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Intraspecific Responses in Flavonoid Contents of 28 Wild Sugarcane Clones to Enhanced Ultraviolet-B Radiation under Field Conditions

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Abstract: Field studies were conducted for two consecutive years to determine the potential for alterations in flavonoid contents and the intraspecific variation in (absorption 305 nm) sensitivity of 28 wild sugarcane (*S. spontaneum* L.) clones to enhanced ultraviolet-B (UV-B, 280-315 nm) radiation. The supplemental UV-B radiation was 5.00 kJ m⁻², simulating a depletion of 20% stratospheric ozone at Kunming (25°N, 1950 m). Across all clones tested in the present study, the effect of UV-B radiation on flavonoid contents in different developmental stages of 28 wild sugarcane clones were observed both in 2003 and 2004. In general, intraspecific responses of flavonoid contents of wild sugarcane clones to enhanced UV-B radiation existed both in 2003 and 2004. Response sensitivities of flavonoid contents of 28 wild sugarcane clones to UV-B radiation in different stages have the following sequence: elongation > flowering > tillering both in 2003 and 2004. So, intraspecific difference to UV-B radiation was indicated by the percentage change of flavonoid contents in elongation of wild sugarcane in 2003 and 2004. According to the ranking of intraspecific sensitivity and hierarchical cluster analysis in elongation, tolerant clones were 83-181, II91-5, 90-22, 93-25, I91-38, II91-13 and 92-11, sensitive clones were 92-26, 92-36, 83-153 and II91-93. 83-181 was the most tolerant clones both in 2003 and 2004. Wild sugarcane clones originating from regions with high ambient UV-B (lower latitude or high elevation) were not necessarily the more tolerant to enhanced UV-B radiation.

Key words: Flavonoid contents, intraspecific responses, *Saccharum spontaneum* L., stratospheric ozone depletion, UV-B radiation

Introduction

Decrease in stratospheric ozone concentrations has been confirmed by satellite measurements. The most pronounced thinning of the ozone layer has been measured over the Antarctic continent, with up to 71% depletion during the Antarctic spring (Kerr, 1993). Recent mathematical models predict a further increase in solar UV-B irradiation in future years (Madronich *et al.*, 1995). UV-B radiation effects on plants have been the subject of considerable research (Caldwell *et al.*, 1995). Flavonoids have been proposed to absorb UV-B radiation in upper tissue layer and preventing damage to sensitive target, so, study on intraspecific differences in flavonoid contents response to UV-B radiation was

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very important. Whilst intraspecific responses in flavonoid metabolism in *Cucumis sativus* (Murali and Teramura, 1986), soybean (D' surney *et al.*, 1993) and *A. thaliana* (Li *et al.*, 1993; Fiscus *et al.*, 1999) and in flavonoid contents in rice (Teramura *et al.*, 1991), in wheat (Li *et al.*, 2000a) and in soybean (Zu *et al.*, 2003; Yao *et al.*, 2005) have been reported, little is known about effect of enhanced UV-B radiation on flavonoid contents and intraspecific differences in flavonoid contents response to UV-B radiation under field conditions (Teramura *et al.*, 1991; Li *et al.*, 2000a; Zu *et al.*, 2003), therefore further studies are required in this area.

In the past two decades, most of the UV-B research has been conducted as short-term experiments in crop growth chambers and greenhouses, where an unnatural spectral balance of radiation may have substantially changed plant sensitivity to UV-B. In growth chamber and greenhouse experiments, the visible and UV-A radiation is usually much less than in sunlight, thus, even if realistic levels of UV-B are used in simulating ozone reduction, the plant response may be exaggerated relative to field conditions. Only field studies can provide realistic assessments of what will happen as the stratospheric ozone layer thins (Caldwell *et al.*, 1995).

At low latitude, the daily UV-B irradiance received at high altitude sites could be nearly six fold greater than the maximum does at arctic latitude. Plants that naturally grow in low latitude may have evolved specific adaptation to protect from the deleterious effects of UV-B radiation. Plant growing in a naturally high UV-B environment may have developed or maintained mechanisms related to reproductive, phenology and ecotypic differentiation may have occurred, intraspecific response to increasing UV-B radiation may exist over an elevational gradient (Ziska *et al.*, 1992; Sullivan *et al.*, 1992). Wild plant sensitivity to UV-B radiation may be related with its ambient UV-B radiation.

Wild sugarcane (*S. spontaneum* L.) is one of the wild parent species of sugarcane and could be used for fodder. The effects of enhanced UV-B radiation on photosynthetic characteristics, growth of sugarcane have been studied (Elawad *et al.*, 1985). Unfortunately, for wild sugarcane, few studies have been conducted with UV-B radiation under field conditions. In the present study, we choice 28 wild sugarcane clones that come from different altitude (from 0 to 1900 m) and latitude (from South China to Northwest China) sites in China and America are chosen. Twenty eight wild sugarcane clones were grown in field under ambient and supplemental levels of UV-B radiation for two consecutive years with the objective to (1) determine if 28 wild sugarcane clones differed in flavonoid contents response to UV-B radiation under field conditions and (2) evaluate intraspecific differences in flavonoid contents of 28 wild sugarcane clones to UV-B radiation in the field. The present study hypothesized: (1) enhanced UV-B radiation would affect flavonoid contents and result in intraspecific differences in flavonoid contents under field conditions and (2). intraspecific differences in flavonoid contents of wild sugarcane clones to UV-B radiation may be related with ambient UV-B radiation of originating region.

Materials and Methods

Plant Materials and Growth Conditions

The field experiment was conducted at Yunnan Agricultural University, Kunming, China for two consecutive years during 2003 and 2004. No fertilization was necessary during the season. The soil properties were pH 7.28, total-N 1.36%, Total-K 0.54%, Total-P 0.83%, available N 150.78 mg kg⁻¹, available K 185.89 mg kg⁻¹ and available P 36.58 mg kg⁻¹.

Twenty eight wild sugarcane (*Saccharum spontaneum* L.) clones were collected from original sites, i.e., Hainan, Guandong, Guanxi, Fujian, Sichuan, Yunnan, Shanxi, Tibet, China and Miami, American. Their origin, altitude and latitude were showed in Table 1. Wild sugarcane clones were planted in Sugarcane Garden of Sugarcane Research institute, Yunnan Agricultural University before this study. In this experiment, seedling of wild sugarcane clones were obtained from Sugarcane Garden and were planted at 0.4 m apart at a density of 15 seedlings m⁻² in 56×3 plots of 2.0×1.0 m each on March 14, 2003,

Table 1: 28 wild sugarcanes (*S. spontaneum* L.) clones together with their origin, altitude and latitude

Clones	Origin	Altitude (m)	Latitude (°N)
88-269	Gejiu, Yunnan	220.0	23
88-270	Gejiu, Yunnan	220.0	23
83-181	Gejiu, Yunnan	1040.0	23
83-153	Gejiu, Yunnan	1250.0	23
83-217	Lushui, Yunnan	1900.0	26
83-157	Pingbian, Yunnan	570.0	23
82-110	Jinghong, Yunnan	570.0	22
I91-91	Zizhong, Sichuan	250.0	30
I91-48	Lianzhong, Sichuan	350.0	37
I91-38	Ziyan, Sichuan	450.0	30
I91-37	Jiayan, Sichuan	550.0	30
92-36	Haikou, Hainan	3.6	20
92-11	Yacheng, Hainan	12.0	18
93-25	Chahe, Hainan	100.0	20
92-26	Zhazhou, Hainan	200.0	20
92-4	Tongshif-Hainan	310.0	18
II91-98	Gaoyao, Guandong	0.0	22
II91-93	Huizhou, Guandong	20.0	23
II91-89	Haifen, Guandong	40.0	23
II91-99	Luodiong, Guandong	80.0	23
II91-126	Tiayang, Guanxi	100.0	23
II91-116	Linming, Guanxi	200.0	23
II91-72	Xianyou, Fujian	30.0	26
II91-81	Zhaocan, Fujian	60.0	26
II91-5	Chenggu, Shanxi	500.0	34
II91-13	Liuba, Shanxi	600.0	34
90-22	Cayu, Tibet	1650.0	28
Holes	Miami, American	4.0	26

an appropriate sowing date for wild sugarcane in Kunming. The overall experimental design was a randomized complete block with two UV-B treatments and 3 replications. At the 45 days after planting, plants were thinned to 10 shoot m⁻² for uniformity in growth. This planting density is within commonly used sowing practices for the Kunming region. Then, supplemental UV-B radiation was provided, leaf samples were collected and flavonoid contents were tested. Shoots were harvested in ripening stage on December 25, 2003. Wild sugarcane was perennial herb. During the early of April, 2004, new shoots of wild sugarcane clones grew from roots. On April 30, 2004, plants were thinned to 10 shoot m⁻² for uniformity in growth. Then, experiments of 2003 were undertaken again.

UV-B Radiation

Supplemental UV-B radiation was provided by filtered Gucun brand (Gucun Instrument Factory, Shanghai, China) 30W sunlamps. The wavelength of sunlamps was 280-315 nm (Li *et al.*, 2000a). Lamps were suspended above and perpendicular to the planted rows (rows oriented in an east-west direction to minimize shading) and filtered with either 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for supplemental UV-B radiation or 0.13 mm polyester plastic films (absorbs all radiation below 320 nm) as a control (Sullivan and Teramura, 1990). Cellulose diacetate filters were presolarized for 8 h and changed weekly to ensure uniformity of UV-B transmission. The spectral irradiance from the lamps was determined with an Optronics Model 742 (Optronics Laboratories Inc. Orlando, FL, USA) spectroradiometer. The spectral irradiance was weighted with the generalized plant response action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain UV-B_{BE}. 6 lamps were installed above each plot. Plants were irradiated for 7 h daily from thinning, April 30 to ripening stage, both in 2003 and 2004, centered around solar noon. Plants under polyester-filtered lamps received only ambient levels of UV-B radiation (10.00 kJ m⁻² UV-B_{BE} during clear sky conditions on the summer solstice). Plants beneath the cellulose diacetate filters received ambient plus supplemental levels of UV-B. The lamp height above the plants was adjusted weekly to maintain a distance of 0.45 m

between the lamps and the top of the plants and provided supplemental irradiances of 5.00 effective $\text{kJ m}^{-2} \text{UV-B}_{\text{BE}}$. This supplemental level was similar to that which would be experienced at Kunming (25°N, 1950 m) with a 20% stratospheric ozone reduction during a clear day on the summer solstice (10.00 $\text{kJ m}^{-2} \text{UV-B}_{\text{BE}}$) according to a mathematical model of Madronich *et al.* (1995). Total daily photosynthetic photon fluence (PPF between 400-700 nm) under lamp fixtures was 90% of that above the lamps.

Measurements and Statistical Analysis

In tillering (July 15), elongation (August 20) and flowering (October 28) in 2003 and 2004. Two samples of leaves were taken from each plot for test of flavonoid contents. Leaf discs of 100 mm^2 were taken from leaves and extracted in 10 ml of acidified methanol (79:20:1 v/v, methanol, water, HCl) for flavonoid measurement, according to the procedure of Mirecki and Teramura (1984). Extract absorbance at 305 nm measured on a spectrophotometer was used as a measure of flavonoid.

Statistical differences between means of control and UV-B radiation treatment of any measured parameter were determined by t-test at the $p < 0.05$ or $p < 0.01$ level, $n = 6$. Relationships between parameters (%change of flavonoid contents, latitude and altitude) of wild sugarcane clones were determined by SPSS at $p < 0.05$ or $p < 0.01$ level, $n = 28$.

Hierarchical cluster analysis was determined by SPSS according to Euclid Method. For test intraspecific differences in overall flavonoid content responses of 28 wild sugarcane clones to UV-B radiation in elongation both in 2003 and 2004, variables selected percentage change of flavonoid contents of 28 wild sugarcane clones in elongation in 2003 and 2004.

Results

Flavonoid Contents in 2003

The effect of UV-B radiation on flavonoid contents in different developmental stages of 28 wild sugarcane clones in 2003 were showed in Table 2. Results showed a significant increase for 4 clones ($p < 0.01$ or 0.05), decreases in 4 clones ($p < 0.01$ or 0.05) and no significant effect on the other 20 clones ($p > 0.05$) in tillering, a significant increase for 4 clones ($p < 0.01$ or 0.05), decreases in 7 clones ($p < 0.01$ or 0.05) and no significant effect on the other 17 clones ($p > 0.05$) in elongation and a significant increase for 6 clones ($p < 0.01$ or 0.05), decreases in 3 clones ($p < 0.01$ or 0.05) and no significant effect on the other 19 clones ($p > 0.05$) in flowering. Response sensitivities of flavonoid contents of 28 wild sugarcane clones to UV-B radiation in different stages have the following sequence: elongation > flowering > tillering in 2003.

Flavonoid Contents In 2004

Table 2 showed flavonoid content responses in different developmental stages of 28 wild sugarcane to enhanced UV-B radiation under field conditions. A significant increase for 5 clones ($p < 0.01$ or 0.05), decreases in 12 clones ($p < 0.01$ or 0.05) and no significant effect on the other 11 clones ($p > 0.05$) in tillering, a significant increase for 16 clones ($p < 0.01$ or 0.05), decreases in 4 clones ($p < 0.01$ or 0.05) and no significant effect on the other 8 clones ($p > 0.05$) in elongation and a significant increase for 8 clones ($p < 0.01$ or 0.05), decreases in 9 clones ($p < 0.01$ or 0.05) and no significant effect on the other 11 clones ($p > 0.05$) in flowering were observed. Response sensitivities of flavonoid contents of 28 wild sugarcane clones to UV-B radiation in different stages have the following sequence: elongation > flowering > tillering in 2004.

Intraspecific Differences In Flavonoid Contents

Table 3 indicated intraspecific differences in flavonoid contents of 28 wild sugarcane clones to UV-B radiation existed under field conditions for two consecutive years, although intraspecific

Table 2: Intraspecific sensitivity to UV-B radiation based on percentage change of flavonoid contents (absorption 305 nm) of 28 wild sugarcane (*S. spontaneum* L.) clones under field conditions in 2003 and 2004

Clones	2003			2004		
	Tillering	Elongation	Flowering	Tillering	Elongation	Flowering
88-269	-8.43	-12.96	-4.99	-56.50**	-28.08**	10.82
88-270	1.69	-23.58	4.54	-13.15	104.73**	-38.76*
83-181	26.84*	72.89*	22.84*	-41.82*	154.05**	-62.51**
83-153	21.48	-44.56*	21.25	-51.38*	29.40	-45.26**
83-217	-2.43	-6.95	105.80**	-28.18**	42.93*	238.64**
83-157	235.51**	-32.29**	-15.50	-29.65*	109.68**	-21.94
82-110	-34.65*	-45.09**	37.67**	39.12**	67.97**	-35.30**
I91-91	-0.59	-4.30	0.56	-75.33**	-63.23**	102.52**
I91-48	-25.25*	-18.38	8.06	-52.49**	31.06**	21.79
I91-38	-12.11	29.87**	-6.35	-41.80*	65.04**	114.41**
I91-37	-3.41	-38.38*	-4.40	41.22*	73.07**	136.76**
92-36	40.26*	-33.46*	-24.80**	-17.75	-17.62	79.39*
92-11	7.30	15.84	-35.20**	21.20	106.27**	-55.10**
93-25	0.72	-3.67	0.85	-1.28	128.33**	-1.27
92-26	-0.59	-31.83*	-6.78	19.64	-51.43**	-70.93**
92-4	13.79	5.83	9.45	92.20**	-23.82	-1.44
II91-98	-9.52	2.02	30.63*	-52.27*	-26.57	-56.02**
II-91-93	-20.51*	-31.76**	8.17	-38.10*	10.39	164.60**
II91-89	-5.13	12.94	-0.47	-37.97**	14.18	-71.67**
II91-99	-14.33	-23.85	15.67*	-42.39**	35.08*	54.09*
II91-126	-0.65	43.10*	-4.69	11.27	-32.00	-8.91
II91-116	-15.55**	10.08	-18.60**	45.14*	-5.56	15.66
II91-72	-22.52	1.28	6.43	47.09**	-54.31*	-8.95
II91-81	21.23*	-21.28	22.93**	23.47	108.30**	-17.68
II91-5	-1.66	1.52	-18.30	10.57	138.20**	-26.42
II91-13	-0.09	13.83	-7.08	18.14	122.48**	102.47**
90-22	-19.20	49.63**	-3.28	4.84	72.94**	-45.69**
Holes	17.97	-34.65	-19.20	-0.62	86.24**	-3.80

**and*, Significant difference between control and UV-B radiation at p<0.01 or p<0.05 according to T-test

Table 3: The ranking of intraspecific sensitivity to UV-B radiation based on percentage change of flavonoid contents (absorption 305 nm) of 28 wild sugarcane (*S. spontaneum* L.) clones under field conditions in 2003 and 2004

Rank	Tillering		Elongation		Flowering	
	2003	2004	2003	2004	2003	2004
1	83-157 (235.51)	92-4 (92.20)	83-181 (72.89)	83-181(154.05)	83-217 (105.80)	83-217 (238.64)
2	92-36 (40.36)	II91-72 (47.09)	90-22 (49.63)	II91-5 (138.20)	82-110 (37.67)	II91-93 (164.60)
3	83-181(26.84)	II91-116 (45.14)	II91-126 (43.10)	93-25 (128.33)	II91-98 (30.63)	I91-37 (136.76)
4	83-153 (21.48)	I91-37 (41.22)	I91-38 (29.87)	II91-13 (122.48)	II91-81 (22.93)	I91-38 (114.41)
5	II91-81 (21.33)	82-110 (39.12)	92-11 (15.84)	83-157 (109.68)	83-181 (22.94)	I91-91 (102.52)
6	Holes (17.97)	II91-81 (23.47)	II91-13 (13.83)	II91-81 (108.30)	83-153 (21.25)	II91-13 (102.47)
7	92-4 (13.79)	92-11 (21.20)	II91-89 (12.94)	92-11 (106.27)	II91-99 (15.67)	92-36 (79.39)
8	92-11 (7.30)	92-26 (19.64)	II91-116 (10.08)	88-270 (104.73)	92-4 (9.45)	II91-99 (54.09)
9	88-270 (1.69)	II91-13 (18.14)	92-4 (5.83)	Holes (86.24)	II91-93 (8.17)	I91-48 (21.79)
10	93-25 (0.72)	II91-126 (11.27)	II91-98 (2.02)	I91-37 (73.07)	I91-48 (8.06)	II91-116 (15.66)
11	II91-13 (-0.09)	II91-5 (10.57)	II91-5 (1.52)	90-22 (72.94)	II91-72 (6.43)	88-269 (10.68)
12	I91-91 (-0.59)	90-22 (4.84)	II91-72 (1.28)	82-110 (67.97)	88-270 (4.54)	93-25 (-1.27)
13	92-26 (-0.59)	Holes (-0.62)	93-25 (-3.67)	I91-38 (65.04)	93-25 (0.85)	92-4 (-1.44)
14	II91-126 (-0.65)	93-25 (-1.28)	I91-91 (-4.30)	83-217 (42.93)	I91-91 (0.56)	Holes (-3.80)
15	II91-5 (-1.66)	88-270 (-13.15)	83-217 (-6.95)	II91-99 (35.08)	II91-89 (-0.47)	II91-126 (-8.91)
16	83-217 (-2.43)	92-36 (-17.75)	88-269 (-12.96)	I91-48 (31.06)	90-22 (-3.28)	II91-72 (-8.95)
17	I91-37 (-3.41)	83-181 (-28.18)	I91-48 (-18.38)	83-153 (29.40)	I91-37 (-4.40)	II91-81 (-17.68)
18	II91-89 (-5.13)	83-157 (-29.65)	II91-81 (-21.28)	II91-89 (14.18)	II91-126 (-4.69)	83-157 (-21.94)
19	88-269 (-8.43)	II91-89 (-37.97)	88-270 (-23.58)	II91-93 (10.39)	88-269 (-4.99)	II91-5 (-26.42)
20	II91-98 (-9.53)	II91-93 (-38.10)	II91-99 (-23.85)	II91-116 (-5.56)	I91-38 (-6.35)	82-110 (-35.30)
21	I91-38 (-12.11)	I91-38 (-41.80)	II91-93 (-31.76)	92-36 (-17.62)	92-26 (-6.78)	88-270 (-38.76)
22	II91-99 (-14.33)	83-181 (-41.82)	92-26 (-31.86)	92-4 (-23.82)	II91-13 (-7.08)	83-153 (-45.26)
23	II91-116 (-15.55)	II91-99 (-42.39)	83-157 (-32.29)	II91-98 (-26.57)	83-157 (-15.50)	90-22 (-45.69)
24	90-22 (-19.20)	83-153 (-51.38)	92-36 (-33.46)	88-269 (-28.08)	II91-5 (-18.30)	92-11 (-55.10)
25	II91-93 (-20.51)	II91-98 (-52.27)	Holes (-34.65)	II91-126 (-32.00)	II91-116 (-18.60)	II91-98 (-56.02)
26	II91-72 (-22.52)	I91-48 (-52.49)	I91-37 (-38.38)	92-26 (-51.43)	Holes (-19.20)	83-181 (-62.51)
27	I91-48 (-25.25)	88-269 (-56.50)	83-153 (-44.56)	II91-72 (-54.31)	92-36 (-24.80)	92-26 (-70.93)
28	82-110 (-34.65)	I91-91 (-75.33)	82-110 (-45.09)	I91-91 (-63.23)	91-11 (-35.20)	II91-89 (-71.67)

Ranking 1-28 is in the order of increasing sensitivity to UV-B radiation. Clone with the same ranking were preceded by a line. Values in parentheses represent (%) change relative to control

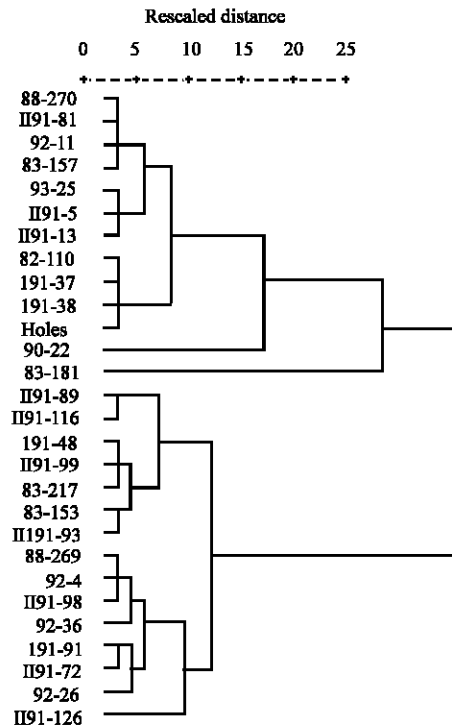


Fig. 1: Hierarchical cluster analysis for intraspecific difference in sensitivity to UV-B radiation based on the percentage change of flavonoid contents in elongation in 2003 and 2004

difference in 2004 were more obvious than that in 2003 (Table 2). However, in 28 wild sugarcane clones, the ranking of flavonoid contents in tillering, elongation or flowering were different in 2003 or 2004 (Table 3). In elongation, response sensitivity of flavonoid contents of 28 wild sugarcane clones to UV-B radiation was the most obvious both in 2003 and 2004. So, intraspecific difference of flavonoid contents of 28 wild sugarcane clones to UV-B radiation was indicated by the percentage change of flavonoid contents in elongation both in 2003 and 2004. According to the ranking of intraspecific sensitivity to UV-B radiation (Table 3) and hierarchical cluster analysis based on the percentage change of flavonoid contents (Fig. 1) in elongation in 2003 and 2004, tolerant clones were 83-181, II91-5, 90-22, 93-25, 191-38, II91-13 and 92-11, sensitive clones were 92-26, 92-36, 83-153 and II91-93. 83-181 was the most tolerant clones both in 2003 and 2004.

Discussion

This is the first study to identify intraspecific responses in flavonoid contents of 28 wild sugarcane clones to enhanced ultraviolet-B radiation under field conditions in different developmental stages for two consecutive years. This is supported by the earlier finding of intraspecific responses in flavonoid metabolism in *Cucumis sativus* (Murali and Teramura, 1986), soybean (D' surney *et al.*, 1993) and *A. thaliana* (Li *et al.*, 1993; Fiscus *et al.*, 1999) and in flavonoid contents in rice (Teramura *et al.*, 1991), in wheat (Li *et al.*, 2000a) and in soybean (Zu *et al.*, 2003; Yao *et al.*, 2005).

Flavonoids have been proposed by some investigators to act as optical filters, by absorbing UV-B radiation in upper tissue layer and thus preventing damage to sensitive targets (Schmitz and

Weissenbock, 2003). In recent years, a series of experiments have provided convincing evidence that plants subjected to UV-B radiation responded by changes in the content and ratios of different flavonoid in leaf epidermal cells, wax, hairs (Harborne and Williams, 2000). Changes in flavonoid contents in leaves of wheat and soybean have been observed under field conditions (Li *et al.*, 2000b; Zu *et al.*, 2003). Recent results from studies with cucumber suggest that intraspecific responses could be at least partly due to inherent intraspecific differences in the accumulation of leaf flavonoids (Teramura and Murali, 1986). The result of Reuber *et al.* (1996) suggests that a flavonoid mutant of barley (*Hordeum vulgare*) exhibits increased sensitivity to UV-B radiation in the primary leaf. The content of flavonoid in barley mutant was only 7% as compared to the mother variety in the primary leaf. Differences in UV-absorption characteristics between the mutant and the mother line were clear from the spectrophotometer analysis (Reuber *et al.*, 1996). In a flavonoid mutant of barley (*H.vulgare*), the lower contents of flavonoid in primary leaves was correlated with a decline in apparent quantum yield and the less robust appearance of the mutant plants as compared to the mother variety (Reuber *et al.*, 1996).

For barley cv. Atlas, L-phenylalanine ammonia-lyase (PAL) was assumed to play a key role in the UV-B acclimatization by flavonoid (Liu and McClure, 1995). In primary leaves of barley cv. Gerbel, the UV-mediated increased accumulation of flavonoid was close correlated with increased activity and amount of immunologically detectable Chalcone Synthase (CHS). In leaves of several plant species, including the gramineous plant rye (*Secale cereale*), CHS was found to play a major role in UV regulation of the flavonoid pathway (Reuber *et al.*, 1996). In another experiment, the substantial suppression of growth of *A. thaliana* flavonoid mutants by UV-B radiation suggests that the disruption of secondary metabolism at the chalcone isomerase site caused an impairment of the normal growth and differentiation process (Fiscus *et al.*, 1999).

The flavonoid content was a good indicator to assess plant sensitivity to enhanced UV-B radiation. In an earlier study, flavonoid contents were different in various experiments. Because plants respond differently to UV-B in each environment, in addition to the inherent genetic differences in different clones, variability between experiments could also be due to differences in growth conditions, length of UV-B radiation, stage of growth and the ratio of incident PPF to UV-B radiation, all of which have been demonstrated to greatly modify UV-B responsiveness (Teramura *et al.*, 1991). In the present study, the flavonoid contents were measured in crops in different developmental stages under field conditions for two consecutive years. So, the results of this study might be reasonable and will provide realistic assessments of intraspecific responses of flavonoid contents in 28 wild sugarcane clones to enhanced UV-B radiation. UV-B radiation may change the synthetic or decomposition processes of flavonoid, then change flavonoid contents. In general, intraspecific differences of flavonoid contents of 28 wild sugarcane clones to UV-B radiation in 2004 were higher than that in 2003. Wild sugarcane was perennial herb, the shoots of 2004 were grown from the roots of 2003, which got UV-B radiation influences and accumulation (Li *et al.*, 1998), may have more adaptation to UV-B radiation.

Ambient UV-B radiation level is great at lower latitude or higher altitude than that at higher latitude or lower altitude. Differences in solar UV-B radiation along latitude or elevation gradients may have result in diverse habitats of wild plants. The habitats were related with plant sensitivities to UV-B radiation. Plant species and even genotypes within species can differ greatly in their responses to UV-B. Reasons for this are related to natural UV-B radiation environment in which these plants grow (Sullivan *et al.*, 1992). It is generally assumed that plants originating near the equator or higher mountain are more tolerant UV-B radiation. The distribution range of wild sugarcane is very wide in China. In this study, wild sugarcane clones were collected from lower latitude (18°N) to higher latitude (37°N) and from lower elevation (0 m) to higher elevation (1900 m). Correlations between latitude or altitude and percentage change of flavonoid contents of 28 wild sugarcane in elongation in 2003 or 2004 were not significant ($p > 0.05$). Wild sugarcane clones originating from regions with high ambient UV-B

(lower latitude or high elevation) were not necessarily the more tolerant to enhanced UV-B radiation. In the previous experiment, the similar result was observed in rice cultivars (Barnes *et al.*, 1993; Dai *et al.*, 1994). Although different results were shown in wheat in field study (Li *et al.*, 2000a). The physiological mechanism and genetic basis for these differences must be further examined.

In conclusion, enhanced UV-B radiation had significant effect on flavonoid contents of 28 wild sugarcane clones in different developmental stages for two consecutive years under field conditions. The response of flavonoid contents of wild sugarcane clones in different developmental stages, in 2003 and 2004 to enhanced UV-B radiation were different. Intraspecific differences in flavonoid contents of 28 wild sugarcane clones in different developmental stages for two consecutive years to enhanced UV-B radiation were complex. Wild sugarcane clones originating from regions with high ambient UV-B (lower latitude or high elevation) were not necessarily the more tolerant to enhanced UV-B radiation. Much is still unknown about the mechanism of intraspecific differences in flavonoid contents of wild sugarcane clones to enhanced UV-B radiation. To better understand these processes, it will be necessary to study a long term and broad range of growth, physiological responses and genetic control to UV-B radiation under field conditions.

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