Changes in defense-related enzymes and phenolics in resistant and susceptible common wheat cultivars under aphid stress

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Abstract

The cereal aphids *Rhopalosiphum padi* L. are serious pests on grain crops, reducing the quality and yield by direct feeding damage and virus transmission. The changes in the physiological and biochemical parameters of two wheat cultivars under aphid stress were investigated to understand aphid-resistance mechanisms. The activity levels of phenylalanine ammonia-lyase (PAL), peroxidase (POD), tyrosine ammonia-lyase (TAL), Ca^{2+} -ATPase and Mg^{2+} -ATPase in resistant cultivar W0923 increased during aphid feeding, and most of them were significantly higher than those in the susceptible cultivar GN21. However, these enzyme activities increased and then decreased in GN21. The polyphenol oxidase (PPO) activity in W0923 was maintained longer than in GN21. The total phenol contents of the two cultivars exhibited significant increases on the 15th day compared with the untreated controls, and the content in W0923 was significantly higher than in GN21 by the 30th day. Most of the POD, PPO, PAL, TAL and Ca^{2+} -ATPase activities and phenolic metabolite contents were higher in W0923 than in GN21 under the untreated control conditions. The Ca^{2+} -ATPase and PAL activities positively correlated with POD, PPO and Mg^{2+} -ATPase activities. Ca^{2+} -ATPase and PAL may be key biochemical markers for evaluating aphid resistance. W0923 had a strong ability to maintain higher enzyme activities and synthesize more phenols and tannins than GN21, which contributed to aphid resistance.

Keywords *Triticum aestivum* · Aphid resistance · Defensive enzymes · Ca^{2+} -ATPase · Phenylalanine ammonia-lyase · Phenolic metabolites

Introduction

Common wheat (*Triticum aestivum* L.) is among the top three food crops in the world and the dominant crop for human consumption in temperate countries (Aradottir et al. 2017).

Rapid population growth, combined with the increase in food consumption per capita, has driven the demand for sustainable wheat production (Shewry 2009; Curtis and Halford 2014). However, aphids can negatively affect wheat yields by direct feeding and vectoring plant viruses (Aradottir et al. 2017; Li et al. 2020). Wheat aphids are common pests, and can cause long and serious harm in wheat (Zhang et al. 2019; Sun et al. 2019). Wheat aphids can infest wheat from the seedling to milk-ripening stages (Naeem et al. 2018). Aphids cause yield losses directly (35–40%) by sucking the sap of plants or indirectly (20–80%) by transmitting fungal and viral pathogens (Aslam et al. 2005). The cereal aphids (*Rhopalosiphum padi* L.) are serious pests on grain crops (Lu et al. 2013). At present, a number of insecticides are available for the control of this pest (Tanguy and Dedryver 2009). Although various insecticides have been advocated by the pest management program, there are serious concerns about the development of acquired resistance in pest species against these insecticides (Batra et al. 2017). However, cultivars resistant to aphids could represent an alternative control

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method in the development of sustainable agriculture (Tanguy and Dedryver 2009; Gatehouse et al. 2011). A better understanding of the mechanisms related to aphid stress is important in designing improved aphid resistance in wheat.

Some enzymes and secondary metabolites take part in plant defenses against pests (Cai et al. 2004). Peroxidase (POD), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) are important biochemical markers in pest-resistant plants (Han et al. 2009; Sha et al. 2015). Chang and co-workers (2008) found that POD and PPO activities increase in different sorghum cultivars under aphid stress. Tyrosine ammonia-lyase (TAL) is induced in responses to biotic and abiotic cues (Khan et al. 2003). TAL and PAL activities are correlated with cellulose, hemicellulose and lignin concentrations (Morrison and Buxton 1993), which can strengthen the structural barrier. The ATPases of plant cells play central roles in plant physiology and can be considered as a “master enzymes” that control many important functions at cellular and organ levels (Serrano 1989). Ca^{2+} -ATPase is believed to transport Ca^{2+} (Axelsen and Palmgren 1998), which also regulates a variety of physiological functions and confers resistance to both abiotic and biotic stresses (Dodd et al. 2010; Steinhorst and Kudla 2014). Mechanical wounding and insect elicitor can induce electrical signals (Mousavi et al. 2013; Salvador-Recatalà et al. 2014) that are potentially connected with intracellular Ca^{2+} waves (Kiep et al. 2015). Mg^{2+} -ATPase is an Mg^{2+} pump (Axelsen and Palmgren 1998). Mg^{2+} is a macronutrient necessary for photosynthesis and other enzymatic reactions, including the regulation of ATPase and kinase activities, induction of protein synthesis machinery and catalysis of DNA replication (Bose et al. 2011). Batra and co-workers (2017) reported that higher PAL and PPO activities, along with higher tannin and total phenol contents, in the flag leaf and grain of tolerant barley might confer resistance to various pests. The aphid resistance levels of different cucumber materials is closely related to the effects of the PPO and PAL activities, as well as the tannin and total phenol contents (Ma et al. 2015). Chen et al. (2003) showed that tannic acid and total phenols have inhibitory effects on aphid survival, growth and development in wheat.

At present, physiological mechanisms of aphid resistance are unclear in wheat. Therefore, in this experiment, resistant and susceptible wheat cultivars were employed to analyze the changes in the levels of defense-related enzymes and phenolic metabolites at three stages of aphid stress. The aim was to investigate the response patterns of leaf physiological and biochemical parameters under aphid stress and aphid defense-related mechanisms. This study provides a valuable reference for the breeding and cultivation of aphid-resistant wheat.

Materials and methods

Plant materials

Cultivars W0923 (highly resistant to aphids) and GN21 (highly susceptible to aphids) are hexaploid common wheat (*Triticum aestivum* L.). Each cultivar was planted in a net-room at Guizhou University (26° 26' N, 106° 38' E and 1120 m altitude), which is situated in Guizhou Province, Southwest China. The plants were spaced 10 cm apart in 20-cm rows. The protective rows were set up around the plot. Wheat was not sprayed with pesticides during this experiment. In total, ten cereal aphids (*Rhopalosiphum padi* L.) per plant were introduced for each treatment, which were performed when wheat flag leaves had just emerged. Untreated plants were used as controls. A randomized complete block design with three replications was employed.

The aphid quantity ratio (AQR) and the physiological and biochemical parameters of the two cultivars were measured on the 15th (early stage), 30th (medium stage) and 45th (late stage) days after the aphid stress.

AQR determination

The AQR was investigated according to the Chinese Agricultural Standard (Chen et al. 2007). Aphid numbers on ten plants per treatment were counted at the sampling stages. The AQR was calculated using the following formula: $\text{AQR} = \text{average aphid number on a cultivar} / \text{average aphid number on all observed cultivars}$. Aphid resistance was assessed using the following scale based on the AQR: (a) level 1 (high resistance), $0.01 < \text{AQR} \leq 0.30$; (b) level 2 (medium resistance), $0.30 < \text{AQR} \leq 0.60$; (c) level 3 (low resistance), $0.60 < \text{AQR} \leq 0.90$; (d) level 4 (low sensitivity), $0.90 < \text{AQR} \leq 1.20$; (e) level 5 (medium sensitivity), $1.20 < \text{AQR} \leq 1.50$; and (f) level 6 (high sensitivity), $\text{AQR} > 1.50$.

Analysis of enzyme activities

Enzyme extraction

The biochemical and physiological parameters of the wheat flag leaves were measured. In total, 0.50 g of leaves were collected and ground in 6 mL of 50 mM phosphate buffer (pH 5.5) containing 5 mM mercaptoethanol, 1 mM EDTA and 1% PVP in an ice bath. After centrifuging at 3550g for 10 min at 4 °C, the supernatant was used as the extract.

Spectrophotometric assay

POD and PPO: A modified method of Zieslin and Ben-Zaken (1993) was used to measure POD activity. Briefly, the assay solution contained 29 mM phosphate buffer (pH 5.5), 120 mM H_2O_2 , 10 mM guaiacol and 0.1 mL of the enzyme extract. The formation of tetraguaiacol was monitored at 470 nm with an ultraviolet–visible spectrophotometer (Thermo Scientific, Evolution 220) after a 5-min incubation period at 30 °C. An enzyme unit was defined as an increase in absorbance by 0.01 at 470 nm $\text{min}^{-1} \text{g}^{-1}$ fresh weight of sample at 25 °C. PPO activity was determined in accordance with the methods of Archana et al. (2011) with slight modifications. The reaction mixture consisted of 39 mM phosphate buffer (pH 5.5), 20 mM catechol and 0.1 mL supernatant was prepared, and then incubated for 10 min at 37 °C. After cooling in an ice bath, the reaction was stopped with 1.22 M trichloroacetic acid (2.0 mL) and read at 525 nm.

PAL and TAL: Supernatants used to measure PAL and TAL activities were prepared as above with modifications, which included using 0.1 M sodium borate buffer (pH 8.8) and centrifuging at 11,833g. PAL activity was examined based on the methods of Assis et al. (2001) with slight modifications. The reaction mixture included 0.2 mL of supernatant, 5 mL of 0.1 M L-phenylalanine in borate buffer (pH 8.8) and 2.8 mL of distilled water. Following incubation at 37 °C for 60 min, the absorbance of the reaction mixture was measured at 290 nm. The method of Khan et al. (2003) was modified for the TAL activity assay. The reaction mixture contained 0.2 mL of supernatant, 2.8 mL of 0.1 M sodium borate buffer (pH 8.8) and 4 mM tyrosine in 0.1 M borate buffer (pH 8.8). After incubation at 30 °C for 30 min, the absorbance of the reaction mixture was recorded at 315 nm.

ATPase: The determination of Ca^{2+} - and Mg^{2+} -ATPase activities were carried out using the methods of Li et al. (2000). However, 4 mL of STN buffer (sucrose, 400 mM; NaCl, 10 mM; and Tris-HCl, 50 mM; pH 7.8) was used instead of phosphate buffer. For the determination of the Ca^{2+} -ATPase activity level, 1 mL of supernatant and 1 mL of activation mixture containing 20 mM EDTA, 10 mM ATP, 5.37 mM trypsin and 250 mM Tris-HCl (pH 8.0) were activated for 10 min in a 20 °C water bath. Then, 0.1 mL of bovine serum albumin (10 mg mL^{-1}) was added to stop the reaction. Afterward, 0.5 mL of reaction mixture [500 mM Tris-HCl (pH 8.0), 50 mM CaCl_2 and 50 mM ATP] was added. Samples were incubated at 37 °C for 10 min, and the reaction was stopped by adding 2.0 mL of 1.22 M trichloroacetic acid. The absorbance was determined at 660 nm. The procedure for determining Mg^{2+} -ATPase activity level

was similar to that of Ca^{2+} -ATPase, but the activation mixture [250 mM Tris-HCl (pH 8.0), 500 mM NaCl, 50 mM MgCl_2 , 50 mM dithiothreitol and 0.5 mM diazoanthracene methylsulfuric acid) and reaction mixture [500 mM Tris-HCl (pH 8.0), 50 mM MgCl_2 and 50 mM ATP] were different.

Phenolic metabolite assays

The flag leaves were oven dried, broken, ground and passed through a 0.4-mm sieve. After refluxing with 5 mL of distilled water in a 100 °C water bath for 30 min, the tissue sample (0.10 g) was cooled and filtered. Total phenolic content of the sample was assessed using the methods of Ramos et al. (2017) with slight modifications. Subsequently, the tissue sample (0.10 g) was refluxed again with 20 mL of H_2O at 60 °C overnight, followed by cooling and centrifuging at 10,000g for 20 min. The supernatant was then collected for the quantification of tannins according to the methods of Sadasivam and Manickam (1992) after dilution to 10 mL with distilled water. The absorbance values were recorded at 770 nm and 660 nm for phenol and tannin contents, respectively. The linear equations obtained from the standard curves were used to determine the concentration of each phenolic metabolite.

Statistical analyses

The data were analyzed using SPSS statistics 19.0 (IBM Corp., Armonk, NY). Statistical differences between means were determined by Duncan's multiple range test at a significance level of $p < 0.05$ after displaying a significant effect during an ANOVA. Pearson's correlation analysis of binary variables was performed, and two variables were considered significantly correlated at the $p < 0.05$ level.

Table 1 The AQR and aphid resistance levels of two wheat cultivars

Stages (days)	Cultivars	AQR	Aphid resistance
15	W0923	0.30	HR
	GN21	1.54	HS
30	W0923	0.25	HR
	GN21	1.69	HS
45	W0923	0.29	HR
	GN21	1.66	HS

HR and HS high aphid resistance and high aphid susceptibility, respectively

Results

Evaluations of aphid resistance

Aphid resistance was classified using the AQR (Chen et al. 2007) (Table 1). The AQR of cultivar W0923 was in the range of 0.25–0.30 at each stage, indicating that it was a highly resistant cultivar to aphid stress. Cultivar GN21 represents a highly susceptible cultivar, because its AQR was more than 1.50 at every stage.

The changes in enzyme activities

POD plays a crucial role in the biosynthesis of plant cell walls, thereby enhancing the defense barriers against biotic invasion (Hiraga et al. 2001). Enzymatic lignification of cell walls has been found in various plant species in responses to biotic stresses (Huang et al. 2016). The levels of POD

activity were remarkably higher ($p < 0.05$) in the two cultivars than in the controls on the 30th day (Fig. 1a). The POD activity in W0923 increased gradually under stress conditions and peaked on the 45th day. The peak value was 20.01% higher than that of GN21. At the same stage, the POD activity of W0923 was markedly ($p < 0.05$) higher than that of GN21 under untreated control conditions. However, the POD activity of GN21 increased dramatically at the beginning and then decreased sharply after 30 days. GN21 was more sensitive to the aphid stress, but showed a less persistent POD activity in comparison with W0923.

PPO diminishes the nutritional quality of the infested plants by converting phenols into quinines that ultimately suppress the digestion of plant proteins in insects (Batra et al. 2017). The PPO activity levels of two cultivars rose initially, reached maximum levels on the 30th day (Fig. 1b), and then fell. Compared with GN21, W0923 exhibited a greater durability, with a significant increase in the PPO activity on the 45th day.

Fig. 1 Effects of aphid stress on the POD (a), PPO (b), PAL (c) and TAL (d) activity levels in two wheat cultivars. Bars show means $\pm$ SDs ($n = 3$). Values with different letters are significantly different at $p < 0.05$

PAL is a vital enzyme in phenylpropanoid pathway, which responsible for catalyzing the formation of t-cinnamic acid (Batra et al. 2017). Furthermore, t-cinnamic acid produces compounds that exhibit some degree of toxicity on herbivores (Morelló et al. 2005). The levels of PAL activity were noticeably higher ($p < 0.05$) in the two cultivars than in the controls on the 15th day (Fig. 1c). The PAL activity of W0923 varied from 6.86 to 8.25 $\text{U min}^{-1} \text{g}^{-1}$ fresh weight under aphid stress. In W0923, the average PAL activity increased by 20.62% and 32.68% than in GN21 under aphid stress and normal conditions, respectively. W0923 showed a continued increase in PAL activity, and the level was obviously ($p < 0.05$) increased compared to GN21 on the 15th and 45th days. The PAL activity in GN21 increased initially, but decreased rapidly after the 30th day, indicating that it was maintained for a shorter period than in W0923.

TAL is a key enzyme of lignin synthesis (Liu et al. 2007), which is particularly important in forming cell walls. The levels of TAL activity in W0923 was significantly (66.70%) higher than in GN21 on the 15th day (Fig. 1d). Significant increases in the TAL activity at three stages occurred in W0923 under the untreated control conditions compared with in GN21. The TAL activity in W0923 increased continuously with the aphid feeding. However, the TAL activity in GN21 rose initially and then fell.

Ca^{2+} -ATPase belongs to the family of P-type ATPases, which involves in the maintenance of intracellular Ca^{2+} homeostasis (Stokes et al. 2005). The levels of Ca^{2+} -ATPase activity were markedly higher ($p < 0.05$) in the two cultivars than in the controls at three stages (Fig. 2a). This enzyme's

activity level significantly increased in W0923 at three stages under the untreated control conditions when compared to that in GN21. The enzyme activity in W0923 increased continually during the aphid infestation and peaked on the 45th day. The peak was 25.30% higher than that of GN21. However, the level in GN21 increased before the 30th day and then dropped rapidly.

Mg^{2+} is the most abundant divalent cation in higher plant cells, and it contributes to plant growth and development (Joshua et al. 2016). Mg^{2+} -ATPase can maintain membrane integrity by regulating Mg^{2+} transport in and out of the cells (Liu et al. 2002). On the 30th day, the levels of Mg^{2+} -ATPase activity were remarkably higher ($p < 0.05$) in the two cultivars than in the controls (Fig. 2b). The activity of this enzyme in W0923 showed a gradual increase during the aphid stress and peaked on the 45th day. The peak value was significantly (31.98%) higher than that of GN21. The Mg^{2+} -ATPase activity in GN21 increased initially, but returned quickly to the control level on the 45th day. W0923 could sustain its Mg^{2+} -ATPase activity for a longer time than GN21.

Changes in phenolic metabolites

Phenols are important secondary compounds involved in wheat resistance to aphids. They not only affect the feeding behavior and physiological metabolism of wheat aphids, but are also the main resistance factors (Ciepiela 1989). The total phenol contents of the two cultivars varied from 2.73 to 3.74 mg g^{-1} under aphid stress (Fig. 3a).

Fig. 2 Effects of aphid stress on the Ca^{2+} -ATPase (a) and Mg^{2+} -ATPase (b) activity levels in two wheat cultivars. Bars show means $\pm$ SDs ($n = 3$). Values with different letters are significantly different at $p < 0.05$

Fig. 3 Effects of aphid stress on the phenol (a) and tannin (b) contents in two wheat cultivars. Bars show means $\pm$ SDs ($n=3$). Values with different letters are significantly different at $p < 0.05$

Compared to the controls, the total phenol contents of the two cultivars exhibited significant increases on the 15th day. The total phenol content in W0923 was markedly ($p < 0.05$) higher than that in GN21 on the 30th day. For the control plants, W0923 displayed remarkable ($p < 0.05$) increases in total phenolic contents compared to GN21 on the 30th and 45th days.

Tannins are secondary metabolites of plants and are natural organic compounds (Zhang and Chen 2008). They can affect the feeding and digestion of nutrients by insects (Yang et al. 2013). The tannin contents of the two cultivars were highest on the 15th day and then decreased slightly (Fig. 3b). The tannin content of W0923 was noticeably ($p < 0.05$) increased compared to GN21 on the 45th day under the untreated control conditions.

Correlations between parameters

As shown in Table 2, the level of Ca^{2+} -ATPase activity was positively correlated with the levels of POD ($r = 0.913$, $p < 0.01$), PPO ($r = 0.740$, $p < 0.05$), PAL ($r = 0.940$,

$p < 0.01$) and Mg^{2+} -ATPase ($r = 0.797$, $p < 0.05$). In addition, the level PAL activity was positively correlated with the levels of POD ($r = 0.836$, $p < 0.01$), PPO ($r = 0.765$, $p < 0.05$) and Mg^{2+} -ATPase ($r = 0.846$, $p < 0.01$). The results indicate that the plant enzymes, especially Ca^{2+} -ATPase and PAL, can assist in defending against aphid stress in common wheat.

Discussion

Aphid stress can trigger a wide variety of plant responses, ranging from physiological metabolisms to molecular processes. The phenylpropanoid pathway contributes to the resistance of wheat against the Russian wheat aphid (Berner and Westhuizen 2010), and PAL and TAL are its key rate-limiting enzymes (Ganapathy et al. 2016). Aphid-resistant wheat cultivars have higher POD, PPO and PAL activities than aphid-sensitive cultivars at the tillering, stem elongation and flag leaf stages (Han et al. 2009). In resistant wheat, Russian wheat aphid infestations

Table 2 Correlations among physiological and biochemical parameters in wheat under aphid stress

Index	PAL	Tannin	Total phenols	PPO	POD	Mg^{2+} -ATPase	TAL
Tannin	-0.292						
Total phenols	-0.347	0.374					
PPO	0.765*	-0.007	0.318				
POD	0.836**	-0.343	-0.263	0.639			
Mg^{2+} -ATPase	0.846**	-0.138	-0.346	0.633	0.641		
Ca^{2+} -ATPase	0.940**	-0.188	-0.254	0.740*	0.913**	0.797*	0.614

*, **Significant correlations at the 0.05 and 0.01 levels, respectively ($n=9$)

rapidly induce the POD activity, resulting in it being levels of magnitude greater than in susceptible wheat (Westhuizen et al. 1998). In tomatoes, PPO and POD activities increase in response to herbivore feeding (Cipollini and Redman 1999). The change in the TAL activity is similar to that of the PAL activity (Ganapathy et al. 2016), which increases the lignin content and inhibits the harm caused by biotic stresses in tobacco (Wang 2018). Results similar to these were obtained in this study (Fig. 1a–d). The POD, PAL and TAL activities in the resistant cultivar W0923 increased with aphid feeding, and most enzyme activity levels were higher than those in the susceptible cultivar GN21. The changes in these enzyme activities peaked on the 30th day, but dropped rapidly later in GN21. The PPO activity in W0923 was maintained for longer than in GN21. During the 45 days of the experiment (wheat progressed from heading, flowering to filling stages), every enzyme activity level in W0923 was higher than in GN21 under the untreated control conditions. Thus, resistant W0923 maintained higher enzyme activities, on the whole, than susceptible GN21 throughout this experiment.

Plant phenolic metabolites can exhibit direct toxicity to insects and/or act through different signal transduction pathways, which in turn generate a variety of secondary metabolites with toxic properties towards insects and trigger the activation of defensive enzymes (Helmi and Mohamed 2016). The defense responses of hard red winter wheat (*T. aestivum* L.) to bird-cherry oat aphid (*R. padi*) infestations trigger biosynthetic pathways that result in enhanced phenolic concentrations in mature wheat grains (Ramos et al. 2017). An enhancement in phenylpropanoid metabolism and the increased levels of phenolic metabolites were found in common wheat under different environmental stresses (Sakihama and Yamasaki 2002). As the total phenol content increases, the aphid resistance of wheat improves (Chen et al. 2003). Compared with the control plants, the total phenol contents of the two cultivars exhibited significant increases on the 15th day in this study (Fig. 3a). The total phenol content in W0923 was markedly ($p < 0.05$) higher than that in GN21 on the 30th day. Among the control plants, W0923 displayed remarkable ($p < 0.05$) increases in total phenolic contents compared to GN21 on the 30th and 45th days. These findings are in good agreement with those of Ramos et al. (2017), Sakihama and Yamasaki (2002) and Chen et al. (2003). Tannins are a group of polyphenolic, anti-nutritional plant metabolites that confer protection against biotic stresses (Barbehenn and Constabel 2011). An increase in tannin level (1.2-fold) was observed in the flag leaves infested by an aphid complex when compared with the controls of six bread wheat varieties (Kaur et al. 2017). The increase in the tannin concentration in manually damaged oak trees to simulate herbivory was not significant, but the number of herbivorous insects on the trees decreased

significantly compared with the control trees (Lauer and Rossi 2011). Often, the tannin differences were subtle; such as the tannin contents in W0923 at the three stages of aphid stress, which were on average only 11.80% higher than those in GN21 in this experiment (Fig. 3b). The tannin content in W0923 was noticeably ($p < 0.05$) higher than that in GN21 under the untreated control conditions on the 45th day (Fig. 3b). Resistant W0923 had a greater ability to synthesize phenols and tannins during the growth process, thereby demonstrating a high resistance to the cereal aphids. A number of natural phenolic compounds in plants, such as gallic acid, pyrogallol, ellagitannins and ellagic acid, have been shown to exert feeding deterrent activity against aphid species (Jones and Klocke 1987). Furthermore, the enhancement of naturally occurring enzymes and phenolic compounds having antioxidant activities in W0923 could potentially increase healthy quality and profitability of wheat crops.

Ca^{2+} -ATPase (calcium pump) participates in dealing with plant stress (Qudeimat and Faltusz 2008). The change in the intracellular free Ca^{2+} level in plants affects many physiological activities (Hepler 2005), such as cell growth and stress response. In this study, the levels of Ca^{2+} -ATPase activity were remarkably ($p < 0.05$) higher in the two cultivars than in the controls at the three stages (Fig. 2a). In W0923, there were significant increases in the enzyme activity levels at the three stages under the untreated control conditions compared with GN21. Mg^{2+} -ATPase plays a significant role in regulating the intracellular Mg^{2+} concentration. In plants, the dynamic Mg^{2+} level in the intracellular environment strongly affects the activities of many key enzymes in vacuoles and chloroplasts (Shaul 2002; Guo et al. 2008). On the 30th day, the levels of Mg^{2+} -ATPase activity levels were noticeably ($p < 0.05$) higher in the two cultivars than in the controls (Fig. 2b). The enzyme activity in W0923 had significantly increased by the 45th day compared with in GN21. Ca^{2+} is a messenger involved in the cellular signaling of all eukaryotes. Ca^{2+} signatures modulate responses to various biotic and abiotic stresses (Pandey and Ray 2017). The Ca^{2+} -ATPase activity was positively correlated with POD, PPO, PAL and Mg^{2+} -ATPase activities (Table 2). When resistant W0923 was infested by the aphids, Ca^{2+} messenger can be triggered and involved in cellular signaling, and then the POD, PPO, PAL, TAL and Mg^{2+} -ATPase activities and phenolic metabolite contents were enhanced to cope with the aphid infestation.

In conclusion, we found that most of the POD, PPO, PAL, TAL, Ca^{2+} -ATPase and Mg^{2+} -ATPase activities and phenolic metabolite contents were higher in resistant W0923 than in susceptible GN21 under both aphid stress and normal conditions. These findings implied that W0923 had a strong ability to maintain higher enzyme activities and synthesize more phenols and tannins, which are closely related to aphid

resistance, than GN21. These results not only provide new insights into the mechanisms of aphid resistance, but also provide useful information for cultivation and breeding in wheat aphid resistance. More persuasive results would be obtained if more wheat genotypes were used in this experiment.

Author contribution statement GDG and SQZ designed the experiments. YX and HG carried out the experiment, analyzed the data and wrote the manuscript. GDG, SQZ and QZZ polished the manuscript. All authors read and approved the manuscript.

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Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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