INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
20–0004/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1556
http://www.fspublishers.org

Full Length Article

Anthocyanin Accumulation and Gene Expression in Maize (*Zea mays*) under Metolachlor Stress

Dongzhi Li[1,2], Li Xu[3], Lin Zhou[2] and Xiling Chen[1*]
[1]*Postdoctoral Research Base, Henan Institute of Science and Technology, Xinxiang, Henan Province, China*
[2]*Postdoctoral mobile stations, Henan Agricultural University, Zhengzhou, Henan Province, China*
[3]*Institute of Pesticide Research, Henan Institute of Science and Technology, Xinxiang, Henan Province, China*
[*]For correspondence: chenxiling456@126.com
Received 02 January 2020; Accepted 24 June 2020; Published _____

Abstract

Anthocyanins are the most conspicuous class of flavonoids and could protect plants from biotic and abiotic stresses. Herbicides are widely used in crops and constitute a common stress to crops. To explore the accumulation of anthocyanins under metolachlor stress, the total phenolics, flavonoids and anthocyanins contents as well as the total antioxidative capacity in metolachlor-susceptible (ZD) and metolachlor-tolerant (ND) maize (*Zea mays* L.) cultivars were measured. The results showed that phenolics contents was not influenced by metolachlor stress in both maize cultivars, while anthocyanins was reduced in both maize cultivars. The flavonoids and the total antioxidant capacity were both increased in ZD under metolachlor stress. Analysis of anthocyanin biosynthesis genes indicated that the expression levels of *PAL, CHS, DFR, ANS, BZ2* and *F3'H* had decreasing tendency under metolachlor stress. These genes were speculated to be the action sites of metolachlor in maize, especially *F3'H. DFR, ANS* and *BZ2* were deduced to be responsible for higher accumulation of anthocyanins in ND compared to ZD, as higher expression levels of these genes were found in ND. This study provided a better understanding on the molecular mechanism of anthocyanins biosynthesis under metolachlor stress in maize. © 2020 Friends Science Publishers

Keywords: Anthocyanins; Metolachlor stresses; Gene expression; Total antioxidative capacity

Introduction

Abiotic stresses, such as high temperature, soil salinity, drought, ultraviolet (UV) radiation and heavy metals, cause significant loss of plant productivity (Farooq *et al*. 2015; Liu *et al*. 2015; Wang *et al.* 2016). Herbicides, which are essential in the modern agricultural production, are becoming a common and severe stress to crops if used improperly. Flavonoids are well known plant secondary products, which are capable to scavenge reactive oxygen species and free radicals (Choi *et al*. 2002; Chang *et al*. 2013; Hideg and Strid 2017). As a result, these compounds serve essential roles in plant by their multiple roles in relieving abiotic stresses (Giovanni and Massimiliano 2010).

Anthocyanins are the most conspicuous class of flavonoids, which are induced in plants to respond to multiple abiotic stresses. The common stress of drought strongly increased the biosynthesis of anthocyanins in *Arabidopsis thaliana* (L.) (Kovinich *et al*. 2015). Overaccumulation of anthocyanin in *A. thaliana* under oxidative and drought stress showed high *in vitro* antioxidative activity, which relieved the accumulation of reactive oxygen species *in vivo* (Nakabayashi *et al*. 2014). The accumulation of anthocyanins was also reported to be related to salt tolerance by stopping the propagation of oxidative chain reactions (Wahid and Ghazanfar 2006; Hichem *et al*. 2009; Singh *et al*. 2014). Leão *et al*. (2013) reported that anthocyanins content was increased with increasing arsenic concentration, suggesting anthocyanins play a role in tolerance to poison arsenic.

The molecular mechanism underlying flavonoids-mediated abiotic stress tolerance has been extensively studied and anthocyanin biosynthesis pathway in plants is well understood now. Anthocyanins are derived from the flavonoid pathway. Most enzymes in this pathway have been characterized, including phenylalanineammonialyase (*PAL*), 4-coumarate-CoA ligase (*4CL*), chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), flavonoid 3'-hydroxylase (*F3'H*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*), *UFGT*, UDP-glucose flavonoid 3-O-glucosyltransferase (*BZ1*) and glutathione *S*-transferase (*BZ2*) (Holton and Cornish 1995) (Fig. 1). The relative transcription levels of genes as *CHS, CHI, F3H, FNS, FLS, DFR* and *ANS* in wheat (*Triticum aestivum* L.) were rapidly increased under drought stress

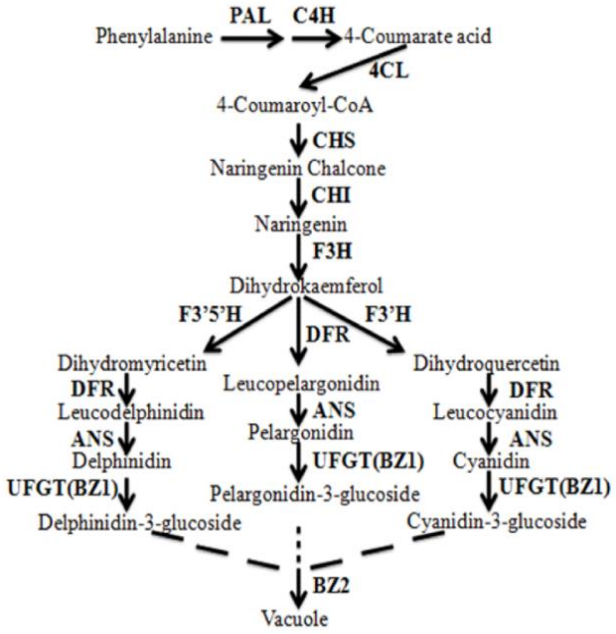

Fig. 1: Overview of the anthocyanin biosynthesis pathway in plants. Broken arrows indicate that the biosynthetic steps are omitted

(Ma *et al*. 2014) and these anthocyanin biosynthesis genes in *Ammopiptanthus mongolicus* (Maxim. ex Kom.) are reported to increase by drought and cold stresses (Wu *et al*. 2014). The expression of *PAL* and *CHS* were induced by UV-B radiation in chili pepper (*Capsicum annuum* L.) (Rodríguez-Calzada *et al*. 2019).

Although herbicides are widely used in agriculture and become a common stress in crop development, studies that explore the mechanism of herbicides stress are still scarce. Molin *et al*. (1986) reported that the accumulation of anthocyanins was inhibited by chloroacetanilide herbicides in etiolated sorghum (*Sorghum bicolor* (L.) Moench). However, there was no information about the effect of chloroacetanilide herbicides on the transcription profiles of anthocyanin biosynthesis pathway genes. Here, for further exploring the possible molecular mechanism of anthocyanin biosynthesis pathway response to metolachlor stress in maize, the relative transcription levels of anthocyanin biosynthesis genes (*PAL, 4CL, CHS, CHI, F3H, F3'H, DFR, ANS, BZ1* and *BZ2*) influenced by metolachlor stress were investigated. Besides, the total accumulation of phenolic, flavonoids and anthocyanins under metolachlor stress between two maize cultivars were compared.

Materials and methods

Plant materials and herbicide treatment

Two widely planted maize cultivars in China, Nongda 86 and Zhengdan 958 (abbreviated as ND and ZD, respectively), were selected to explore the influence on the total accumulation of anthocyanins under metolachlor stress and ND had 4-fold metolachlor tolerance than ZD (Li *et al*. 2017a). The concentration of metolachlor (97% pure, provided by College of Science, China Agricultural University) used was 30 μmol L^{-1}. At this concentration, slight inhibition was showed in maize seedlings. Greater concentration could cause visible symptom of growth inhibition (Li *et al*. 2017b).

Seeds of ND and ZD cultivars were soaked 12 h before transferred to the artificial climate chamber (RXZ-3808) to germinate. The germination condition was 28°C, 75% RH and 16 h/8 h day/night cycle. Sand was sterilized at 160°C for 3 h and then used as culture medium. Metolachlor solution (30 μmol L^{-1}, 60 mL) was applied to the sterilized sand before seeds sown and set as six a pot with three biological replicates. Equal volume of solvent without metolachlor was applied for control samples. The pots were then kept in artificial climate chamber with the same conditions as germination. Leaves were harvested 60, 78 and 96 h after metolachlor treatment for RNA isolation. For the measurement of total content of phenolics, flavonoids and anthocyanins, maize leaves were harvested after 96 h.

Extraction

The extraction method for total phenolics and total flavonoids was conducted according to Cai *et al*. (2004) and Bao *et al*. (2005) with minor modifications. Fresh leaves (1 g) of each sample was ground into powder by liquid nitrogen and extracted with 20 mL ethanol (60%) in a shaker (200 r min^{-1}) at room temperature for 2 h. The mixture was centrifuged at 8000× *g* for 10 min and the supernatant was collected for the determination of total accumulation of phenolic, flavonoid and radicals Folin-Ciocalteu reagent and 2,2-dipheny-1-(2,4,6-trinitrophenyl) -hydrazyl ($DPPH^{\cdot}$, purchased from J&K Scientific) and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) cation ($ABTS^{\cdot+}$) scavenging tests.

The extraction method for anthocyanins was according to Ma *et al*. (2014). Leaf samples were ground into powder in liquid nitrogen. Each sample weighed 0.5 g was extracted with 1.5 mL solvent mixed by methanol, hydrochloride acid and water with volume ratio of 25:5:70 in a shaker at 32°C and 150 r min^{-1} for 4 h. The mixture was centrifuged at 10,000× *g* for 20 min and the supernatant was obtained for anthocyanin content determination. Three biological samples were conducted for all the extractions above.

Determination of total phenolic, flavonoids and anthocyanin content

The total phenolic content of the samples was estimated by the Folin-Ciocalteu colorimetric method with minor modifications (Zheng and Wang 2001; Liu *et al*. 2002). 0.3 mL extractions or standard gallic acid (Sigma-Aldrich)

solutions were oxidized by 2 mL Folin-Ciocalteu reagent and saturated sodium carbonate (1.6 mL, 10%) were used to neutralize the reaction. The mixture was incubated for 2 h at 30°C. PerkinElmer Lambda 35 spectrophotometer (California, U.S.A.) was used to measure the absorbance at 760 nm. Quantification was calculated based on the standard curve of gallic acid with the concentration of gallic acid at the range of 12.5, 25, 50, 100 and 200 μg mL^{-1}.

Colorimetric method with minor modification was chosen to determine the total content of flavonoid (Bao *et al*. 2005; Ma *et al*. 2014). Diluted or standard rutin (Sigma-Aldrich) solutions at 0.5 mL were transferred into a tube containing 2 mL ddH_2O and mixed with 0.15 mL of 5% $NaNO_2$. The mixture was kept for 5 min followed by the addition of 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$ solution. Another 5 min later, the mixture was added with 1 mL of 1 mol L^{-1} NaOH. The mixture was mixed well and incubated at room temperature for 15 min and then the absorbance was measured at 415 nm with PerkinElmer Lambda 35 spectrophotometer. Total flavonoids content was calculated using the standard curve of rutin with the concentration of rutin at the range of 50, 100, 200, 400 and 800 μg mL^{-1}.

The content of anthocyanins was estimated by spectroscopic methodology according to Ma *et al.* (2014). The absorbance of the extractions was determined at 525 nm and 657 nm with PerkinElmer Lambda 35 spectrophotometer and the content of anthocyanin was calculated according to the following equation: content of anthocyanin = (OD525-OD657×0.25)/fresh weight of leaves.

Radical cation $ABTS^{\cdot+}$ scavenging activity

The determination of total antioxidant capacity of the two maize cultivars was carried out using PerkinElmer Lambda 35 spectrophotometer according to Total Antioxidant Capacity Assay Kit (Beyotime, Nanjing, China). Briefly, 10 μL extractions or standard trolox solutions (0, 0.3, 0.6, 0.9, 1.2 and 1.5 mmol L^{-1}) was added to 20 μL peroxidase solution. Then it was mixed with 170 μL $ABTS^{\cdot+}$ working solution and incubated at room temperature for 6 min. The absorbance was determined at 414 nm. Ethanol (60%) was used as blank solution for control sample (170 μL of $ABTS^{\cdot+}$ + 20 μL peroxidase solution + 10 μL 60% ethanol). Quantification was calculated according to a standard curve of trolox. The activity of $ABTS^{\cdot+}$ scavenging was expressed as trolox equivalent antioxidant capacity (TEAC).

Radical $DPPH^{\cdot}$ scavenging activity

$DPPH^{\cdot}$ method with minor modifications was used to estimate the free radical scavenging activity of the extractions (Brand-Williams *et al*. 1995). An aliquot of the extractions (0.1 mL) was added to 1.9 mL $DPPH^{\cdot}$ (300 μmol L^{-1}) and mixed well. The mixture was kept for 30 min at room temperature and then used to measure its absorbance at 515 nm with PerkinElmer Lambda 35 spectrophotometer. Ethanol (60%) was used as blank solution, and $DPPH^{\cdot}$ solution (1.9 mL $DPPH^{\cdot}$ mixed with 0.1 mL 60% ethanol) was used as control. The inhibition percent of $DPPH^{\cdot}$ absorbance was used to express the antioxidant activity and calculated according to the following equation = (Absorbance of control – Absorbance of test)×100%/Absorbance of control. A high inhibition rate indicates a high radical scavenging activity.

RNA isolation and Quantitative real-time PCR analysis

Total RNA was extracted from the frozen leaves ground in liquid nitrogen with RNAprep pure Plant Kit (Tiangen, Beijing, China) according to the manufacturer's protocols and then used to synthesize the first strand cDNA by the Fast Quant RT Kit (Tiangen, Beijing, China). Quantitative real-time PCR (qRT-PCR) was conducted using SuperRealPreMix Plus (Tiangen, Beijing, China). Mixtures were run in 20 μL system including 10 μL 2× SuperRealPreMix solution, 1 μL cDNA template, 0.6 μL of each forward and reverse primer, 0.4 μL 50×ROX Reference Dye and 7.4 μL redistilled H_2O. Sequence for all the primer sets used is listed in Table 1. Multiple reference genes *18S rRNA, EF1α* and *GAPDH* of maize were used to normalize the expression of each gene across all the samples. A melt curve was used to check the primer specificity. Three independent RNA samples were collected as biological replicates and the relative expression folds of each gene were obtained by $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen 2001).

Statistical analysis

The final results were presented as mean ± SD and one-way analysis of variance (ANOVA), completed with Duncan's post-hoc comparison tests, were employed to analyze the statistical differences by S.P.S.S. 16.0 (S.P.S.S., Chicago, U.S.A.). Values of $P < 0.05$ were considered significant.

Results

Accumulation of phenolics, flavonoids and anthocyanins in two maize cultivars in response to metolachlor stress

Results indicated that phenolics, flavonoids and anthocyanins contents had no significant difference between control plants of the two maize cultivars (Fig. 2). The total content of anthocyanins was reduced and the total phenolics was not influenced by metolachlor treatment in both cultivars, whereas the total content of flavonoids in ZD was observed to have a slight increase (Fig. 2). ZD was more sensitive to be influenced by metolachlor stress than ND.

The radical scavenging activity of maize leaves in response to metolachlor stress

The $DPPH^{\cdot}$ system and the $ABTS^{\cdot+}$ cation method of

Table 1: Primers of reference genes and anthocyanins biosynthesis genes

Genes	Sequence (5'-3', forward-reverse)	
EF1α	TGGGCCTACTGGTCTTACTACTGA	ACATACCCACGCTTCAGATCCT
GAPDH	CCATCACTGCCACACAGAAAAC	AGGAACACGGAAGGACATACCAG
18SrRNA	GCTCTTTCTTGATTCTATGGGTGG	GTTAGCAGGCTGAGGTCTCGTTC
PAL	CACATCGAGGAGAACGTCAAG	GATCTCCTGGAGCAGGTCCTTC
4CL	TCACGCACCCGGAGATCAAG	TCCTTGGCGACGAATTGCTTG
CHS	CGTCCGTGAACCGCCTGATG	TCCGAGCACACCACCAGGAC
CHI	TTGAGAAGAGTGTGGGAAGTAG	CCCTCGAAAGCTCAATCACAG
F3H	CGAGGACTGGGGCATCTTC	CTTCTTCCCGCCGGACATG
F3'H	CGACACTAGGTTCAACGAGAC	CTTCCTGAGCACGTCCGGATG
DFR	GCGCATCGTCTTCACTTCCTC	GAAGTACATCCATCCGGTCATC
ANS	GCATCTCCTGGGTCGTCTTC	GCTTTGTGCTGCTGTTTCTTC
BZ1	CGACCAGGCAGCAAACAGGG	GTGGACGAGGAGGTTGATGACG
BZ2	GGGAGGTCAGCCCGTTCA	GAGCCTGTCGCTTTTCTTGG

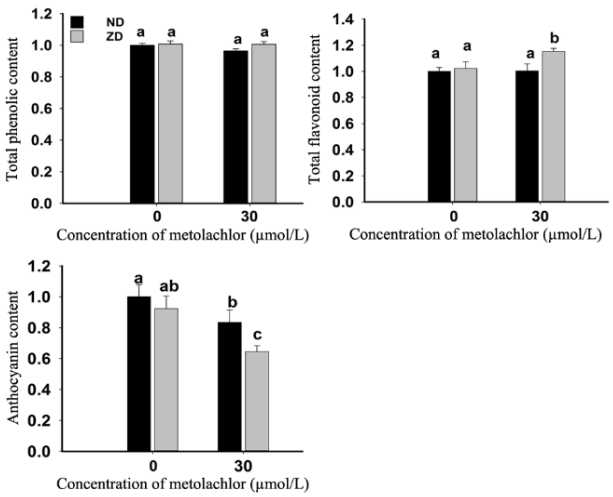

Fig. 2: Relative content of phenolics, flavonoids and anthocyanins in maize leaves after 96 hours metolachlor treatment. Data are expressed as fold change relative to the control sample of ND. 0 denotes control; 30 represents plants treated with 30 μmol L^{-1} metolachlor; error bars are standard deviations (n=3). With each figure, columns with different letters are significantly different ($P < 0.05$)

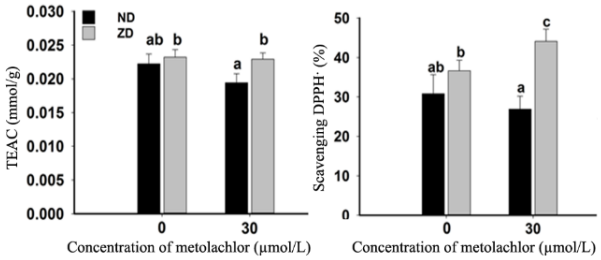

Fig. 3: The radical scavenging activity of maize leaves under metolachlor stress. 0 denotes control; 30 represents plants treated with 30 μmol L^{-1} metolachlor 96 h; error bars indicate standard deviations (n=3). Columns within each figure with different letters were significantly different ($P < 0.05$)

measuring free radical scavenging activity generated a similar result after metolachlor treatment. The free radical scavenging activity was comparable between control plants of the two maize cultivars, while a significant increase of the free radical scavenging activity in ZD was found under metolachlor stress (Fig. 3).

Expression analysis of anthocyanin biosynthesis genes in maize leaves

The relative transcription folds of genes *PAL*, *4CL*, *CHS*, *CHI*, *F3H*, *F3'H*, *DFR*, *ANS*, *BZ1* and *BZ2* in anthocyanin biosynthesis pathway in maize leaves were analyzed by qRT-PCR (Fig. 4). When compared with control samples, the expression level of *CHI* and *F3H* were not changed by metolachlor. While the expression level of *PAL*, *CHS*, *F3'H*, *DFR*, *BZ2* and *ANS* showed decreasing tendency after metolachlor treatment in both cultivars. The expression of *PAL* was significantly decreased after metolachlor treatment for 60 and 96 h in both cultivars, while only decreased in ZD at 78 h. *CHS* is the first gene encoding the early, un-branched segment of the flavonoid's biosynthesis pathway. The expression of *CHS* was significantly decreased at 60 h after metolachlor treatment in both cultivars and only significantly decreased in ND at 96 h, while no significant change was observed after metolachlor treatment for 78 h in both cultivars. *F3'H* catalyze the formation of cyaniding-3-glucoside, which was one of the branch of anthocyanins biosynthesis. The expression of *F3'H* was significantly decreased under the stress of metolachlor in both cultivars at 60, 78 and 96 h. *DFR* and *ANS* are the last two genes responsible for the formation of pro-anthocyanidins. Their expression levels were significantly decreased in ND after metolachlor treatment for 60 and 96 h, while significant decrease was found in ZD at 78 h. Besides, the expression level of *DFR* was significantly decreased in ZD after 96 h metolachlor exposure. The expression of *4CL* was only significantly increased in ZD after 96 h metolachlor exposure. The expression of *BZ1* was significantly increased in ND and decreased in ZD after 96 h metolachlor exposure. *BZ2* is the last gene of anthocyanins biosynthesis pathway exporting

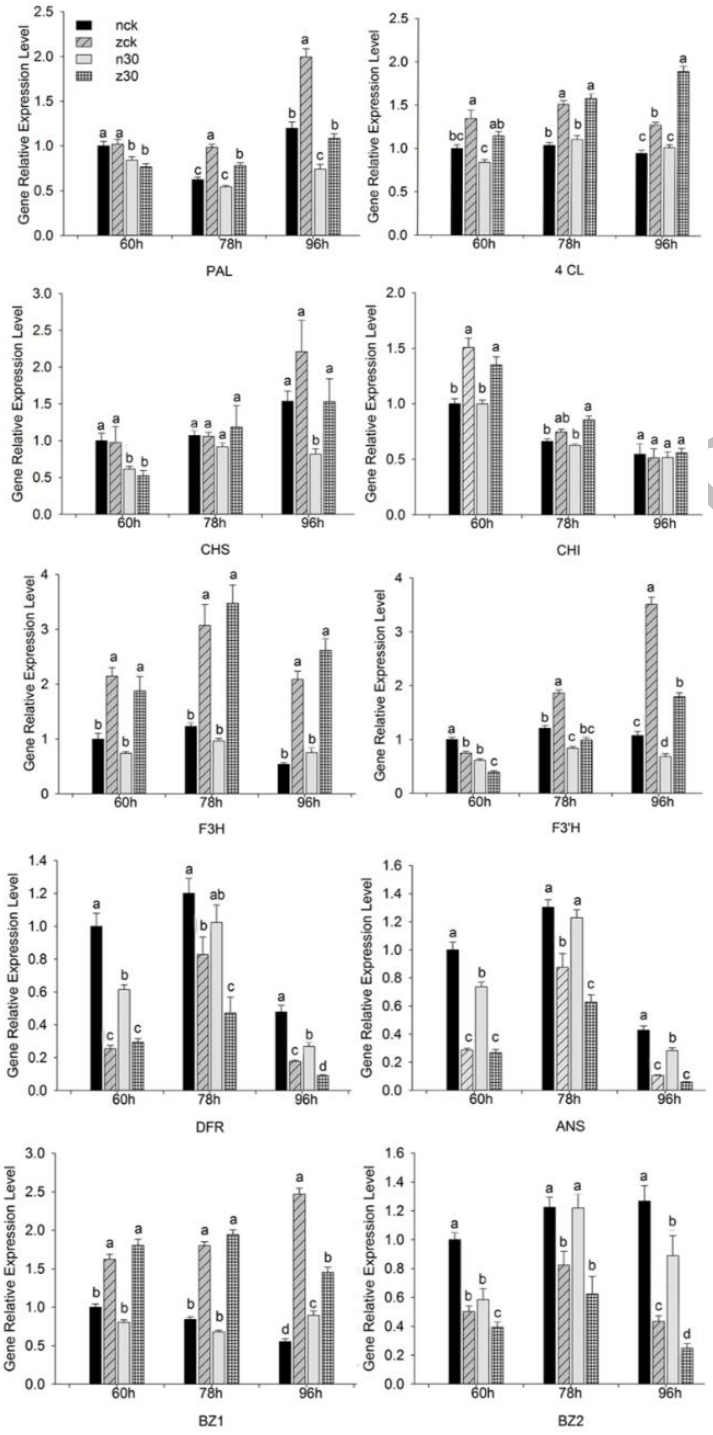

Fig. 4: Relative expression level of anthocyanin biosynthesis genes between two maize leaves under metolachlor stress. nck denotes control samples of ND; zck denotes control samples of ZD; n30 represents ND plants treated with 30 μmol L^{-1} of metolachlor; z30 represented ZD plants treated with 30 μmol L^{-1} of metolachlor; error bars indicate standard deviations (n=3). Columns within each treatment with different letters were significantly different ($P < 0.05$)

anthocyanins to vacuole. The expression level of *BZ2* was significantly decreased at 60 and 96 h after metolachlor treatment in both ND and ZD.

The relative expression level of *DFR, ANS* and *BZ2* showed higher expression level in ND than ZD with or without metolachlor treatment, whereas other genes (*PAL, 4CL, F3H, F3'H* and *BZ1*) showed higher expression in ZD with or without metolachlor treatment.

Discussion

This study investigated the effect of metolachlor stress on maize anthocyanins accumulation and the related molecular mechanisms. The results showed that metolachlor reduced the total content of anthocyanins and inhibited the expression of most anthocyanins biosynthesis genes.

Flavonoids accumulation could increase radical scavenging activity *in vitro*, which enhanced the ability of anti-oxidation (Nakabayashi *et al*. 2014). In this study, the metolachlor-susceptible cultivar ZD showed more accumulation of flavonoids than the metolachlor-tolerant cultivar ND after 96 h metolachlor treatment (Fig. 2). And our results of radical scavenging activity in both $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ methods were in agreement with the total content of flavonoids between the two cultivars, which suggested that flavonoids play an important role in response to metolachlor stress (Fig. 3). The phenomenon that flavonoids accumulated more in ZD than ND was possibly due to the first line of defense against ROS in ZD was less effective under metolachlor stress and the oxidative stress stimulating accumulation of antioxidant in ZD was more severe. This had been observed in many other plants when response to different stresses (Giovanni *et al*. 2012).

Multiple reports had suggested that the content of anthocyanins was highly increased under abiotic stresses (Paolacci *et al*. 2001; Gonzalez *et al*. 2008; Ma *et al*. 2014) and the expression of genes involved in anthocyanin biosynthesis pathway could be increased by abiotic stress (Castellarin *et al*. 2007; Vasquez-Robinet *et al*. 2008; Yuan *et al*. 2012; Liu *et al*. 2012; Kim *et al*. 2012). Comparing with other abiotic stress, herbicides stress of isoproturon and diuron significantly decreased the content of anthocyanins in wheat and maize, respectively (Kim *et al*. 2006; Alla and Hassan 2014). In etiolated sorghum seedlings, anthocyanins and lignin synthesis were inhibited by alachlor, as well as metolachlor. Since anthocyanins and lignin were derived from coumaric acid, it could be deduced that the inhibition site by alachlor might be before coumaric acid synthesis. However, when phenylalanine, coumaric acid and cinnamic acid were added, the synthesis of anthocyanins could not be recovered, which suggested that the inhibition of anthocyanins biosynthesis by alachlor might be multi-sites (Molin *et al*. 1986). In the current study, the total content of anthocyanins was decreased in both maize cultivars after 96 h metolachlor exposure, Molin *et al*. (1986) also reported that chloroacetanilide herbicides could inhibit the biosynthesis of anthocyanins in etiolated sorghum. Among the tested genes in anthocyanin biosynthesis pathway, only *PAL*, *CHS*, *DFR*, *ANS*, *BZ2* and *F3'H* showed decreasing tendency compared with control samples after 60, 78 and 96 h, especially *F3'H*, which was significantly decreased in both cultivars after 60, 78 and 96 h metolachlor treatment. Thus, it was speculated that *F3'H* should be the main inhibition site of metolachlor in anthocyanins biosynthesis, while *PAL*, *CHS*, *DFR*, *BZ2* and *ANS* might be the other target sites. Comparing the expression level of the tested genes between the two maize cultivars, only *DFR*, *ANS* and *BZ2*, catalyzing dihydrokaemferol into stable anthocyanins, showed higher expression level in ND than ZD after 96 h treatment. Therefore, these three genes might be responsible for the higher content of anthocyanins in ND than ZD.

Conclusion

This study showed that metolachlor stress increased the total flavonoids in ZD, while decreased the total content of anthocyanins in both cultivars after 96 h. *F3'H* were thought to be the main inhibition site by metolachlor in anthocyanins biosynthesis pathway, and *PAL*, *CHS*, *DFR*, *BZ2* and *ANS* might be other target sites. *DFR*, *ANS* and *BZ2* were responsible for the different total content of anthocyanins between ND and ZD cultivars.

Acknowledgements

This work was supported by Henan Institute of Science and Technology Postdoctoral Research Base and National Key R&D Program of China (2018YFD0200600).

Author Contributions

This work was supported by Henan Institute of Science and Technology Postdoctoral Research Base and National Key R&D Program of China (2018YFD0200600). Xiling Chen designed and coordinated the experiment. Dongzhi Li carried out the experiment. Li Xu performed statistical analysis and formulated the manuscript. Lin Zhou revised and improved the manuscript.

References

Alla MMN, NM Hassan (2014). Alleviation of isoproturon toxicity to wheat by exogenous application of glutathione. *Pest Biochem Physiol* 112:56–62

Bao JS, YZ Cai, M Sun, GY Wang, H Corke (2005). Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myric rubra*) extracts and their color properties and stability. *J Agric Food Chem* 53:2327–2332

Brand-Williams W, ME Cuvelier, C Berset (1995). Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 28:25–30

Cai Y, Q Luo, M Sun, H Corke (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci* 74:2157–2184

Castellarin SD, MA Matthews, GD Gaspero, GA Gambetta (2007). Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta* 227:101–112

Chang H, Q Xie, QY Zhang, XL Peng, JD Zhu, MT Mi (2013). Flavonoids, flavonoid subclasses and breast cancer risk: A meta-analysis of epidemiologic studies. *PLoS One* 8; Article e54318

Choi CW, SC Kim, SS Hwang, BK Choi, HJ Ahn, MY Lee, SH Park, SK Kim (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci* 163:1161–1168

Farooq M, M Hussain, A Wakeel, KHM Siddique (2015) Salt stress in maize: Effects, resistance mechanisms and management. A review. *Agron Sustain Dev* 35:461–481

Giovanni A, T Massimiliano (2010). Multiple functional roles of flavonoids in photoprotection. *New Phytol* 186:786–793

Giovanni A, A Elisa, P Susanna, T Massimiliano (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Sci* 196:67–76

Gonzalez A, M Zhao, JM Leavitt, AM Lloyd (2008). Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings. *Plant J* 53:814–827

Hichem H, D Mounir, EA Naceur (2009). Differential responses of two maize (*Zea mays* L.) varieties to salt stress: Changes on polyphenols composition of foliage and oxidative damages. *Ind Crop Prod* 30:144–151

Hideg É, Å Strid (2017). The effects of UV-B on the biochemistry and metabolism of plants. *In: UV-B Radiation and Plant Life: Molecular Biology to Ecology*, pp:90–110. CABI Publishers, Wallingford, UK

Holton TA, EC Cornish 1995. Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* 7:1071–1083

Kim BG, ER Lee, JH Ahn (2012). Analysis of flavonoid contents and expression of flavonoid biosynthetic genes in *Populus euramericana* guinier in response to abiotic stress. *J Kor Soc Appl Biol* 55:141–145

Kim JS, BH Lee, SH Kim, KH Oh, KY Cho (2006). Responses to environmental and chemical signals for anthocyanin biosynthesis in non-chlorophyllous corn (*Zea mays* L.) leaf. *J Plant Biol* 49:16–25

Kovinich N, G Kayanja, A Chanoca, MS Otegui, E Grotewold (2015). Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. *Plant Signal Behav* 10; Article e1027850

Leão GA, JAD Oliveira, RTA Felipe, FS Farnese, GS Gusman (2013). Anthocyanins, thiols, and antioxidant scavenging enzymes are involved in *Lemna gibba* tolerance to arsenic. *J Plant Interact* 9:143–151

Li DZ, QG Gao, L Xu, S Pang, ZQ Liu, CJ Wang, WM Tan (2017a). Characterization of glutathione S-transferases in the detoxification of metolachlor in two maize cultivars of differing herbicide tolerance. *Pest Biochem Physiol* 143:265–271

Li DZ, L Xu, S Pang, ZQ Liu, K Wang, CJ Wang (2017b). Variable levels of glutathione *S*-transferases are responsible for the differential tolerance to metolachlor between maize (*Zea mays*) shoots and roots. *J Agric Food Chem* 65:39–44

Liu DG, SZ He, XJ Song, H Zhai, N Liu, DD Zhang, ZT Ren, QC Liu (2015). IbSIMT1, a novel salt-induced methyltransferase gene from *Ipomoea batatas*, is involved in salt tolerance. *Plant Cell Tiss Org* 120:701–715

Liu ML, XR Li, YB Liu, B Cao (2012). Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*. *Plant Physiol Biochem* 73:161–167

Liu M, XQ Li, C Weber, CY Lee, J Brown, RH Liu (2002). Antioxidant and antiproliferative activities of raspberries. *J Agric Food Chem* 50:2926–2930

Livak KJ, TD Schmittgen (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle CT}$ Method. *Methods* 25:402–408

Ma D, D Sun, C Wang, Y Li, T Guo (2014). Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress. *Plant Physiol Biochem* 80:60–66

Molin WT, EJ Anderson, CA Porter (1986). Effects of alachlor on anthocyanin and lignin synthesis in etiolated sorghum (*Sorghum bicolor* (L.) Moench) mesocotyls. *Pest Biochem Physiol* 25:105–111

Nakabayashi R, K Yonekura-Sakakibara, K Urano, M Suzuki, Y Yamada, T Nishizawa, F Matsuda, M Kojima, H Sakakibara, K Shinozaki, AJ Michael, T Tohge, M Yamazaki, K Saito (2014). Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. *Plant J* 77:367–379

Paolacci AR, R D'Ovidio, R Marabottini, C Nali, G Lorenzini, MR Abenavoli, M Badiani (2001). Research note: Ozone induces a differential accumulation of phenylalanine ammonia-lyase, chalcone synthase and chalcone isomerase RNA transcripts in sensitive and resistant bean cultivars. *Funct Plant Biol* 28:425–428

Rodríguez-Calzada T, M Qian, A Strid, S Neugar, M Schreiner, I Torres-Pacheco, RG Guevara-González (2019). Effect of UV-B radiation on morphology, phenolic compound production, gene expression, and subsequent drought stress responses in chili pepper (*Capsicum annuum* L.). *Plant Physiol Biochem* 134:94–102

Singh DP, R Prabha, KK Meena, L Sharma, AK Sharma (2014). Induced accumulation of polyphenolics and flavonoids in cyanobacteria under salt stress protects organisms through enhanced antioxidant activity. *Amer J Plant Sci* 5:726–735

Vasquez-Robinet C, SP Mane, AV Ulanov, JI Watkinson, VK Stromberg, DD Koeyer, R Schafleitner, DB Willmot, M Bonierbale, HJ Bohnert, R Grene (2008). Physiological and molecular adaptations to drought in Andean potato genotypes. *J Exp Bot* 59:2109–2123

Wahid A, A Ghazanfar (2006). Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *J Plant Physiol* 163:723–730

Wang FB, WJ Tong, H Zhu, WL Kong, RH Peng, QC Liu, QH Yao (2016). A novel Cys2/His2 zinc finger protein gene from sweet potato, IbZFP1, is involved in salt and drought tolerance in transgenic *Arabidopsis*. *Planta* 243:783–797

Wu YQ, W Wei, XY Pang, XF Wang, HL Zhang, B Dong, YP Xing, XG Li, MY Wang (2014). Comparative transcriptome profiling of a desert evergreen shrub, *Ammopiptanthus mongolicus*, in response to drought and cold stresses. *BMC Genomics* 15; Article 671

Yuan Y, YJ Liu, C Wu, SQ Chen, ZY Wang, ZC Yang, SS Qin, LQ Huang (2012). Water deficit affected flavonoid accumulation by regulating hormone metabolism in *Scutellaria baicalensis* georgi roots. *PLoS One* 7; Article e42946

Zheng W, XY Wang (2001). Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 49:5165–5170