamination reaction in vitro, that is α-ketoglutarate (α-KG) + NH_4^+ + dihydronicotinamide adenine dinucleotide (NADH) ↔ glutamate (Glu) + H_2O + nicotinamide adenine dinucleotide (NAD^+) [1]. There are two opinions about the reaction catalyzed by GDH in plants [2, 3]. One view supports that GDH catalyzes the deamination reaction in vivo. One key piece of evidence is that GDH purified from various plant organs exhibits high Km values (> 1 mM) for NH_4^+ [4], with some additional evidence coming from isotope-labeled Glu feeding trial [5, 6]. Another view supports that GDH catalyzes amination reaction in vivo, and the supporting evidence comes from studies under stress conditions. Under the stress of viruses [7], drought [8], high salt [9-11] and alkali [12], GDH aminating activity increased, which was an important supplement to nitrogen (N) metabolism [3]. On the whole, there are few studies on GDH in plants. One reason is that the study of N metabolism is mainly focused on glutamine synthetase (GS) and glutamate synthase (GOGAT), which have been identified as the main enzymes of N metabolism in plants. The other reason is that the role of GDH in plants remains to be clarified. As far as the existing studies are concerned, the reports supporting the

deaminating activity of GDH come from in vitro experiments, while the reports supporting the aminating activity of GDH come from in vivo experiments of vegetative organs such as rice seedlings or tobacco leaves [7-12]. These studies on rice or tobacco have shown that, at least under stress conditions (virus infection, drought, high salt and high alkali, etc.), GDH catalyzes ammonia assimilation, which plays an important role in N metabolism. However, there are few reports on the role of GDH in plants, especially in reproductive organs such as grains under natural growth conditions.

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a serious airborne fungal disease of wheat (*Triticum aestivum* L.) [13]. It can reduce wheat quality [14, 15]. Under powdery mildew stress, the activities of two N metabolism enzymes, GS and GOGAT, were inhibited [16], and the oxidation level in grain cells increased [17]. There are few reports on the effect of powdery mildew on GDH in wheat grains. In this study, the changes of *GDH* mRNA expression and enzyme activity under natural and powdery mildew stress conditions were analyzed, and the role of GDH in N metabolism was discussed.

2. Materials and Methods

2.1. Materials

Two winter wheat cultivars Xi'nong 979 (susceptible) and Zhengmai 103 (resistant) were used as plant materials.

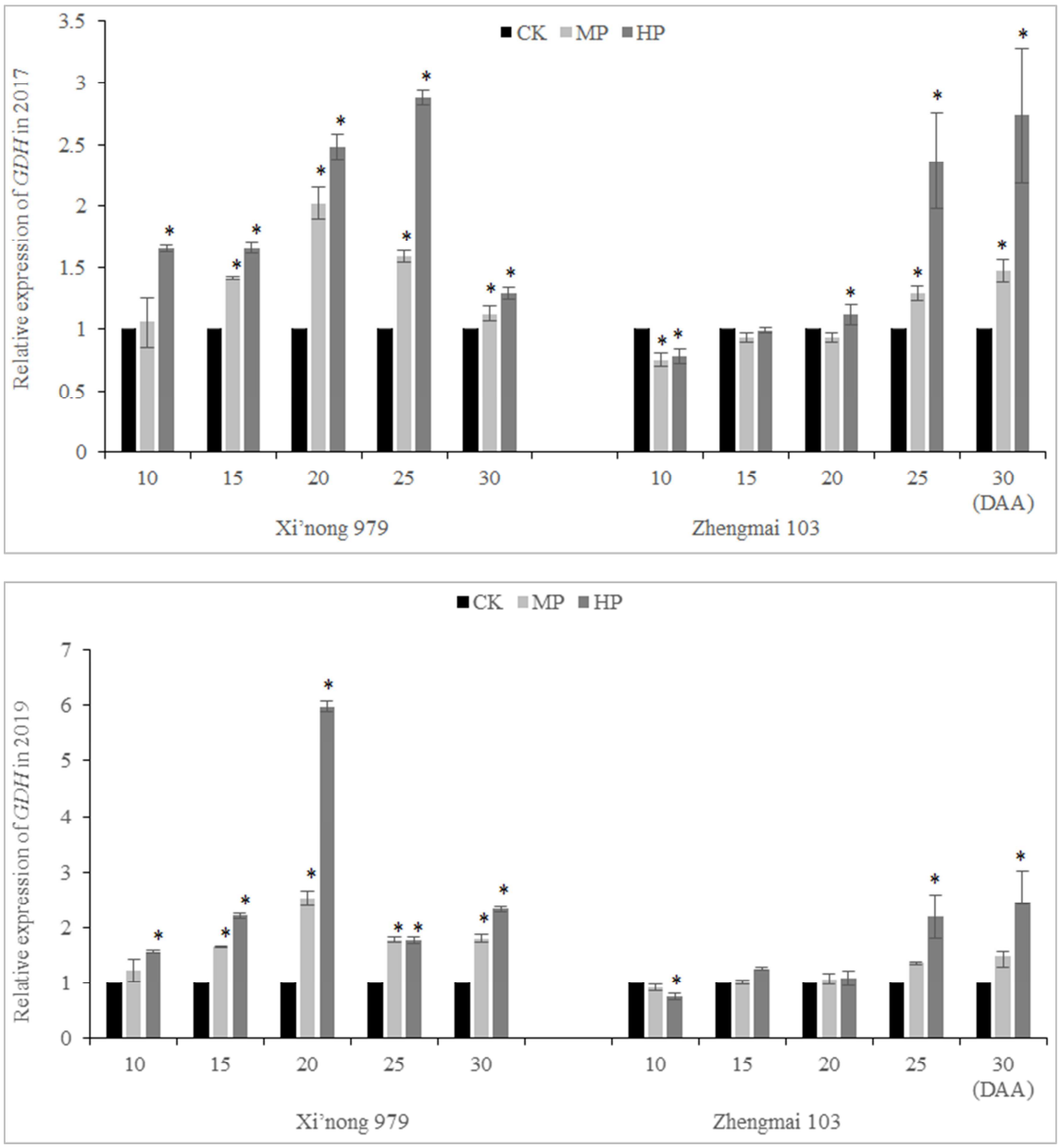


Figure 1. *Relative gene expression of glutamate dehydrogenase (GDH) in grains of Xi'nong 979 (susceptible) and Zhengmai 103 (resistant) in response to inoculation of Blumeria graminis f. sp. tritici in two years. CK, no inoculation; MP and HP, one and nine inoculations of Blumeria graminis f. sp. tritici, respectively. Error bars represent S. E. M. (n=3) "*" indicates a significant level at the 0.05 level.*

2.2. Pathogen Source

The pathogen of *Bgt* isolate E20 was propagated on susceptible wheat (Xi'nong 979) in greenhouse.

2.3. Experimental Design

Split plot design, 3 replicates, 3 main treatments were respectively marked as CK (un-inoculated control), MP (inoculated once) and HP (inoculated nine times), 2 vice treatments were two wheat cultivars. During 2016 – 2017 and 2018 – 2019 growing seasons (hereafter signed as 2017 and 2019), field experiments and field management were the same as Gao et al. [16].

2.4. Powdery Mildew Assessment and Sampling Methods

The Disease assessment and sample collection were the same as Gao et al. [16].

2.5. Gdh Expression Analysis by Quantitative Real-Time Rt-Pcr (Qrt-Pcr) Analysis

Quantitative real-time PCR (Qrt-Pcr) was performed on a Roche Light Cycler*480 Real-Time PCR Detection System using the gene-specific primers of *GDH* (KU869729.1) (Table 1), 3 replicates. The expression analysis of *GDH* was conducted using the $2^{-\Delta\Delta Ct}$ method [19]. The transcription level was normalized to the expression of *β*-actin [16].

2.6. Enzyme Activity Analysis

The extraction of crude enzyme solution was the same as Gao et al. [16]. GDH activity was determined according to a reported method [21]. In the amination reaction, GDH activity was determined in the presence of 100 mM NH_4Cl or 50 mM $(NH_4)_2SO_4$, 13 mM α-KG, 0.25 mM NADH, and 1 mM $CaCl_2$ in 100 mM Tris-HC1, pH 8.0, and the decrease in absorbance value at 340 nm was recorded for 3 min. In the deamination reaction, GDH activity was determined in the presence of 35 mM Glu, 0.25 mM NAD, and 1 mM $CaCl_2$ in 100 mM Tris-HC1, pH 9.3, and the increase in absorbance value at 340 nm was recorded for 3 min. One unit of GDH activity was defined as the reduction or oxidation of 1 μM of coenzyme (NAD or NADH, respectively) per min per g of grain fresh weight at 30°C.

2.7. Statistical Methods

Analysis of variance (ANOVA) was performed using SPSS 17.0 (SPSS Institute Inc.). Pearson's correlation tests were used to examine the possible relationships among *GDH* expression, GDH activity and disease index (DI).

Table 1. *The DNA sequences of the primers used in real-time PCR.*

Gene symbol	GeneBank Accession	Primer orientation	Primer sequences (5' - 3')	Product length (bp)
GDH	KU869729.1	Forward Reverse	TGCCACTGAAGCCCTACTTG AGCCCAGGAGCCAACATTAC	91
β-actin	AB181991	Forward Reverse	CCAATCTATGAGGGATACACGC AGCGGTTGTTGTGAGGGAG	123

3 Results

3.1. Disease Assessment

In both years, the DI of Xi'nong 979 was significantly different among three treatments, showing a trend of HP > MP > CK (Table 2). The DI of Zhengmai 103 was zero in three treatments at 7 and 14 DAA, and was very low (less than 10) at 21 and 28 DAA, showing a trend of HP > MP > CK. Overall, the DI of Zhengmai 103 was lower than that of Xi'nong 979.

Table 2. *Disease index under varying degrees of Bgt infection in two years.*

Year	Treatment	Xi'nong 979				Zhengmai 103			
		7	14	21	28	7	14	21	28 (DAA)
2017	CK	6.2	12.5	21.7	60.6	0	0	0.83	2.95
	MP	12.0	17.8	26.2	74.1	0	0	2.73	3.90
	HP	27.8	31.3	53.2	87.4	0	0	3.29	7.63
	S. E. M	0.327	0.823	0.598	0.787	0	0	0.396	1.177
	p	0.000	0.000	0.000	0.000			0.002	0.016
2019	CK	6.5	11.8	21.3	65.1	0	0	0.70	2.78
	MP	17.3	20.1	24.8	70.9	0	0	1.11	5.38
	HP	25.8	32.5	54.3	79.3	0	0	2.32	8.07
	S. E. M	0.387	0.681	1.628	1.746	0	0	0.211	0.416
	p	0.000	0.000	0.000	0.000			0.001	0.000

Note: DAA, days after anthesis.

3.2. Changes of Gdh Expression in Grains

For Xi'nong 979, the expression of *GDH* increased with the increase of DI in two years, the symptoms of powdery mildew appeared earlier (earlier than 7 DAA), and the expression of *GDH* also increased earlier (from 10 DAA or

earlier) (Figure 1). For Zhengmai 103, there was no significant difference in *GDH* expression among the three treatments at 10 – 20 DAA, but there was a significant increase in *GDH* expression at 25 and 30 DAA in both years, which occurred after the powdery mildew symptoms appeared (21 DAA). The correlation analysis between *GDH* expression of Xi'nong 979 and DI of powdery mildew showed that there was a significant positive correlation between them (Table 3). It showed that *GDH* expression increased with *Bgt* infection in the susceptible cultivar, and the earlier powdery mildew symptoms appeared, the earlier *GDH* expression was induced.

Table 3. *Correlation between GDH expression and powdery mildew disease index, and correlation between GDH activity and powdery mildew disease index in Xi'nong 979 (susceptible) in two years.*

Year		Xi'nong 979				
		10	15	20	25	30 (DAA)
2017	*GDH* expression	0.94**	0.91**	0.89**	1.00*	0.95**
	NADH-GDH activity	0.97**	0.99**	0.99**	0.97**	0.98**
	NAD^+-GDH activity	0.90**	0.74*	-0.97**	-0.87**	-0.97**
2019	*GDH* expression	0.90**	0.99**	0.99**	0.87*	0.97**
	NADH-GDH activity	0.98**	0.97**	0.94**	0.96**	0.98**
	NAD^+-GDH activity	0.97**	0.95**	-0.95**	-0.88**	-0.94**

Note: DAA, days after anthesis; "*" and "**" indicate a significant level correlation at the 0.05 and 0.01 levels, respectively.

Table 4. *Ratios of the NADH-GDH/NAD+-GDH activities in Xi'nong 979 (susceptible) and Zhengmai 103 (resistant) under varying degrees of Bgt infection in two years.*

Year	Treatment	Xi'nong 979					Zhengmai 103				
		10	15	20	25	30	10	15	20	25	30 (DAA)
2017	CK	4.34	3.33	3.08	9.56	20.15	2.80	2.70	5.52	11.64	16.78
	MP	3.46	4.07	5.48	23.01	24.96	2.94	2.72	5.59	13.48	18.89
	HP	3.50	4.92	25.74	42.24	60.01	3.16	2.91	5.64	17.13	20.02
	S. E. M	0.162	0.483	0.428	1.552	2.723	0.426	0.150	0.091	1.705	1.324
	p	0.003	0.045	0	0	0	0.716	0.367	0.461	0.015	0.002
2019	CK	4.87	3.88	4.15	12.49	21.13	2.97	2.56	6.40	16.14	17.30
	MP	4.75	3.12	8.37	17.15	34.83	2.86	2.58	6.67	21.01	21.89
	HP	4.67	3.16	23.41	22.68	51.87	2.79	2.69	6.70	21.23	24.66
	S. E. M	0.181	0.211	2.169	1.112	1.396	0.157	0.169	0.656	1.237	1.269
	p	0.554	0.019	0	0	0	0.559	0.728	0.894	0.168	0.442

Note: DAA, days after anthesis.

3.3. Changes of Gdh Activity in Grains

In CK, GDH aminating activity of Zhengmai 103 was higher than that of deaminating activity, which indicated that GDH in wheat grain mainly assimilated ammonia under normal growth conditions (Figures 2 and 3). Meanwhile, GDH aminating activity was low at 10 – 15 or 20 DAA and high at 20 or 25 – 30 DAA, indicating that GDH aminating activity increased gradually with grain development. In MP and HP, GDH aminating activity was significantly higher than that of deaminating activity in Xi'nong 979 at 10 – 30 DAA and Zhengmai 103 at 21 and 28 DAA, which indicated that GDH mainly assimilated ammonia under powdery mildew stress.

For Xi'nong 979, GDH aminating activity increased significantly with the increase of DI (HP > MP > CK) during 10 – 30 DAA in both years, which was consistent with the expression of *GDH* (Figure 2). For Zhengmai 103, GDH aminating activity changed little among different treatments at 10 – 20 DAA, which was due to almost no change in its DI. However, it increased significantly with the increase of DI at 25 and 30 DAA (HP > MP > CK). Thus, both mild and severe powdery mildew could induce GDH aminating activity, similar to the induction of *GDH* expression. The deaminating activity of Xi'nong 979 increased (10 – 15 DAA) with the increase of DI, and then decreased (20 – 30 DAA) (Figure 3). For Zhengmai 103, there was no significant difference in GDH deaminating activity among three treatments at 10 – 20 DAA, but it increased with the increase of DI at 25 – 30 DAA. Therefore, the change of GDH deaminating activity was consistent with *GDH* expression when the DI of powdery mildew was low, but it was inconsistent when the DI was high. As for the ratio of GDH aminating activity to deaminating activity, there was no significant difference at 10 – 15 DAA (in Xi'nong 979) and 10 – 20 DAA (in Zhengmai 103), and then it increased with the increase of DI. The results showed that *Bgt* infection induced *GDH* expression and aminating activity.

4. Conclusion

Whether under natural or powdery mildew stress conditions, the deaminating activity of GDH in wheat grains was very low, while the aminating activity of GDH was high, so the main function of GDH was to assimilate ammonia. Under powdery mildew stress conditions, the GDH aminating activity and *GDH* expression level of wheat grains were significantly increased.

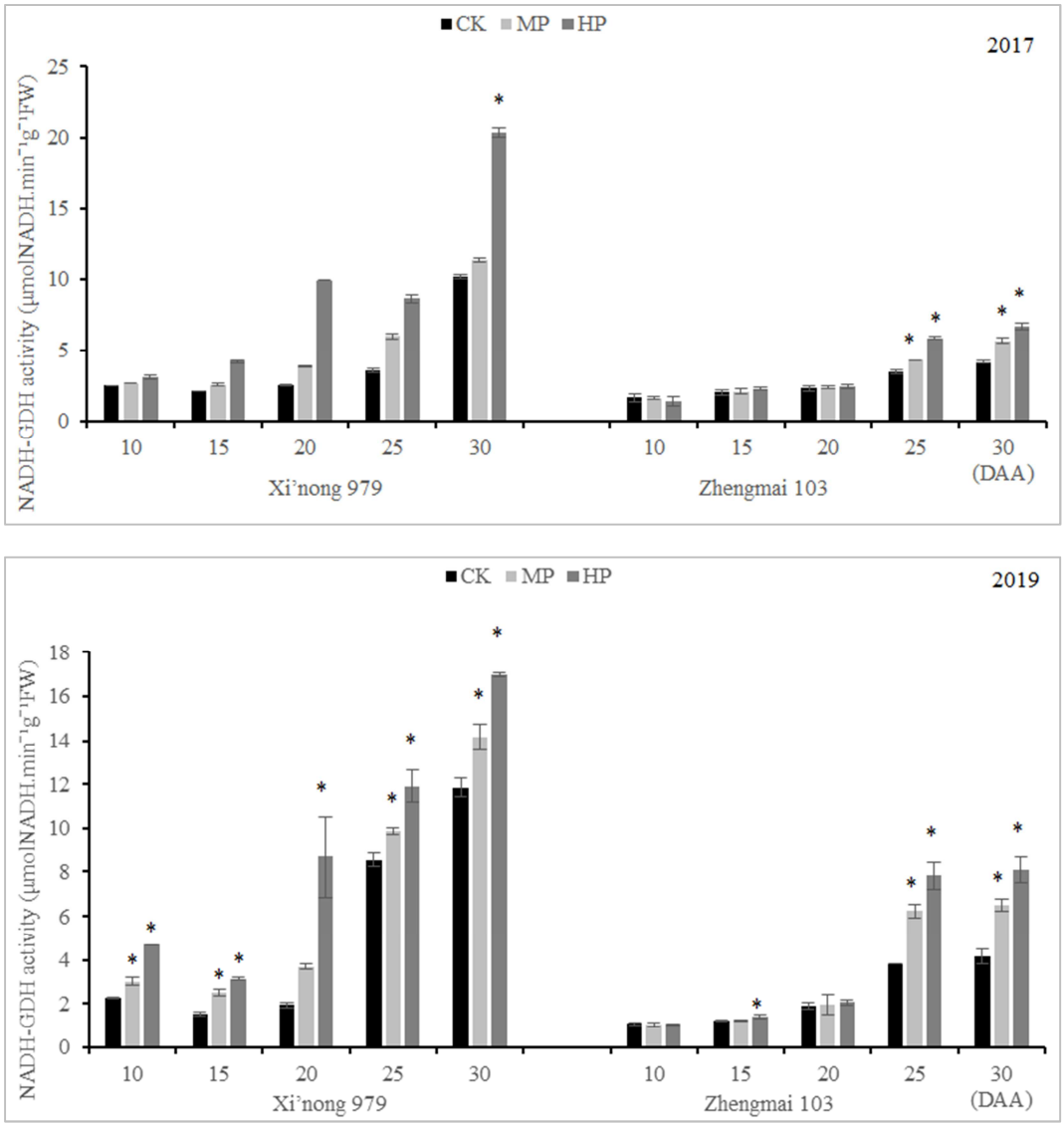
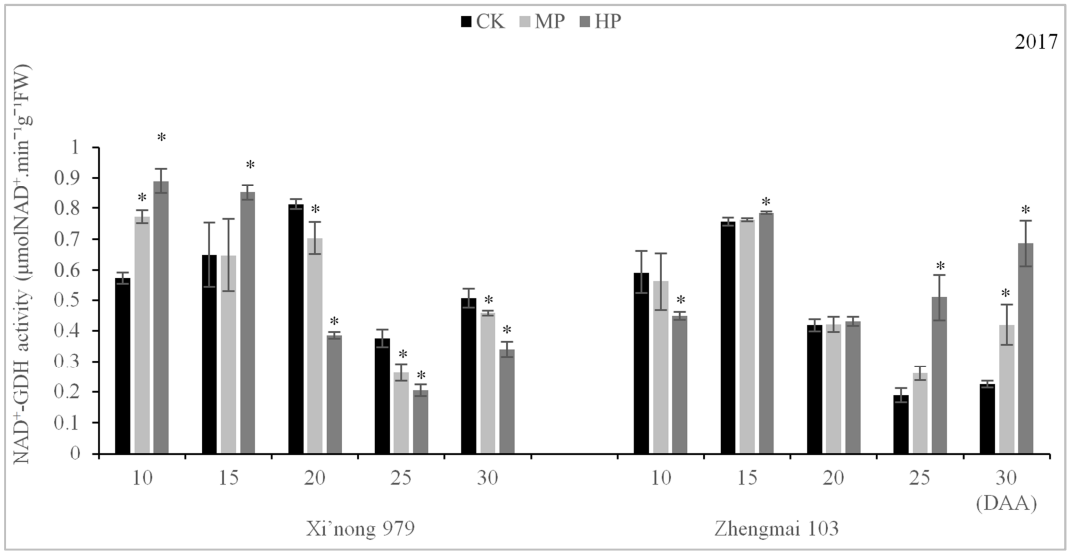


Figure 2. *NADH-dependent glutamate dehydrogenase (GDH) aminating activity in grains of Xi'nong 979 (susceptible) and Zhengmai 103 (resistant) in response to inoculation of Blumeria graminis f. sp. tritici in two years. CK, no inoculation; MP and HP, one and nine inoculations of Blumeria graminis f. sp. tritici, respectively. Error bars represent S. E. M. (n=3) "*" indicates a significant level at the 0.05 level.*



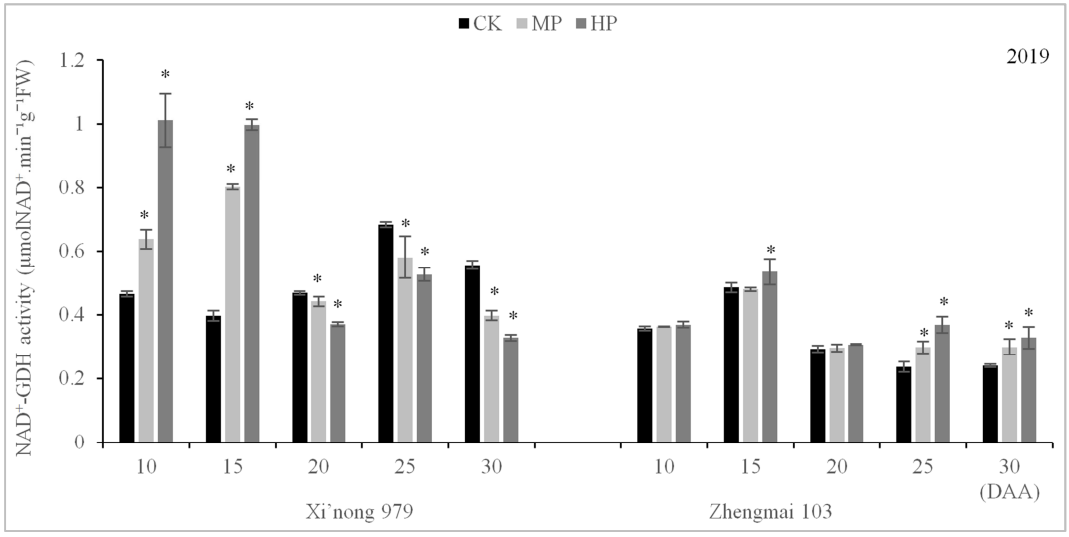


Figure 3. *NAD^+-dependent glutamate dehydrogenase (GDH) deaminating activity in grains of Xi'nong 979 (susceptible) and Zhengmai 103 (resistant) in response to inoculation of Blumeria graminis f. sp. tritici in two years. CK, no inoculation; MP and HP, one and nine inoculations of Blumeria graminis f. sp. tritici, respectively. Error bars represe nt S. E. M. (n=3) "*" indicates a significant level at the 0.05 level.*

5. Discussion

In this study, in CK (natural state), GDH aminating activity was higher than that of deaminating activity during grain development of Zhengmai 103, indicating that the main function of GDH in vivo was to synthesize Glu by amination reaction. Meanwhile, GDH aminating activity was low at 10 – 15 or 20 DAA and high at 20 or 25 – 30 DAA, indicating that GDH aminating activity increased gradually with grain development. One study of soybean also indicated that GDH activity was 12- to 50-fold higher in the direction of amination than in the direction of the deamination reaction [21]. Recently, it was found that GDH aminating activity in wheat grains increased continuously from 8 DAA [22], which was similar to the present study.

In MP and HP, DI of Xi'nong 979 at 7 and 14 DAA, and Zhengmai 103 at 21 and 28 DAA were lower (under mild powdery mildew), and they were under mild powdery mildew conditions. Their *GDH* expression, aminating and deaminating activities were induced, indicating that mild powdery mildew could induce *GDH* expression and activity. In MP and HP, Xi'nong 979 had higher DI at 21 and 28 DAA, and was under severe powdery mildew conditions. Its *GDH* expression and GDH aminating activity significantly increased, while GDH deaminating activity decreased. It indicated that severe powdery mildew could induce *GDH* expression and aminating activity, and inhibit deaminating activity. Pathogenic viruse, *Pseudomonas syringae* pv. *syringae* and pv. *tabaci* strains inoculated tobacco plants also showed that both *GDH* expression and GDH aminating activity were induced in infection leaves [7]. Under other stress conditions such as drought, high salinity and high alkalinity, GDH aminating activity also increased, and GS and/or GOGAT activity decreased [8, 11, 12]. It is suggested that the enhancement of GDH aminating activity, which plays an auxiliary role in N metabolism, is a kind of compensation for the weakening of GS/GOGAT cycle under various biotic and abiotic stress conditions [8-12]. In wheat grains, powdery mildew inhibited GS/GOGAT cycle [16]. In the present study, results showed that *GDH* expression and aminating activity increased significantly with the increase of DI under powdery mildew stress, indicating that GDH aminating activity was significantly induced. The induction of GDH was an important complement to N metabolism.

Authors' Contribution

Hongyun Gao designed and carried out experiments, wrote the manuscript. Jishan Niu revised the manuscript. Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare there are no conflicts of interest.

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