Genomic identification, Characterization and Expression Analysis of Glyoxalase Gene Family in *Brassica Napus* L.

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Abstract

Background Glyoxalase I (GLYI) and glyoxalase II (GLYII), two enzymes of the glyoxalase pathway, are responsible for the detoxification of a cytotoxic metabolite methylglyoxal (MG) into the nontoxic S-D-lactoylglutathione, which play crucial roles in stress tolerance in various plant species. Considering the roles of glyoxalases, the *GLY* gene families have been analyzed in higher plant, such as rice, soybean and Chinese cabbage; however, little is known about them in rapeseed (*Brassica napus* L.). The *B. napus* glyoxalase pathway is worth an in-depth investigation about the presence, distribution, localizations, and expression of glyoxalase genes.

Result In this study, a total of 35 *BnaGLYI* and 30 *BnaGLYII* genes were identified in the *B. napus* genome, and they were clustered into six and eight subfamilies, respectively. The classifications, chromosomal distributions, gene structures, and conserved motifs were predicted and analyzed. Importantly, these genes were mainly localized in chloroplast and cytoplasm. Moreover, their expression profiles varied among different tissues. For example, *BnaGLY* genes are expressed in most organs but tend to be highly expressed in a single organ, such as *BnaGLYI27* and *BnaGLYII19*; while some members are only expressed in specific tissues, e.g., *BnaGLYI32* only expressed in silique pericarps. Some genes were induced at different germination stages. Notably, a number of *BnaGLY* genes showed responses to *Plasmodiophora brassicae* infection.

Conclusion This study systematically identifies *BnaGLYI* and *BnaGLYII* gene families in *B. napus*. The different sub-cellular organelles and expression analysis offer insight into their biological roles and function in plant development and stress resistance.

Background

Glyoxalase pathway, the dominant methylglyoxal (MG) scavenging pathways of plant, includes two enzymes, GLYI and GLYII[1]. GLYI catalyses the MG conversion into S-D-lactoylglutathione in the presence of reduced glutathione (GSH) [2]. Subsequently, GLYII hydrolyzes S-D-lactoylglutathione into non-toxic D-lactic acid and GSH [3]. The depletion of GSH and the activity of GLYI lead to the toxicity of MG[4]. While, GLYII is a metal-beta-lactamase family, which is the second rate-limiting enzyme in the system[5]. Lack of GLYII leads to the accumulation of lactoylglutathione. Recently, the third enzyme GLYIII, found to convert MG directly into D-lactose, is far less efficient than GLYI and GLYII enzymes [6].

In plants, the roles of GLY system in resistance to biotic and abiotic stresses have been getting more attention recently. More reports are about the role of *GLY* system in resisting salt, extreme temperature and heavy metal stresses [7–11]. Previous studies found that the *GLYI* and *GLYII* can be induced by stresses at different levels. The transcript expression level of *GLYII* in *Brassica juncea* L. was significantly increased under zinc stress[12]. GLYI protein in rice roots at seedling stage was significantly induced by low temperature stress[13]. The protein level of GLYI in tomato roots was significantly increased under heavy metal Al stress[14]. Similarly, GLYI and GLYII activities in onion were induced by low temperature[15]. The level of transcript, protein, and specific activity of GLYI was markedly enhanced under water stress [7]. In addition to abiotic stress responses, *GLY* genes are also regulated by biotic stresses. The expression of *GLYI* was induced in *Brassica* after infection by *Sclerotinia sclerotiorum* [16]. In *B. rapa*, *BrGLYI1*, *BrGLYI6*, *BrGLYI11* and *BrGLYI16* were up-regulated after inoculation of *Plasmodiophora brassicae* in both clubroot-resistant and susceptible lines. *BrGLYII13* was more highly induced in the resistant line than susceptible line at 12 hour after inoculation [17]. Overall, the enzyme system is considered as a biomarker of plant stress tolerance[18].

Plants product MG under stress conditions [19]. High concentration of MG can impair plant cells or cell components and can even destroy DNA or cause mutation, resulting in the death of plant cells and tissues [11]. Overexpression or higher activity of glyoxalase enzymes eliminate MG toxicity and confer stress tolerance [18]. For example, overexpression of the *GLYI* gene in tobacco showed better stress tolerance than the normal plant to MG and high salinity [8]. The transgenic mustard overexpressing of *GLYI* gene enabled the tolerance to salt, heavy metals and drought stresses[19]. Overexpressing of wheat *GLYI* gene also enhanced the tobacco tolerance to $ZnCl_2$ [20]. Double transgenic tomato plants had also been raised harboring the glyoxalase pathway, which showed improved salinity resistance, probably by decreasing the oxidative stress [10]. The transgenic tobacco overexpressing of both *GLYI* and *GLYII* genes showed significantly resistance to $ZnCl_2$ [21]. Therefore, over-expression of the glyoxalase pathway or individual genes harbors the potential to confer tolerance to multiple stresses. Moreover, *GLYI* and *GLYII* genes were present as multi-gene families. To date, genome-wide identification of glyoxalase family has been carried out in six plant genomes *Arabidopsis thaliana*, *Oryza sativa*, *Glycine max*, *Medicago truncatula*, *Brassica rapa* and *Vitis vinifera* L. [17, 22–26]. There are at least 11 *GLYI*/homologous genes in *A. thaliana* and rice, twenty-four *GLYI* members in soybean genome, and 15 in *B. rapa*. According to *GLYII* genes, five *GLYII* genes are in *A. thaliana*, three in rice, twelve *GLYII* members in soybean genome, and 16 *GLYII* in *B. rapa* [17, 23]. Moreover, some *GLYI* and *GLYII* family genes have been proved to play an important role in response to drought, heavy metals and other stress responses [27–29].

Rapeseed (*Brassica napus* L.) is one of the most important oil crops in the world. It not only provides high quality edible oil for human beings, but also is an important industrial raw material. However, rapeseed is often severely damaged by adverse stresses, such as low temperature, pathogen infection and so on. All these stresses severely affect the overall development stages of rapeseed, which not only seriously reduce the yield, but also threatens the health of animals and human beings when it enters the food chain[30]. Thus, exploring tolerant genes in rapeseed is of great theoretical and practical significance for the cultivation of resistant varieties. *GLYI* and *GLYII* family genes as biomarkers for plant stress resistance have not been analyzed in *B. napus*. Here, the members of *GLYI* and *GLYII* families were identified from the *B. napus* database by bioinformatics analysis, and their genetic structure, evolutionary relationships, conserved motifs, chromosome localization and expression in different tissues and response to stresses were also studied. The study will provide an important basis for clarifying the evolutionary level and functional differentiation of *GLYI* and *GLYII* family genes in *Brassica* crops, and laid a theoretical foundation for further understanding the response mechanisms of stress in *Brassica*.

Results

Identification of GLYI and GLYII genes in the *B. napus* genome

Glyoxalase proteins (GLYI and GLYII) had been identified in Brassicaceae plant *A. thaliana* and *B. rapa*. Thus, *AtGLYI//AtGLYII* and *BrGLYI//BrGLYII* sequences were used as quires to blast genome database of *B. napus* and the sequences with E-value under 1E-10 were screened out. Then proteins that included the glyoxalase domain (PF00903) and the metallo-beta-lactamase domain (PF00753) were classified as BnaGLYI and BnaGLYII proteins, respectively. In this study, thirty-five *BnaGLYI* and 30 *BnaGLYII* genes were identified in *B. napus*. Table 1 summarized the gene ID, chromosomal distribution, number of introns, CDS and amino acid length, predicted sub-cellular localization, calculated molecular weight, and isoelectric points of the putative *BnaGLYI* and *BnaGLYII*.

Table 1
Detail information of *BnaGLYI* genes identified in *Brassica napus* L. genomes

Gene Symbol	Locus Identifier	Location	Gene Start(bp)	Gene Stop(bp)	Strand	No. of Introns	CDS Length(bp)	PP Length(aa)	MW(kDa)	pI	Localization
BnaGLYI01	BnaA02g19970D	A02	12337272	12338438	-	2	504	168	18818.68	5.81	Cy ^a , Nu ^b
BnaGLYI02	BnaA03g10440D	A03	4700661	4701804	+	3	582	194	21785.91	8.43	Cy ^a , Mt ^b
BnaGLYI03	BnaA05g35240D	A05	670044	672049	-	3	414	138	15252.22	5.46	Cy ^a , Nu ^b
BnaGLYI04	BnaA06g04170D	A06	2547123	2548306	-	3	516	172	19274.7	7.77	Cy ^a , Ch ^b
BnaGLYI05	BnaA06g04580D	A06	2708014	2710119	+	7	714	238	26635.17	8.33	Cy ^a ;Om ^a ,Ch ^{b,c}
BnaGLYI06	BnaA06g07360D	A06	3915659	3923909	+	26	3648	1216	134984.62	8.86	Cy ^{a,b}
BnaGLYI07	BnaA06g10060D	A06	5342612	5343786	+	2	525	175	19778.79	5.68	Cy ^a , Ch ^b
BnaGLYI08	BnaA07g13890D	A07	12263440	12263994	+	0	555	185	20887.37	4.78	Cy ^{a,b}
BnaGLYI09	BnaA07g26290D	A07	19358162	19361477	-	8	1026	342	37894.44	6.48	Cy ^a , Ch ^{b,c}
BnaGLYI10	BnaA08g23870D	A08	16843513	16844540	-	2	525	175	19846.96	5.88	Cy ^a , Ch ^b
BnaGLYI11	BnaA08g25110D	A08	17357805	17359871	-	7	852	284	31872.41	5.26	Cy ^{a,b}
BnaGLYI12	BnaA09g49270D	A09	32814199	32815336	+	2	414	138	15394.22	5.84	Cy ^a , Ch ^b
BnaGLYI13	BnaA09g49870D	A09	33093200	33095241	+	1	1323	441	48072.12	5.72	Cy ^a ;Om ^a ,Cysk
BnaGLYI14	BnaA09g56790D	A09	3889968	3891223	+	2	504	168	18943.89	5.86	Cy ^a , Ch ^b
BnaGLYI15	BnaA10g04310D	A10	2272334	2273834	-	1	1338	446	48973.02	5.45	Om ^a , Cy ^b
BnaGLYI16	BnaA10g11070D	A10	9346288	9347612	-	3	588	196	21959	6.71	Cy ^a , Mt ^b
BnaGLYI17	BnaC02g23290D	C02	20281012	20282133	-	2	504	168	18762.53	5.66	Cy ^a , Nu ^b
BnaGLYI18	BnaC02g46640D	C02	2394713	2395834	+	2	504	168	18804.61	5.66	Cy ^a , Nu ^b
BnaGLYI19	BnaC03g13130D	C03	6313042	6314254	+	3	582	194	21712.86	7.77	Cy ^a , Ch ^b
BnaGLYI20	BnaC03g51010D	C03	35479070	35480133	-	4	453	151	16992.13	5.25	Cy ^a , Nu ^b
BnaGLYI21	BnaC05g04530D	C03	2231485	2233489	-	1	1338	446	48857.97	5.65	Cy ^{a,b} ,Om ^c
BnaGLYI22	BnaC05g05340D	C05	2609787	2610529	-	2	417	139	15596.41	5.94	Cy ^{a,b}
BnaGLYI23	BnaC05g05770D	C05	2848240	2850316	+	7	708	236	26438.95	8.74	Cy ^a ; Pe ^a ,Ch ^{b,c}
BnaGLYI24	BnaC05g08770D	C05	4659651	4665014	+	19	2175	725	80672.51	7.49	Cy ^{a,b} ,Om ^c
BnaGLYI25	BnaC05g11680D	C05	6800739	6802181	+	2	525	175	19733.75	5.68	Cy ^a , Ch ^b
BnaGLYI26	BnaC06g28360D	C06	29576211	29578606	-	8	1038	346	38368.97	6.19	Cy ^a ,Ch ^{b,c}
BnaGLYI27	BnaC08g15100D	C08	19658835	19661229	+	7	852	284	31814.27	5.26	Cy ^{a,b}
BnaGLYI28	BnaC08g16660D	C08	20576966	20578018	+	2	525	175	19821.86	5.89	Cy ^a , Ch ^b
BnaGLYI29	BnaC08g38920D	C08	34930459	34931660	-	2	522	174	19568.62	5.86	Cy ^a , Mt ^b
BnaGLYI30	BnaC08g44820D	C08	37849659	37851160	-	1	1323	441	48162.21	5.46	Cy ^a , Cysk ^b

Abbreviations: CDS: coding DNA sequence, PP: polypeptide length, MW: molecular weight, PI: isoelectric point, bp: base pair, aa: amino acid, kDa: kilodalton, Ch: chloroplast, Cy: cytosol, Mt: mitochondria, Nu: nucleus, Pe: periplasm, Ou: outer membrane, Ec: extracellular matrix.

a Localization prediction by pSORT (<http://www.genscript.com/wolf-psort.html>)

b Localization prediction by TargetP 1.1 Server (<http://www.cbs.dtu.dk/services/TargetP/>)

c Chloroplast localization signal confirmed by ChloroP (<http://www.cbs.dtu.dk/services/ChloroP/>)

Gene Symbol	Locus Identifier	Location	Gene Start(bp)	Gene Stop(bp)	Strand	No. of Introns	CDS Length(bp)	PP Length(aa)	MW(kDa)	pI	Localizati
BnaGLYI31	BnaC09g53670D	C09	3644531	3644929	+	0	399	133	15196.72	7.77	Cy ^{a, b}
BnaGLYI32	BnaCnng38880D	Cnn	37521585	37522696	-	2	414	138	15502.36	6.2	Cy ^a ; Ch ^b
BnaGLYI33	BnaCnng47290D	Cnn	46764251	46765432	-	5	432	144	16180.32	5.39	Cy ^{a, b}
BnaGLYI34	BnaCnng47280D	Cnn	46758036	46759221	-	5	324	108	12264.1	5.01	Cy ^a ; Nu ^b
BnaGLYI35	BnaCnng59150D	Cnn	58862580	58863862	-	3	414	138	15253.16	5.45	Cy ^a ; Nu ^b
Abbreviations: CDS: coding DNA sequence, PP: polypeptide length, MW: molecular weight, PI: isoelectric point, bp: base pair, aa: amino acid, kDa: kilodalton, Ch: chloroplast, Cy: cytosol, Mt: mitochondria, Nu: nucleus, Pe: periplasm, Ou: outer membrane, Ec: extracellular matrix.											
a Localization prediction by pSORT (http://www.genscript.com/wolf-psort.html)											
b Localization prediction by TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/)											
c Chloroplast localization signal confirmed by ChloroP (http://www.cbs.dtu.dk/services/ChloroP/)											

The CDS length of *BnaGLYI* members ranged from 324 bp (*BnaGLYI34*) to 3648 bp (*BnaGLYI06*), and the average CDS length is 785 bp. Accordingly, *BnaGLYI06* encodes for the largest protein with a polypeptide length of 1216 aa and molecular weight of 134.9 kDa; while *BnaGLYI34* encodes for the smallest protein with a polypeptide length of 108 aa and molecular weight of 12.2 kDa (Table 1). The isoelectric point (pI) of *BnaGLYI* proteins vary from 4.78 (*BnaGLYI08*) to 8.86 (*BnaGLYI06*). Most of the *BnaGLYI* members showed acidic pI value, and only four genes (*BnaGLYI02*, *BnaGLYI05*, *BnaGLYI06* and *BnaGLYI23*) showed obvious alkalinity pI value (around 8.0) (Table 1). Moreover, there were also three members (*BnaGLYI04*, *BnaGLYI19* and *BnaGLYI31*) showing basic pI value (Table 1). Sub-cellular localization of all identified *BnaGLYI* proteins were analyzed using three tools (CELLO, Wolf-pSORT and ChloroP). The results predicted that almost all *BnaGLYI* expect *BnaGLYI15* were localized in cytoplasm. However, the Wolf-pSORT predicted that most of *BnaGLYI* were localized in the chloroplast, followed by the cytoplasm, nucleus, mitochondrion and cytoskeleton (Table 1).

Similarly, the CDS length of *BnaGLYII* genes also had very wide ranges, from 777 bp (*BnaGLYII09*) to 5745 bp (*BnaGLYII24*). Accordingly, *BnaGLYII09* was the smallest protein (259 aa, 28.57 kDa); while *BnaGLYII24* was the largest protein (1915 aa, 212.36 kDa) (Table 2). The pI value of *BnaGLYII* proteins varied from 5.21 (*BnaGLYII29*) to 9.04 (*BnaGLYII04*). Most of *BnaGLYII* proteins showed alkalinity with pI value around or more than 7.0, and only seven *BnaGLYII* proteins had an acidic pI value, including *BnaGLYII09*, *BnaGLYII12*, *BnaGLYII13*, *BnaGLYII15*, *BnaGLYII17*, *BnaGLYII29* and *BnaGLYII30* (Table 2). Sub-cellular locations of *BnaGLYII* proteins were different from *BnaGLYI* proteins. *BnaGLYII* proteins members were localized in different sub-cellular compartments, such as cytoplasm, chloroplast, extracellular matrix, outer membrane, periplasm, nucleus and mitochondrion (Table 2).

Table 2
Basic characteristics of *BnaGLYI* genes identified in *Brassica napus* L. genomes

Gene Symbol	Locus Identifier	Location	Gene Start (bp)	Gene Stop (bp)	Strand	No. of Introns	CDS Length (bp)	PP Length (aa)	MW (kDa)	pI	Localization
BnaGLYII01	BnaA01g03370D	A01	1597636	1600850	-	10	1056	352	39338.98	7.93	Cy ^a ; Ch ^{b,c}
BnaGLYII02	BnaA02g25890D	A02	18962115	18965111	+	12	1848	616	68076.64	6.62	Cy ^{a,b}
BnaGLYII03	BnaA03g14380D	A03	6611723	6613870	+	7	987	329	36085.29	8.81	Cy ^a ; Ch ^{b,c}
BnaGLYII04	BnaA03g20230D	A03	9624333	9627034	-	8	1026	342	37496.84	9.04	Ec ^a ; Ch ^{b,c}
BnaGLYII05	BnaA03g33050D	A03	15994860	15997418	+	10	1074	358	39988.96	7.17	Cy ^a ; Ch ^{b,c}
BnaGLYII06	BnaA04g25070D	A04	18183840	18186578	-	7	993	331	36397.62	8.82	Ec ^a ; Ch ^{b,c}
BnaGLYII07	BnaA05g03320D	A05	1854330	1860877	-	20	2868	956	106401.82	8.51	Ou ^a ; Ch ^{b,c}
BnaGLYII08	BnaA05g11390D	A05	6376663	6378817	-	7	981	327	35883.92	8.07	Pe ^a ; Ch ^{b,c}
BnaGLYII09	BnaA05g28080D	A05	20057585	20059255	+	6	777	259	28689.47	6.1	Pe ^a ; Cy ^b
BnaGLYII10	BnaA06g00850D	A06	603532	606586	+	8	1167	389	43530.78	7.74	Cy ^a ; Pe ^a ; Nu ^b
BnaGLYII11	BnaA06g22690D	A06	15848234	15855367	+	23	3450	1150	126907.01	9.02	Cy ^a ; Ch ^{b,c}
BnaGLYII12	BnaA08g01070D	A08	806559	808627	+	7	786	262	28574.49	6.19	Pe ^a ; Nu ^b
BnaGLYII13	BnaA09g28480D	A09	21351908	21354940	-	11	1566	522	57465.81	6.19	Cy ^{a,b}
BnaGLYII14	BnaA09g50050D	A09	33176404	33178430	+	7	969	323	35263.31	7.16	Cy ^a ; Ch ^{b,c}
BnaGLYII15	BnaAnng27360D	Ann	31283476	31286304	-	4	2085	695	77448.79	5.81	Cy ^a ; Nu ^b
BnaGLYII16	BnaC01g04650D	C01	2443496	2446457	-	10	1059	353	39822.41	6.96	Cy ^a ; Ch ^{b,c}
BnaGLYII17	BnaC01g28980D	C01	27137154	27139952	-	4	2076	692	77157.53	5.81	Cy ^a ; Mt ^b
BnaGLYII18	BnaC02g47740D	C02	3827265	3830275	+	12	1848	616	68049.53	6.43	Cy ^{a,b}
BnaGLYII19	BnaC03g17440D	C03	8920776	8922877	+	7	975	325	35687.84	8.85	Cy ^a ; Mt ^b ; Ch ^c
BnaGLYII20	BnaC03g24200D	C03	13557749	13560223	-	7	981	327	35840.97	8.85	Ec ^a ; Pe ^a ; Ch ^{b,c}
BnaGLYII21	BnaC03g38140D	C03	23395036	23397633	+	10	1077	359	39993.97	7.14	Cy ^a ; Ch ^{b,c}
BnaGLYII22	BnaC03g50930D	C03	35336691	35341520	-	16	2682	894	98725.64	8.7	Cy ^a ; Ch ^{b,c}
BnaGLYII23	BnaC03g69760D	C03	59489758	59491865	-	7	870	290	31928.74	8.57	Cy ^a ; Ch ^{b,c}
BnaGLYII24	BnaC04g02920D	C04	2066379	2078825	-	31	5745	1915	212357.58	8.24	Ou ^a ; Ch ^{b,c}
BnaGLYII25	BnaC04g48930D	C04	47330538	47333344	-	7	996	332	36386.59	8.7	Ec ^a ; Ch ^{b,c}
BnaGLYII26	BnaC05g20710D	C05	14195563	14198830	+	11	1566	522	57524.85	6.47	Cy ^{a,b}
BnaGLYII27	BnaC06g06420D	C06	6895047	6896947	-	7	909	303	33196.01	7.21	Pe ^a ; Ch ^{b,c}
BnaGLYII28	BnaC08g44630D	C08	37747241	37749209	-	7	984	328	35631.65	6.83	Pe ^a ; Ch ^{b,c}
BnaGLYII29	BnaCnng30690D	Cnn	29167588	29174293	+	10	2448	816	93535.33	5.21	Cy ^{a,b}

Abbreviations: CDS: coding DNA sequence, PP: polypeptide length, MW: molecular weight, PI: isoelectric point, bp: base pair, aa: amino acid, kDa: kilodalton, Ch: chloroplast, Cy: cytosol, Mt: mitochondria, Nu: nucleus, Pe: periplasm, Ou: outer membrane, Ec: extracellular matrix.

a Localization prediction by pSORT (<http://www.genscript.com/wolf-psort.html>)

b Localization prediction by TargetP 1.1 Server (<http://www.cbs.dtu.dk/services/TargetP/>)

c Chloroplast localization signal confirmed by ChloroP (<http://www.cbs.dtu.dk/services/ChloroP/>)

Gene Symbol	Locus Identifier	Location	Gene Start (bp)	Gene Stop (bp)	Strand	No. of Introns	CDS Length (bp)	PP Length (aa)	MW (kDa)	pI	Localization
BnaGLYII30	BnaCnng46950D	Cnn	46379687	46381389	-	6	777	259	28653.35	5.72	Pe ^a ; Ch ^b ; c
Abbreviations: CDS: coding DNA sequence, PP: polypeptide length, MW: molecular weight, PI: isoelectric point, bp: base pair, aa: amino acid, kDa: kilodalton, Ch: chloroplast, Cy: cytosol, Mt: mitochondria, Nu: nucleus, Pe: periplasm, Ou: outer membrane, Ec: extracellular matrix.											
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Chromosome localization and gene duplication analysis of BnaGLYI and BnaGLYII

In order to get a more intuitive view of the distribution of *GLY* genes in *B. napus* chromosomes, the information of their starting and ending position in chromosomes were collected (Table 1), and chromosome distribution map was drawn on the MG2C (Map Gene2 Chromosome v2) website (Fig. 1). For *BnaGLYI* gene family, there were 31 genes were unevenly distributed on 14 chromosomes and no genes were found on chromosomes A01, A04, C01, C04 and C07. Four genes (*BnaGLYI32-35*) were located in the unmapped scaffolds in the Ann_random and Cnn_ random genome (Fig. 1a). Chromosomes A06, C05 and C08 contained the maximum number of *BnaGLYI* genes, four genes each; while five chromosomes (A02, A03, A05, C06 and C09) had only one *BnaGLYI* gene (Fig. 1a). Totally, 16 *BnaGLYI* genes distributed on the An-subgenome and 19 were from the Cn-subgenome. Similarly, it has been found that 27 *BnaGLYII* genes were unevenly located in 15 different chromosomes (Fig. 1b). There were four chromosomes that contained no *BnaGLYII* genes (A07, A10, C07 and C09) (Fig. 1b). Chromosome C03 harbored five *BnaGLYII* genes, which contained most *BnaGLYI* genes. However, most of chromosomes, like A01, A02, A04, A08, C02, C05, C06 and C08, contained only one *BnaGLYII* gene (Fig. 1b). The remaining three genes could not be mapped into chromosomes of the rapeseed genome, which were distributed on Ann and Cnn (Fig. 1b). Moreover, BnaGLYII proteins were mainly localized in the chloroplast and cytosol.

Gene replication is the main way of gene family expansion. This study also analyzed the gene replication of *GLY* gene family in *B. napus*. Among BnaGLYI proteins, there were 15 pairs duplicated genes. Three genes categorized into one group that formed three gene pairs (*BnaGLYI01/17/18*). Other gene pairs all included two genes (*BnaGLYI02/19*, *BnaGLYI03/35*, *BnaGLYI04/22*, *BnaGLYI05/23*, *BnaGLYI07/25*, *BnaGLYI09/26*, *BnaGLYI10/28*, *BnaGLYI11/27*, *BnaGLYI12/32*, *BnaGLYI13/30*, *BnaGLYI14/29*, *BnaGLYI15/21*). Moreover, it was found that nine gene pairs with over 95% sequence similarity in BnaGLYII proteins. They were *BnaGLYII01/16*, *BnaGLYII02/18*, *BnaGLYII03/19*, *BnaGLYII04/20*, *BnaGLYII05/21*, *BnaGLYII06/24*, *BnaGLYII08/29*, *BnaGLYII11/22*, *BnaGLYII12/23*, respectively (Fig. 1b). Such high level of sequence similarities implied the possibility of segmental duplication of those genes. And there were no tandem duplication found in both the BnaGLYI and BnaGLYII proteins.

Gene structure and phylogenetic analysis of the BnaGLYI and BnaGLYII

To further investigate the gene structure, the CDS and genomic sequences of the *BnaGLY* genes were aligned. It was shown that one to 26 introns varied from *BnaGLYI* genes and there were no introns in the *BnaGLYI08* and *BnaGLYI31* (Fig. 2, Table 1). *BnaGLYI13*, *BnaGLYI15*, *BnaGLYI21* and *BnaGLYI30* only contained one intron. *BnaGLYI06* gene had the largest number of introns (26). Similarly, the *BnaGLYII* genes also contained varied numbers of introns (4–31); e.g., thirty-one introns were identified in *BnaGLYII17*, and 4 introns were predicted in *BrGLYII24* (Fig. 2, Table 2). In addition, the GLY proteins that clustered in the same group possessed a similar structure.

To understand the evolutionary relationships and functions among the predicted BnaGLY proteins, a phylogenetic tree was drawn with all BnaGLY protein sequences by MEGA6.0. The 35 BnaGLY proteins were resolved into six distinguishing groups (group A-F; Fig. 3a). The largest clade Group F, containing 31 GLYI members, included 19 GLYI in Brassicaceae; whereas the smallest group (Clade B) containing 10 members, included 6 GLYI in Brassicaceae (Fig. 3a). AtGLYI4 and AtGLYI7 were in group F, whose transcription level could be induced by osmotic, extreme temperature and wounding [23]. BnaGLYI01 showed a high similarity to AtGLYI7. It was hypothesized that BnaGLYI01 may play similar roles in the response to stresses. Clade A included 15 members of Brassicaceae, one protein from Arabidopsis and one protein from rice. Group C included 27 proteins, which contained 12 members of Brassicaceae (six *BnaGLYI*, three *BnaGLYI* and three *BolGLYI*). Group D and E contained 12 and 14 members, respectively. Generally, BnaGLYI members first clustered with proteins from *B. rapa* and *B. oleracea*, followed by AtGLYIs, forming a small Brassicaceae clade, and subsequently further clustered with GLYI members from rice (Fig. 3a). It was reported that *OsGlyI* overexpressing rice line improved tolerance to NaCl, ZnCl₂ and mannitol, and performed higher seed setting rate and yield compared to wild-type plants[31]. In Group F, BnaGLYI01, BnaGLYI17 and BnaGLYI18 that were highly similar to *OsGlyI* may have the similar roles in stress response.

Similarly, the BnaGLYII proteins formed eight distinct clades (Fig. 3b). Two BnaGLYII proteins were clustered in groups A, B, C, D, and F, respectively; four BnaGLYII proteins were classified in group E and G, and twelve BrGLYII proteins were in Group F (Fig. 3b).

Ka/Ks Ratio of BnaGLY genes

To explore the selective pressure on *BnaGLY* genes, we calculated the non-synonymous/synonymous mutation ratio (K_a/K_s); $K_a/K_s > 1$ indicates positive selection, $K_a/K_s = 1$ indicates neutral selection, and $K_a/K_s < 1$ indicates purifying selection[32]. The K_a/K_s ratio for 10 *BnaGLYI* genes was > 1 , ranging from 1.0664 (*BnaGLYI11*) to 6.3118 (*BnaGLYI02*), which indicated that these genes were experienced higher positive selection pressure during the evolutionary history of *B. napus*. The other genes showed $K_a/K_s < 1$, which experienced higher purifying selection pressure (Table 3). Moreover, most of the *BnaGLYII* genes had a $K_a/K_s < 1$, only five *BnaGLYII* genes experienced positive selection pressure (Table 4). A comparison of K_a/K_s ratios among the *BnaGLYI* and *BnaGLYII* indicated that the average K_a/K_s ratio of *BnaGLYI* was higher than that of *BnaGLYII* genes. These results suggested that the *BnaGLYI* gene family may have suffered robust purifying selective pressure during evolution.

Table 3
Ka and Ks values for *BnaGLYI* paralogous gene pairs

No	Locus 1	Locus 2	Ks	Ka	Ka/Ks	Selection pressure	Average Ka/Ks
1	BnaGLYI11	BnaGLYI27	0.0482	0.0514	1.0664	Positive selection	1.8559
2	BnaGLYI14	BnaGLYI29	0.0977	0.0384	0.3930	Purifying selection	
3	BnaGLYI30	BnaGLYI15	0.036	0.1499	4.1639	Positive selection	
4	BnaGLYI30	BnaGLYI21	0.0362	0.1173	3.2403	Positive selection	
5	BnaGLYI32	BnaGLYI04	0.0744	0.1663	2.2352	Positive selection	
6	BnaGLYI32	BnaGLYI22	0.0728	0.1182	1.6236	Positive selection	
7	BnaGLYI03	BnaGLYI35	0.079	0.0134	0.1696	Purifying selection	
8	BnaGLYI33	BnaGLYI05	0.1198	0.2261	1.8873	Positive selection	
9	BnaGLYI33	BnaGLYI23	0.1184	0.2442	2.0625	Purifying selection	
10	BnaGLYI19	BnaGLYI02	0.0418	0.013	0.3110	Purifying selection	
11	BnaGLYI07	BnaGLYI10	0.2567	0.1386	0.5399	Purifying selection	
12	BnaGLYI07	BnaGLYI28	0.2567	0.1236	0.48150	Purifying selection	
13	BnaGLYI25	BnaGLYI10	0.3206	0.1386	0.4323	Purifying selection	
14	BnaGLYI25	BnaGLYI28	1.3206	0.1236	0.0936	Purifying selection	
15	BnaGLYI23	BnaGLYI05	0.0928	0.066	0.7112	Purifying selection	
16	BnaGLYI22	BnaGLYI12	0.0364	0.2007	5.5137	Positive selection	
17	BnaGLYI10	BnaGLYI28	0.0448	0.0128	0.2857	Purifying selection	
18	BnaGLYI13	BnaGLYI15	0.0358	0.1339	3.7402	Positive selection	
19	BnaGLYI12	BnaGLYI04	0.0372	0.2348	6.3118	Positive selection	

Table 4
Ka and Ks values for *BnaGLYII* paralogous gene pairs

No	Locus 1	Locus 2	Ks	Ka	Ka/Ks	Selection pressure	Average Ka/Ks
1	BnaGLYII09	BnaGLYII30	0.12470	0.05000	0.4010	Purifying selection	
2	BnaGLYII24	BnaGLYII04	0.05800	0.06350	1.0948	Positive selection	
3	BnaGLYII24	BnaGLYII07	0.02070	0.02720	1.3140	Positive selection	
4	BnaGLYII29	BnaGLYII03	0.11980	0.08840	0.7379	Purifying selection	
5	BnaGLYII29	BnaGLYII08	0.03070	0.03670	1.1954	Positive selection	
6	BnaGLYII25	BnaGLYII06	0.11250	0.03930	0.3493	Purifying selection	
7	BnaGLYII19	BnaGLYII08	0.12060	0.07830	0.6493	Purifying selection	
8	BnaGLYII19	BnaGLYII03	0.04170	0.02420	0.5803	Purifying selection	
9	BnaGLYII10	BnaGLYII27	0.08610	0.03340	0.3879	Purifying selection	
10	BnaGLYII10	BnaGLYII12	0.19390	0.10200	0.5260	Purifying selection	
11	BnaGLYII02	BnaGLYII18	0.01030	0.00890	0.8641	Purifying selection	
12	BnaGLYII28	BnaGLYII14	0.07520	0.05860	0.7793	Purifying selection	0.7978
13	BnaGLYII23	BnaGLYII12	0.05060	0.03380	0.6680	Purifying selection	
14	BnaGLYII08	BnaGLYII03	0.13160	0.07190	0.5464	Purifying selection	
15	BnaGLYII11	BnaGLYII22	0.04280	0.03320	0.7757	Purifying selection	
16	BnaGLYII15	BnaGLYII17	0.05250	0.01200	0.2286	Purifying selection	
17	BnaGLYII13	BnaGLYII26	0.06540	0.04560	0.6973	Purifying selection	
18	BnaGLYII20	BnaGLYII04	0.01020	0.03350	3.2843	Positive selection	
19	BnaGLYII20	BnaGLYII24	0.06890	0.07010	1.0174	Positive selection	
20	BnaGLYII27	BnaGLYII12	0.18670	0.08020	0.4296	Purifying selection	
21	BnaGLYII01	BnaGLYII16	0.06060	0.04020	0.6634	Purifying selection	
22	BnaGLYII21	BnaGLYII05	0.03470	0.00580	0.1672	Purifying selection	
23	BnaGLYII07	BnaGLYII04	0.08040	0.07980	0.9925	Purifying selection	

Conserved motif and the enzyme activity of BnaGLY

Based on the conserved metal binding sites H/QEH/QE of GLYI proteins and multiple alignment results, the expected enzyme activity of the putative BnaGLYI proteins was predicted. Overall, out of a total 35 putative BnaGLYI proteins, sixteen have all the four conserved residues and are expected to have functional GLYI enzyme activity (Fig. 4 and Table 5). The other 19 BnaGLYI proteins may have variant function. Additionally, GLYII from microorganism and plant contained the well conserved metal binding motif (THXHXDH) and active site motif (C/GHT) [26, 33], which play an important role in the GLYII enzyme activity. Therefore, based on protein sequence alignment results, the presence of enzymatic activity of the putative BnaGLYII proteins were evaluated (Fig. 5, Table 6). Fourteen of 30 putative BnaGLYII proteins possess all the conserved metal binding residues and may have the functional GLYII enzyme activity (Table 6).

Table 5
Analysis of the putative BnaGLYI for their activity and metal ion dependency

Proteins	Metal binding sites Expected GLYI				Expected GLYI enzyme activity	Length of GLYI domain (aa)
	H/Q	E	H/Q	E		
BnaGLYI1	✓	✓	✓	✓	Yes	
BnaGLYI2	✓	—	✓	✓	No	
BnaGLYI3	✓	—	✓	✓	No	
BnaGLYI4	—	✓	—	—	No	
BnaGLYI5	✓	✓	✓	✓	Yes	
BnaGLYI6	✓	✓	✓	✓	Yes	
BnaGLYI7	✓	✓	✓	✓	Yes	
BnaGLYI8	✓	—	✓	✓	No	
BnaGLYI9	✓	✓	✓	✓	Yes	
BnaGLYI10	✓	✓	✓	✓	Yes	
BnaGLYI11	✓	✓	✓	✓	Yes	
BnaGLYI12	—	✓	—	—	No	
BnaGLYI13	✓	—	—	—	No	
BnaGLYI14	✓	✓	✓	✓	Yes	
BnaGLYI15	✓	—	—	—	No	
BnaGLYI16	✓	—	✓	✓	No	
BnaGLYI17	✓	✓	✓	✓	Yes	
BnaGLYI18	✓	✓	✓	✓	Yes	
BnaGLYI19	✓	—	✓	✓	No	
BnaGLYI20	—	✓	—	✓	No	
BnaGLYI21	✓	—	—	—	No	
BnaGLYI22	—	✓	—	—	No	
BnaGLYI23	✓	✓	✓	✓	Yes	
BnaGLYI24	✓	—	✓	✓	No	
BnaGLYI25	✓	✓	✓	✓	Yes	
BnaGLYI26	✓	✓	✓	✓	Yes	
BnaGLYI27	✓	✓	✓	✓	Yes	
BnaGLYI28	✓	✓	✓	✓	Yes	
BnaGLYI29	✓	✓	✓	✓	Yes	
BnaGLYI30	✓	—	—	—	No	
BnaGLYI31	✓	—	✓	—	No	
BnaGLYI32	—	✓	—	—	No	
BnaGLYI33	—	✓	✓	✓	No	
BnaGLYI34	—	—	—	—	No	
BnaGLYI35	✓	—	✓	✓	No	
BnaGLYI6	✓	✓	✓	✓	Yes	
BnaGLYI7	✓	✓	✓	✓	Yes	
BnaGLYI8	✓	✓	✓	✓	Yes	
BnaGLYI9	✓	✓	✓	✓	Yes	
BnaGLYI10	✓	✓	✓	✓	Yes	

Table 6
Analyses of the putative BnaGLYII proteins for their activity and conserved motifs

Proteins	Conserved metal binding motif (THHHXDH)	Active site motif (C/GHT)	Proteins Expected GLYII enzyme activity
BnaGLYII1	—	✓	No
BnaGLYII2	—	—	No
BnaGLYII3	✓	✓	Yes
BnaGLYII4	✓	✓	Yes
BnaGLYII5	—	—	No
BnaGLYII6	✓	—	No
BnaGLYII7	✓	✓	Yes
BnaGLYII8	✓	✓	Yes
BnaGLYII9	—	✓	No
BnaGLYII10	—	✓	No
BnaGLYII11	—	—	No
BnaGLYII12	—	✓	No
BnaGLYII13	✓	✓	Yes
BnaGLYII14	✓	✓	Yes
BnaGLYII15	—	—	No
BnaGLYII16	—	✓	No
BnaGLYII17	—	—	No
BnaGLYII18	—	—	No
BnaGLYII19	✓	✓	Yes
BnaGLYII20	✓	✓	Yes
BnaGLYII21	—	—	No
BnaGLYII22	—	—	No
BnaGLYII23	—	✓	No
BnaGLYII24	✓	✓	Yes
BnaGLYII25	✓	✓	Yes
BnaGLYII26	✓	✓	Yes
BnaGLYII27	—	✓	No
BnaGLYII28	✓	✓	Yes
BnaGLYII29	✓	✓	Yes
BnaGLYII30	✓	✓	Yes

To better understand the protein sequence features of BnaGLY, the conserved motifs of each protein were also identified using MEME program (<http://meme-suite.org/tools/meme>) (**Additional file 1 : Fig. S1**). We found that most proteins in the same group had similar motifs, and the LOGOs of these protein motifs were obtained by MEME (**Additional file 2 : Fig. S2**). In BnaGLYI proteins, motifs 1 were observed in all the 34 BnaGLYI proteins except the BnaGLYI20. Motif 1, 2, 3 and 4 contained the conserved sites of H/QEH/QE. Similarly, motifs 1 were detected in most BnaGLYII proteins except the BnaGLYI16 and BnaGLYI26. Motif 1 and 3 contain the metal binding site (THXHXDH) and active site (C/GHT), respectively. Moreover, we also found that most proteins in the same group had similar motifs (**Fig. S2**).

Tissue expression profiles of BnaGLYI and BnaGLYII

To investigate the tissue-specific expression profiles of the *BnaGLY* genes, we selected 20 types of tissues in database (<https://biodb.swu.edu.cn/brassica/>) to analysis, including 10 tissues at the initial flowering stage and 10 tissues at the different development stages of the podding. The results indicated that the expression levels of the *BnaGLY* genes showed diverse expression patterns (Fig. 6). Six *BnaGLYI* members (*BnaGLYI09*, *BnaGLYI11*, *BnaGLYI16*, *BnaGLYI26*, *BnaGLYI27* and *BnaGLYI35*) were highly expressed in most of the tissues. Their average fragments per kilo base of exon sper million fragments mapped (FPKM) values were over 25 and the value in single tissue was at least over 3. In contrast, the expression levels of *BnaGLYI22*, *BnaGLYI29*, *BnaGLYI32* and *BnaGLYI34* were not detected in most tissues. They only showed faint expression in one to three tissues, e.g., the expression of *BnaGLYI32* was only detected in siliques pericarps (SP) at 24 and 27 days after flowering (DAF). The expression of *BnaGLYI14* was not detected in all the 10 tissues (Fig. 6a). In *BnaGLYII*

genes, *BnaGLYII28* were only weakly expressed in SP at 27 DAF, while the FPKM values of *BnaGLYII18* and *BnaGLYII29* were Less than 1. The other *BnaGLYII* genes were constitutive expression. However, some genes were expressed at higher levels in specific tissues, e.g., *BnaGLYII19* were highly expressed in anther (Fig. 6b). These results indicated that most *BnaGLY* genes were expressed at different levels in different tissues.

Moreover, the expression levels of *BnaGLY* in developing seeds at two, four, six and eight weeks after pollination (WAP) were analyzed using the gene expression omnibus (GEO) database (GSE77637) (Fig. 7). The expression of *BnaGLYI11* and *BnaGLYI27* gene was high in the four stages and the FPKM values were all over 2000. The *BnaGLYI01*, *BnaGLYI04*, and *BnaGLYI32* showed high expression level at eight WAP. *BnaGLYI10*, *BnaGLYI14*, *BnaGLYI28*, *BnaGLYI29* and *BnaGLYI34* showed low expression levels in all the seed development stages (Fig. 7a). In the case of *BnaGLYII*, the expression of *BnaGLYII09*, *BnaGLYII14* and *BnaGLYII28* was upregulated at eight WAP. The expression of *BnaGLYII03*, *BnaGLYII06*, *BnaGLYII24*, *BnaGLYII13*, *BnaGLYII25*, *BnaGLYII19*, *BnaGLYII20* and *BnaGLYII26* were gradually decreased in the four development stages (Fig. 7b).

The expression of the BnaGLY during seed germination

To dissect the influence of *GLY* pathways in seed germination of *B. napus*, the expression level of *GLY* genes was analyzed using the data in GEO (GSE13723). The expression level of *BnaGLYI* in winter oilseed rape (WOSR) accessions with different germination rates (high, medium and low; C129, C033 and C032, respectively) at different germination times showed similar expression pattern, e.g., the expression of *BnaGLYI11* and *BnaGLYI27* were significantly up-regulated at 72 hour after imbibition (hai) in all the three accessions, and the expression of *BnaGLYI04*, *BnaGLYI07*, *BnaGLYI17*, *BnaGLYI21* and *BnaGLYI25* obviously decreased at 36 hai and 72 hai. Meanwhile, the *BnaGLYI06*, *BnaGLYI10*, *BnaGLYI14*, *BnaGLYI24*, *BnaGLYI28*, *BnaGLYI29* and *BnaGLYI34* showed weak expression in all the accessions at the four germination stages (Fig. 8a). In contrast, the average expression level of *BnaGLYII* genes was much higher than that of *BnaGLYI*. *BnaGLYII5*, *BnaGLYII11*, *BnaGLYII21* and *BnaGLYII22* showed significantly up-regulation at 36 hai and 72 hai in all the three accessions; while *BnaGLYII1* were only up-regulated at 72 hai in the three accessions. *BnaGLYII9* showed high expression at early stage of germination, however, sharply was decreased at 36 hai, and then was significantly up-regulated at 72 hai. *BnaGLYII3* and *BnaGLYII19* were up-regulated at the three germination time points in the three accessions. *BnaGLYII17* and *BnaGLYII25* showed very low expression levels in accession C033 from 0 to 72 hai (Fig. 8b). The results demonstrated that *BnaGLY* showed diverse expression patterns, though some genes were clustered within the same group.

The response of the BnaGLYI and BnaGLYII on pathogen infection

The RNA-seq data sets available in GEO (GSE81545) were used for analysis the *BnaGLY* gene expression in response to the fungal pathogen [34]. In *BnaGLYI* gene, the expression level of *BnaGLYI07*, *BnaGLYI24* and *BnaGLYI25* were significantly up-regulated after *S. sclerotiorum* pathogen infection in the resistant cultivar (ZY821) and sensitive cultivar (Westar). *BnaGLYI02*, *BnaGLYI19*, *BnaGLYI31* and *BnaGLYI35* were significantly down-regulated after pathogen infection in both cultivars. While the expression of *BnaGLYI08* and *BnaGLYI10* were down-regulated in sensitive cultivar; however, their expression were up-regulated in resistant cultivar. Most interestingly, the *BnaGLYI17* and *BnaGLYI18* gene were up-regulated in the susceptible cultivar; however, they were down-regulated in the resistant cultivar (Fig. 9a).

Different from the *BnaGLYII* genes, most *BnaGLYII* genes showed significantly decreased expression levels after pathogen infection, e.g., *BnaGLYII06*, *BnaGLYII08*, *BnaGLYII14*, *BnaGLYII23*, *BnaGLYII25*, *BnaGLYII28* and *BnaGLYII30* were significantly down-regulated in both cultivars. Only *BnaGLYII02*, *BnaGLYII03*, *BnaGLYII18* and *BnaGLYII19* were significantly induced in both cultivars after infected by pathogen (Fig. 9b). Moreover, *BnaGLYII16* was up-regulated in the susceptible cultivars, and down-regulated in the resistant cultivars.

Discussions

Identification of glyoxalase genes in plants

Abiotic and biotic stresses severely affect the overall growth of plants and reduce their total productivity. MG is a cytotoxic metabolite, which is produced more under abiotic and biotic stresses [18]. Excessive accumulation of MG results in the disruption of the antioxidant defense system, biomembrane structures and cellular functions including the peroxidation of lipids, the oxidation of protein and fatty acids and other metabolic dysfunctions [18, 35]. The glyoxalase pathway as the main MG detoxification system could protect DNA and protein by converting MG into D-lactate and improve plant adaptation to multiple stresses. Many studies have reveal that the glyoxalase-overexpressing transgenic plants restrict MG accumulation and confer stress tolerance [18]. Therefore, genome-wide analysis of the glyoxalase enzymes have been carried out previously in rice, *Arabidopsis*, soybean, *B. rapa*, grape and *M. truncatula* based on their conserved metal ion and substrate binding sites [22–24, 26, 36]. However, these families have not been studied in *B. napus*. In this study, we identified 30 *BnaGLYI* and 35 *BnaGLYII* in *B. napus* genome. *B. napus* (AACC) originates from natural crossing of *B. rapa* (AA) and *B. oleracea* (CC) [37]. Sixteen *BnaGLYI* and 14 *BnaGLYII* were located in the AA subgenome. While, 15 and 13 *BnaGLYI* and *BnaGLYII* were located in the CC subgenome, which indicated that *GLY* genes might play a similarly important functional role in the both two ancestral species. The number of *BnaGLYI* gene number was much higher than the ancestral species plants *B. rapa* [17] and *B. oleracea* (data not published). The *GLY* families display varied sequences, which might lead to diversity in their biochemical functions. Furthermore, phylogenetic relations, gene structures, protein motifs, Ks/Ka, and the expression pattern of *BnaGLY* gene families were systematically investigated in the study.

According to the recent *GLYI* screening criterias, not only metal binding sites but also GSH binding sites, active sites and dimer interfaces should be considered [38]. Meanwhile, conserved 14 amino acids of *GLYIIs* that were important for substrate binding, metal binding and catalytic activity [39]. Using the criteria, only three *A. thaliana* genes (*AtGLYI2*, *AtGLYI3* and *AtGLYI6*) and two rice genes (*OsGLYI8* and *OsGLYI11.2*) were functional and contained all the binding sites. In

this study, different BnaGLYs subfamily proteins shared the different type of conserved domains, which suggested their functional diversity. Analysis of all BnaGLYI proteins revealed that 16 BnaGLYI possessed conserved metal binding site (H/QEH/QE) and may have expected enzyme activity of the putative BnaGLYI proteins along with the catalytic domain (PF00903). In addition, fourteen of 30 putative BnaGLYII proteins contained metal binding motif (THHHXDH) and active site motif (C/GHT), which were similar to *Escherichia coli*, *Saccharomyces cerevisiae*, *Salmonella typhimurium*, *L. infantum*, *Homo sapiens* and higher plant (*A. thaliana*, *B. juncea*, *O. sativa*) [33].

Different expression patterns of glyoxalase genes

Previous reports indicated that genes with less introns showed rapid gene activation and timely response to various stresses [40]. In our study, *BnaGLYI* and *BnaGLYII* genes showed significantly varied gene length and exon-intron structure. However, the genes with less or no introns did not show higher expression levels as reported [40–41], e.g., *BnaGLYI08* and *BnaGLYI31* containing no introns showed very low level of expression, which did not show significant expression response to the stresses conditions. Meanwhile, we found some tissue-specific expression genes, such as *BnaGLYI29*, were only detected weak expression in carpel (0.305), and *BnaGLYII28* was only detected a very faint expression in SP at 27 DAF. While some genes expressed higher in specific tissues, such as *BnaGLYII19*. These results indicate that most *BnaGLY* genes were expressed at different levels in different tissues.

There is a firm link between the GLY enzymes and stress tolerance in plants. The expression level of GLYI and GLYII can be induced under various stress treatments in different plants [27]. The transcripts of *GLYI* in tomato were up-regulated under salinity stress and phytohormonal and osmotic stimulation [27]. In pumpkin seedlings, transcription level of *GLYI* was induced by salinity, heavy metal, white light, and MG treatments [42]. Therefore, the expression patterns of the *BnaGLYI* and *BnaGLYII* genes were analyzed using publicly available expression data firstly in the study. The expression of *BnaGLYI14* was undetected. It was speculated that *BnaGLYI14* had no expression or had spatial and temporal expression patterns. The *BnaGLY* did not show any significant effect on winter oilseed rape germination rates, because the genes showed similar expression pattern in accessions with different germination rates during germination. In recent study, the glyoxalase enzyme of rice not only increased tolerance abiotic stresses (salinity, drought and extreme temperatures) but also reduced damage from biotic stresses (*Rhizoctonia solani*) [43]. Moreover, in grape, most glyoxalase genes had two periods of high expression after downy mildew inoculation [22]. In our study, the induced genes after infection may have response on the *S. sclerotiorum* pathogen, e.g., *BnaGLYI07*, *BnaGLYI24*, *BnaGLYI25*, *BnaGLYII02*, *BnaGLYII03*, *BnaGLYII18* and *BnaGLYII19* genes, whose expression were significantly up-regulated in both cultivars. Furthermore, the genes were only up-regulated in resistant cultivar may have related to disease resistances of plants, such as *BnaGLYI08* and *BnaGLYI10*. In addition, the genes up-regulated in the susceptible cultivar and down-regulated in the resistant cultivar require further study, such as the *BnaGLYI17* and *BnaGLYI18*. However, whether glyoxalase proteins participate in *S. Sclerotiorum* pathogen response require further investigations.

The function of the BnaGLYI genes

Increasing or overexpressing of *GLYI* or *GLYII* as well as both in transgenic tobacco conferred better stress tolerance than the normal plant to several abiotic and biotic stresses, e.g., MG, high salinity, high heavy metal and hormone levels, and pathogenic infection [8–10]. Our another study found that *BnGLYI-3* (*BnGLYI05* in this study) transgenic yeast cells enhanced their tolerance to extreme temperature stress [25]. In study of *A. thaliana*, overexpression of *AtGLYI2*, *AtGLYI3* and *AtGLYI6* in *E. coli* provides multi-stress tolerance (including salinity, exogenous MG, oxidative, mannitol and heat stress) [38]. In our study, certain *BnaGLY* genes shared a high sequence similarity with previously reported genes, which may show significant function similarity with known genes; e.g., *BnaGLYI11* and *BnaGLYI7* shared approximately 96% identity with *ATGLYI3*. Similarly, *BnaGLYI5* and *BnaGLYI23* showed 98% identity with *ATGLYI2*. In addition, further investigations should explore the mechanism of the response of the glyoxalase pathway to stress tolerance in plants to generate more stress-tolerant varieties using molecular approaches.

Conclusion

GLY genes play important roles in responses to biotic and abiotic stresses in plants. This study provides a systematic knowledge of *BnaGLY* gene family in *B. napus*. A total of 35 *BnaGLYI* and 30 *BnaGLYII* genes were identified. The genes in the same subfamily showed similar structure and motif composition. Phylogenetic comparison and synteny analysis of *BnGLY* genes provide valuable clues for their evolutionary characteristics. Moreover, the gene expression pattern and their responses to seed germinate rate and *S. Sclerotiorum* pathogen were also determined. These results provide an important foundation for further understanding biological functions of *BnaGLY* genes. However, the study only provided preliminary characterization of *BnaGLY* genes and further functional validation work need to be done to understanding the roles of these genes.

Methods

Identification of the GLYI and GLYII genes family in *B. napus*.

Firstly, AtGLYI and AtGLYII protein sequences were acquired from the Arabidopsis Information Resource - TAIR (<http://www.arabidopsis.org>). The genes and proteins of *B. napus* were downloaded at the website of the *B. napus* Genome Browser (http://www.genoscope.cns.fr/brassicana_napus/) databases. Secondly, Arabidopsis GLY protein sequences were used as query to perform the Blast P against *B. napus* genome. Then the protein sequences with the e-value under 1e-10 were confirmed again by the Pfam protein family database according to the particular domains (PF00903) in GLYI proteins and metallo-beta-lactamase domain (PF00753) in the GLYII proteins. All identified putative GLY proteins were designated as *BnaGLYI01* to *BnaGLYI35* and *BnaGLYII01* to *BnaGLYII30* according to their location in chromosome. Localization of proteins were predicted using CELLO v.2.5 website (<http://cello.life.nctu.edu.tw/>) [44] and WOLF PSORT website (<https://www.genscript.com/wolf-psort.html>) [45]. Chloroplast localization was confirmed by ChloroP (<http://www.cbs.dtu.dk/services/ChloroP/>) [46]. The isoelectric points (pI) and molecular weights (Mw) were calculated using the ExPASy proteomics server database (<http://www.expasy.org/tools/>)

Chromosomal location, duplication and Ka (non-synonymous mutation rate)/Ks (synonymous mutation rate) analysis

The location information of *GLY* genes on chromosomes were acquired from the website of the *B. napus* Genome Browser. Mapping of these *GLY* genes was performed using the online website MG2C (http://mg2c.iask.in/mg2c_v2.0/). Gene duplication was defined according to the criteria described in previous studies [47, 48]: the aligned region of two sequences covers over 70% of the longer sequence and the similarity of the aligned region is over 95%. In addition, the Ka and Ks values of the repeating gene pairs were calculated using DnaSp software, and then evolutionary rate and the type of selection pressure on the gene were determined based on the Ka/Ks ratio [32].

Phylogenetic analysis, gene structure and conserved motif

Cluster X program was used to perform multiple sequence alignments with default parameters[49]. Phylogenetic trees were subsequently constructed by the MEGA 6.0 software using the Neighbor-Joining (NJ) [50]. Bootstrap analysis was conducted with 1000 replications. The iTOL website (<https://itol.embl.de/>) was used to better visualize the phylogenetic tree [51].

The exon/intron of *GLY* genes were inferred through comparison of genomic sequences and CDS sequences in the gene structure display server (<http://gsds.cbi.pku.edu.cn/index.php>)[52].

The MEME online tool (<http://meme-suite.org/>)[53] was employed to identify conserved motifs in *BnaGLY* genes with the following parameters: distribution of motifs, the optimum width of motif, 6–50; the maximum number of motif, 5; the number of repetitions, any. Only motifs with an e-value < 1e-10 were retained for further analysis.

Expression analysis of *BnaGLY* genes at different developmental stages and under stress treatments

The temporal and spatial expression patterns of *BnaGLY* expression were analyzed using the RNA-seq data online (<https://biodb.swu.edu.cn/brassica/>), including roots, stems, leaves, flowers, seeds and siliques tissues, and germination, bolting, initial flowering and podding stages. Stem-1 was sampled at bolting stage. Stem-2, anther, filament, pedicel, inflorescence tip, mature leaf, young leaf, petal and carpel root were sampled at initial flowering stage. Embryo, inner integument, outer integument, seed, silique pericarp, embryo and seed coat were sampled at podding stage. All the materials were planted in the field.

The publicly available data of the gene expression omnibus (GEO) database (GSE13723) was used to reveal the probable function of *BnaGLY* genes in germination of winter oilseed rape with different germination rate [54]. The expression of all *BnaGLY* genes in response to *Sclerotinia* infection was analyzed using the reported RNA-seq data (GSE81545). The inoculated leaves in both susceptible (Westar) and tolerant (ZY821) genotypes of *B. napus* were analyzed [34].

The data was used to generate a heatmap using the Heat map Illustrator (Heml, <http://hemi.biocuckoo.org/down.php>) package [45].

Abbreviations

aa: amino acid; bp: base pair; Br: *Brassica rapa*; BRAD: *Brassica* database; CdE: cadmium exposure; CDS: Coding DNA sequence; Ch: chloroplast; Cy: cytosol; Ec: extracellular matrix; FPKM: Fragments Per Kilo base of exon per Million fragments mapped; GLYI: glyoxalase I; GLYII: glyoxalase II; GSH: Glutathione (reduced); hai: Hours after inoculation; Kb: Kilo base pair; MG: Methylglyoxal; Mt: mitochondria; MW: molecular weight; NJ: Neighbor-joining; Nu: nucleus; GEO: gene expression omnibus; ROS: Reactive oxygen species; Os: *Oryza sativa*; Ou: outer membrane, Pe: periplasm; *Pg. Pennisetum glaucum*; PI: isoelectric point; PP: polypeptide length; ROS: Reactive oxygen species; hai: hour after imbibition

Declarations

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Author Contributions

Designed the experiments: GX Y and XM W. Analyzed the data: GX Y, ML Z, FG Z, WJ G, LL Y, and GZ G, K X, BY C, and LX L. Wrote the paper: GX Y. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

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Figures

Figure 1

Chromosomal localization of BnaGLYI (a) and BnaGLYII (b) genes in the *B. napus* chromosomes. Duplicated genes are connected by black lines.

Figure 2

Phylogenetic relationship and gene structure of BnaGLYI and BnaGLYII. (A) BnaGLYI, (B) BnaGLYII. An unrooted tree was generated by MEGA 7.0 software (the bootstrap value is 1000) using the full-length amino acid sequences. Exons and untranslated regions (UTR) are indicated by rectangles (red and blue) and

Figure 3

Phylogenetic relationships of BnaGLYI and BnaGLYII from various plant species. A phylogenetic tree was constructed based on the multiple alignments results using MEGA 7.0 software with the Neighbor-Joining method. Bootstrap support from 1,000 reiterations is indicated above the branches. “At”, “Br”, “Gm” and “Os” refer to the proteins in *A. thaliana*, *B. rapa*, *G. max* and *O. sativa* (only the first splice variants were considered in the case of multiple members), respectively.

Figure 5

Multiple sequence alignment of BnaGLYII proteins. The red boxes indicate the most conserved metal binding motif (THHHXDH) and active site motif (G/CHT)

a

b

Figure 6

Expression patterns of the BnaGLY genes across various tissues and organs. (a) The expression profiles of the BnaGLYI genes; (b) The expression profiles of the BnaGLYII genes. The RNA-seq data was obtained from BrassicaEDB database (<https://biodb.swu.edu.cn/brassica/>). The Heatmap was generated by Heatmap Illustrator (HemI, <http://hemi.biocuckoo.org/down.php>) package. The color bar at the upper right side of the figure represents Log2 (FPKM + 1), with

blue representing little or no expression. Note: root (Ro), stem (St), young leaf (Yl), mature leaf (Ml), pedicel (Ped), petal (Pet), filament (Fi), anther (An), inflorescence tip (It), and carpel (Ca).,silique pericarp-24 DAF (Sp-24 D), seed-24 DAF (Se-24 D), outer integument-24 DAF (Oi-24 D), inner integument-24 DAF (Ii-24 D), embryo-24 DAF (Em-24 D), silique pericarp-27 DAF (Sp-27 D), seed coat-27 DAF (Sc-27 D), seed-27 DAF (Se-27 D), embryo-27 DAF (Em-27D), embryo-30 DAF (Em-30 D)

Figure 7
The expression patterns of the BnaGLY genes at different seed development times. a: The expressions of the BnaGLYI genes, b: The expressions of the BnaGLYII genes

Figure 8

The expressions profiles of the BnaGLY genes during seed germination. a: The expressions of the BnaGLYI genes, b: The expressions of the BnaGLYII genes

Figure 9

Expression profiles of the BrGLYI genes in response to *P. brassicae* infection. Relative expression data of available BnaGLYI (a) and BnaGLYII (b) genes under *P. brassicae* infection were obtained from BrassicaEDB database (<https://biodb.swu.edu.cn/brassica/>). The expression level of the inoculated leaves in susceptible (Westar) and tolerant (ZY821) genotypes were analyzed. Note: W.C. : Control of Westar; W.T. : Treatment of Westar; Z. C. : Control of ZY821; Z.T. : Treatment of ZY821.

Supplementary Files

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