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Characterization of biogenic volatile organic compounds and membrane lipid peroxidation during leaf ageing in maize (*Zea mays*)*

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Plants evolved a series of morphological and physiological adaptation mechanism to cope with various biotic and abiotic stresses. The secondary metabolism substance played important roles in these processes. Based on this, McKey (1974) put forward the optimal defense theory (ODT), which assumed that it is valuable for plant to produce these substances to cope with neighbour plants competition, herbivores and pathogen infection (Strauss *et al.* 2002). Recently, the physiological and ecological effects of low molecular weight biogenic volatile organic components (BVOCs) were intensively investigated and the mysterious of BVOCs were unveiled (Vickers *et al*. 2009, Loreto and Schnitzler 2010). For example, the universal green leaf volatiles (GLVs) can induce direct and indirect defense response (Bate and Rothstein 1998, Kishimoto *et al.* 2005). Terpenoid (Terp), another group of universal BVOCs, had antioxidant function (Peñuelas and Llusià 2002, Vickers *et al.* 2009) and could protect leaves against singlet oxygen (Velikova *et al.* 2004).

Given the important role of BVOCs in plant life, the spatial-temporal distribution of these metabolism substances was uneven. Besides the external environment, such as light (Rasulov *et al*. 2009), temperature (Velikova *et al.* 2009), water and nitrogen availability (Gouinguene and Turlings 2002, Penuelas *et al.* 2009; Ormeño *et al.* 2009) had great influence on the biosynthesis and emission rate of BVOCs; the developmental and senescence process also had significant effect on BVOCs biosynthesis and emission (Ucar and Ucar 2010, Niinemets *et al.* 2010), which may the cause of age-dependent resistance, as the un-senescence tissue or organs are more important for the plant based on the ODT (Radhika *et al.* 2008). However, most of these studies were focused on perennial woody plant. For annual cereal crops, researchers pay more attention to BVOCs ecological function, and crop seedlings were used frequently in their studies. While BVOCs emission pattern during the late growth stage of these cereal crops, which might be really different from seedling stage and play a more important role in their life-cycle, is neglected. Here, we used the most important cereal crop maize (*Zea mays* L.) to explore BVOCs emission pattern during leaf senescence in parallel with monitoring leaf chlorophyll (Chl) degradation and malonyldiadehyde (MDA) accumulation.

Leaves used in this study were harvested in the scientific and education park of Henan Agricultural University in Zhengzhou city (China) in 2010. To create different light environment, high and low plant population densities were selected in parallel. Row-to-row distance was 0.7m for both plant population densities; while plant-to-pant distances were 0.16m and 0.32m for high and low plant population, respectively. The mid part of the second uppermost leaf (Up), ear leaf (Mid) and the seventh leaf (Down)were sampled at tasselling stage (T_1), 10 days after tasselling (DAT) (T_2), 20 DAT (T_3), 30 DAT (T_4) and 45 DAT (T_5). Sun irradiance at each sampling canopy level were measured by using SunScan System and photosynthetically active radiation (PAR) distribution character at each canopy level were presented by relative light transmittance in Fig 1.

Leaf volatile were collected by 100 μm PDMS SPME fiber (Supelco) as described by Kollner (2008). Chl and MDA content were determined spectrophotometrically by the method of Lichtenthaler (1987) and Du *et al.* (2011) respectively. Hydrogen peroxide (H_2O_2) was measured according to Alexieva *et al.* (2001). All the quantification was based on three replications. Statistics were done by using SPSS 16.0. The effects of population density, leaf position and leaf age were assessed with ANOVA separately, and the interaction among them was analyzed using a GLM-univariate (P=0.05). Post hoc analyses were done by Duncan's multiple-range test at 0.05 probability levels.

*Short note

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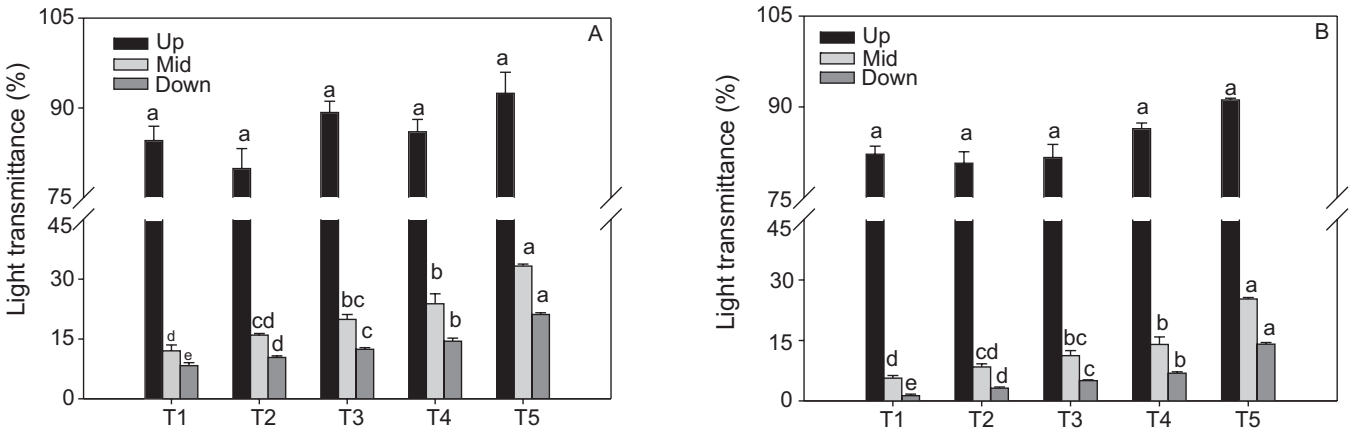

Fig 1 Relative light transmittance of low (*A*) and high (B) population density at different canopy level. Different letters above the columns indicate statistically different means at $P < 0.05$

Light transmittance decreased dramatically along the vertical gradient in the canopy. And light transmittance of high population density decreased more than that of low population density. At T_1 stage, light transmittances of low population density and high population density were 12.03% and 5.63% at ear level; and were 8.31% and 1.29% at the seventh leaf level respectively. At T_5 stage, light transmittances of low population density and high population density were 33.26% and 25.27% at ear level and were 21.12% and 14.06% at the seventh leaf level respectively (Fig 1). Leaves at lower canopy level received less PAR than upper level leaves, and at the same canopy level, leaves in low population density received more PAR than high population density.

GLVs and Terp were two major compositions of total BVOCs in the present study. Total GLVs emission rate of the second uppermost leaves and ear leaves in both low and high density populations changed little with leaf aging. There are significant interaction between sampling stage and leaf position (P<0.001, Table 1). Total GLVs emission rate of the seventh leaf decreased significantly with leaf aging. GLVs emission rate of the seventh leaf was significantly lower than that of the second uppermost and ear leaves in both low and high density populations at the same sampling time (P<0.01, Table 1). This might be partially caused by the reduction of PAR (Gouinguene and Turlings 2002).

Terp emission rate of the second uppermost and ear leaves in both low and high density populations first slightly increased and then decreased with leaf aging. Terp emission rate of the seventh leaf in low density population decreased rapidly with leaf aging, while kept rarely low in a similar emission rate in the high density population (Table 1).Terp emission rate of ear leaves was highest among the three leaf position, and then the second uppermost leaves, the seventh leaves comes the last (Table 1). Terp can protect leaves by scavenging reactive oxygen and reduce pest damage via tritrophic interactions. Ear leaf is closest to the reproductive organ. By the economic analogy, ear leaf is more important for kernel growth and development at the reproduce stage to keep that species passed on (Bloom *et al.* 1985). Invest in synthetic these volatiles to protect the ear leaf are valuable based on ODH (McKey 1974) with enough substrate and energy for the metabolism.

Variation of Chl *a* and Chl *b* content exhibited a similar character as total GLVs emission rate. Chl *b* decreased more rapidly than Chl *a*, which led a decrease of Chl *a/b*. MDA and H_2O_2 content show an opposite regularity (Table 1). In general, Chl content decreased with leaf aging. On the contrary, the content of MDA and H_2O_2, which caused by the excess reactive oxygen species and reflect senescence, increased with leaf aging, is in accordance with Hung (1997) and Prochazkova *et al.* (2001). Lipid peroxidation aggravation is accompanied by the decrease of BVOCs emission rate. Besides antioxidant enzyme system, several BVOCs composition also have effect on reactive oxygen species quenching (Affek and Yakir 2002, Velikova *et al.* 2004, Vickers *et al.* 2009). BVOCs emission profile from deferent development stage and the decline of BVOCs emission rate of plants with aging process has been recognized (Zhang *et al.* 2008, Ucar and Ucar 2010, Niinemets *et al.* 2010).

For the low density population, GLVs and Terp emission rate of ear leaves was higher than the second uppermost leaves. However, for the high density population, GLVs and Terp emission rate of ear leaves were a little lower than the second uppermost leaves at the late stage (Table 1). The difference of BVOCs emission patterns of the second uppermost and ear leaves between low and high population density indicate higher emission rate is probably caused by the sufficient PAR. Insufficient PAR led a rapid senescence of lower canopy position leaves (Monneveux *et al.* 2005). As we can see that ear leaves from high density population exhibit relatively low Chl content and high MDA and H_2O_2 content at all sampling stage (Hung and Kao 1997). BVOCs emission rate of leaves at the lower canopy level was

Table 1 BVOCs emission rate, Chl, MDA and H_2O_2 content at different canopy level with leaf aging

		Low density			High density		
		Up	Mid	Down	Up	Mid	Down
GLVs	T_1	16.6±0.7a	20.0±0.2a	16.1±0.7a	15.9±0.6b	18.9±0.6a	14.3±0.9a
	T_2	19.1±0.8a	19.0±0.3a	14.2±2.3ab	19.5±1.4a	19.6±0.6a	13.1±1.1ab
	T_3	19.9±0.6a	20.2±1.1a	12.8±1.6abc	19.8±0.4a	19.9±0.5a	10.7±1.3ab
	T_4	20.7±1.0a	21.9±1.1a	10.7±1.5bc	20.5±0.5a	19.8±1.5a	8.9±1.3b
	T_5	17.8±1.9a	18.2±0.7a	9.5±0.6c	18.1±1.1ab	17.1±0.6a	
Terp	T_1	38.4±2.8c	72.9±6.7ab	37.1±2.9a	36.9±4.6c	43.7±2.0c	28.8±5.9a
	T_2	68.3±5.2ab	85.6±13.4a	30.8±4.2a	57.2±2.0b	55.7±3.6ab	26.1±3.2a
	T_3	82.2±3.9a	75.3±1.3ab	27.2±2.2ab	78.5±0.7a	61.0±3.4a	16.9±5.0a
	T_4	65.2±10.1ab	69.1±6.6ab	17.5±0.2bc	55.9±4.8b	47.6±1.5bc	9.2±3.2a
	T_5	58.7±4.4b	58.0±6.1b	12.5±6.2c	53.5±4.7b	39.3±3.3c	
Chl *a*							
(mg/g FW)	T_1	2.3±0.0b	3.4±0.1a	2.3±0.1a	2.2±0.1b	3.2±0.0a	1.7±0.0a
	T_2	3.1±0.4a	3.4±0.0a	2.0±0.0b	2.9±0.1a	2.8±0.0b	1.4±0.0b
	T_3	3.2±0.0a	3.1±0.4ab	1.8±0.1b	2.8±0.2a	2.7±0.0c	1.2±0.0c
	T_4	2.9±0.2a	2.9±0.1b	1.6±0.2c	2.4±0.0b	2.5±0.0d	1.1±0.0d
	T_5	2.0±0.2b	2.3±0.1c	1.2±0.0d	1.7±0.0c	1.9±0.1e	
Ch *b*							
(mg/g FW)	T_1	0.7±0.0c	1.1±0.1a	0.8±0.0a	0.6±0.0c	0.9±0.0a	0.6±0.0a
	T_2	1.0±0.1a	1.1±0.1a	0.7±0.0b	0.9±0.0ab	0.9±0.0b	0.6±0.0a
	T_3	1.0±0.0a	1.0±0.1ab	0.6±0.0c	0.9±0.1a	0.8±0.0c	0.5±0.0b
	T_4	0.9±0.0b	1.0±0.0b	0.6±0.0c	0.8±0.0b	0.8±0.0c	0.5±0.0b
	T_5	0.7±0.0c	0.8±0.0c	0.5±0.0d	0.6±0.0c	0.7±0.0d	
Chl *a/b*	T_1	3.4±0.0a	3.1±0.3a	2.9±0.1a	3.5±0.2a	3.5±0.0a	2.8±0.2a
	T_2	3.2±0.3a	3.1±0.2a	2.9±0.1a	3.3±0.1ab	3.2±0.0b	2.5±0.0bc
	T_3	3.2±0.1a	3.1±0.2a	3.0±0.2a	3.1±0.1b	3.2±0.1b	2.6±0.1ab
	T_4	3.2±0.2a	3.0±0.1a	2.7±0.3a	2.9±0.1c	3.0±0.0c	2.4±0.1bc
	T_5	3.1±0.3a	2.9±0.1a	2.6±0.1a	2.7±0.0d	2.8±0.1d	
MDA							
(ìmol/g FW)	T_1	24.5±2.1c	36.5±3.4d	37.0±2.8d	23.0±3.9d	38.2±2.8d	40.6±1.5c
	T_2	31.7±3.8b	39.2±2.6cd	41.7±3.5cd	34.6±1.5c	39.3±3.1cd	42.5±2.4bc
	T_3	39.0±1.7a	42.0±3.8bc	43.9±1.9bc	42.5±2.3b	45.0±4.6bc	47.3±4.4ab
	T_4	41.5±1.4a	45.4±2.2ab	47.0±1.6b	47.4±4.9b	49.7±3.0ab	53.0±2.7a
	T_5	42.4±2.1a	48.2±0.5a	53.4±3.0a	53.7±2.4a	53.1±1.6a	
H_2O_2							
(ìmol/g FW)	T_1	146.3±15.4d	196.5±11.2c	285.7±16.7c	164.7±4.9d	274.6±25.4c	332.4±21.8c
	T_2	236.2±7.1c	304.8±15.5b	336.8±12.1b	369.1±11.4c	389.6±15.9b	412.0±31.6b
	T_3	328.9±30.1b	318.6±24.9b	360.5±21.4b	411.8±28.2b	419.3±10.1b	451.0±16.2ab
	T_4	356.6±18.0b	365.3±22.0a	422.0±46.0a	425.0±24.7b	464.4±24.1a	472.0±35.2a
	T_5	395.1±17.8a	391.0±31.5a	437.1±24.5a	501.1±30.8a	474.3±26.7a	

Different letters within the same row indicate mean values statistically different at $P < 0.05$

significantly lower than the mid and up leaves also provide evidence for this. This is in agreement with the prior results that some BVOCs emission is light dependent (Schuh *et al.* 1997, Gouinguene and Turlings 2002, Rasulov *et al.* 2009).

Our result also indicates that leaf aging also remarkably affects BVOCs emission pattern. On one hand, juvenile leaves metabolism are sharper, which can provide sufficient substrate for BVOCs synthesis; on the other hand, juvenile leaves is the growth center, more assimilates redistribution

or translocation to this center organ (Bloom *et al.* 1985, Radhika *et al.* 2008).

SUMMARY

A study was conducted to access BVOCs emission pattern during leaf senescence in parallel with monitoring leaf Chl degradation and MDA accumulation. BVOCs emission rate and Chl content of leaves in the lower canopy position were significantly lower than leaves in the mid and up positions. While MDA and H_2O_2 content of the lower position leaves were significantly higher than the up and mid position leaves. Positive correlation between leaf volatile emission rate and chlorophyll content and negative correlation between leaf volatile emission rate and MDA and H_2O_2 content were observed. VOCs emission pattern during the cereal crop aging could provide more evidence for age-related resistance though the results here we show are *in vitro*.

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