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Seasonal Variations in Total Antioxidant Capacity and Total Phenolics Content of Leaves of *Phyllostachys* Taxa Using Different Extraction Methods

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Abstract

Changes in total phenolic content (TP) and total antioxidant capacity (AC) during the vegetation period (April-November) were analysed in *Phyllostachys aureosulcata* f. *aureocaulis (PAA), P. flexuosa (PF), P. humilis (PH), P. sulphurea* var. *sulphurea (PSS).* Different extraction methods were compared: infusion or decoction with water or aqueous methanol. The highest significant value for TP was measured in the case of infusion at 90 °C for 5 min, which lead to the highest value for AC. During the vegetation period the highest values of TP were measured in April and May in the case of *PAA* (409.5; 314.9 and 258.7; 119.0 μg GA/ml) and *PH* (388.4; 411.6 and 252.9; 253.3 μg GA/ml). There was a clear trend in the changes of TP, with high values in April and November and a peak during August-September. Similar to TP, the highest values of AC were measured in April and May in *PH* (519.7; 566.3 and 513.5; 510.4 μg AA/ml) and *PAA* (534.5; 337.8 and 394.9; 275.4 μg AA/ml). We compared the change of TP in all taxa with stress index values derived from daily maximum and minimum air temperature, cumulative precipitation plus irrigation and cumulative evapotranspiration values. A close correlation $R^2 = 0.32$ ($p=0.001$) was found between stress index values and the change in TP of all taxa. The correlation is even closer $R^2 = 0.52$ ($p=0.001$) with *PF, PH* and *PSS*. Our results with *PAA* can be explained by possible inter/intra-specific differences in freeze tolerance and cold-acclimation.

Keywords: bamboo, *Bambusoideae, herba*, *Poaceae*, tea

Introduction

The bamboo genus *Phyllostachys* originated in the warm temperate regions of SE China and has long been cultivated and has naturalized in neighbouring countries such as Korea and Japan (Ohrnberger, 2002). *Phyllostachys* taxa have significant importance in the agriculture of Asian countries, mainly in forestry and olericulture (Kleinhenz and Midmore, 2001), but are also used in traditional Korean herbal medicine (Kim *et al.*, 1995) and as ornamentals (Ohrnberger, 2002). The determination of human health promoting properties and phytochemicals of horticultural crops, including the measurement of antioxidant activity or total phenolics has been one of the targets of horticultural research in the past decades (Kwon *et al.*, 2008; Im *et al.*, 2010). The leaves of bamboo (*Herba Phyllostachys*) are used in traditional Chinese and Korean medical pharmacology (Kim *et al.*, 1995; Chen and Chen, 2004), but also other *herba* from other bamboo taxa are used in Korea (Park and Lim, 2009; Hwang *et al.*, 2007; Park *et al.*, 2007). These *herba* have high antioxidant properties (Hu *et al.*, 2012; You *et al.*, 2010; Park *et al.*, 2007) and contain flavonoids (Kim *et al.*, 2010), phenolic acids (Park *et al.*, 2007), chlorogenic acid derivatives (Kweon *et al.*, 2001), phenolic and flavone glycosides (Li *et al.*, 2008; Park *et al.*, 2007). Leaf extracts have been shown to have anticarcinogenic (Lin *et al.*, 2008; Shin *et al.*, 2003), cardioprotective (Fu *et al.*, 2006) effects, relieve lipotoxicity (Kim *et al.*, 1995; Panee *et al.*, 2008; Ryou *et al.*, 2012), protect against oxidative stress-induced glaucoma (Lee *et al.*, 2010; Kim *et al.*, 2013). Leaf extracts can synergistically be used in combination with other compounds in the treatment of immune/allergic diseases related to mast cells (Kim *et al.*, 2010), treatment of leukaemia (Kim *et al.*, 2007), in the prevention and treatment of diabetic complications (Jung *et al.,* 2007) and autoimmunity (Kim *et al.*, 2007). Bamboo leaves are also used as food additives (Oh and Lim, 2010), and bamboo leaf extracts also exhibit antimicrobial activity during food storage (Park and Lim, 2010). The studies of Jeong *et al.* (2008), In *et al.* (2010) and Kim *et al.* (2012) have evaluated the nutritional components and antioxidant activities of *Sasa borealis*, maengjong-juk (*Phyllostachys pubescens*) and ojuk (*Phyllostachys nigra*) leaf water extracts prepared by

traditional tea manufacturing processes. Therefore, the aim of the present study was to investigate the seasonal variations in antioxidant activity and total phenolic content of different *Phyllostachys* bamboo taxa leaves using different extraction methods.

Materials and methods

Plant material and sample preparation

For the comparison of different extraction methods, a sample comprised of 30 leaves that were randomly collected from a similar exposure and position within the canopy of *Phyllostachys flexuosa,* on the 30th of November 2010. The bamboos are growing in the Botanical Garden of Eötvös Lóránd University (BG ELTE), Budapest, Hungary (47°29" N latitude, 19°05'02" E longitude and 108 m altitude). Four repetitive samples were collected.

For the examination of seasonal effect, 30 leaves per each sample from a similar exposure and position within the canopy were randomly collected on the last day of each month, from April to November 2011, from the following *Phyllostachys* taxa: *P. aureosulcata f. aureocaulis, P. flexuosa, P. humilis, P. sulphurea var. sulphurea*. Four repetitive samples were collected each month while winter sampling (from December to March) was neglected to avoid the effect of unpredictable leaf tissue freeze damage.

Measurement of environmental parameters and irrigation

Air temperature (°C) and precipitation (mm) were recorded during the experiment (Fig. 1). Air temperature was measured six times per hour by a meteorological instrument. Plants were irrigated by overhead sprinklers between April 1 and October 10 every 14 days with 40 mm of water. Potential evapotranspiration (ET_0) was calculated from that used for other vegetable crops (Helyes and Varga, 1994; Helyes *et al.*, 2013); the amount of daily water demand was calculated based on the daily average temperature (in °C) divided by five and was expressed in millimetres:

$$I_d = \left(\frac{T_{\min} + T_{\max}}{2} \right) / 5$$

Stress index (SI) was calculated for each sampling date (month) from monthly cumulative values of daily minimum air temperature Tmin <= 5 °C (SI value 5/0) + daily maximum air temperature Tmax <= 5 °C (SI value 5/0) + daily average air temperature Tave <= 5 °C (SI value 5/0) + cumulative precipitation plus irrigation < cumulative potential evapotranspiration (SI value 1/0).

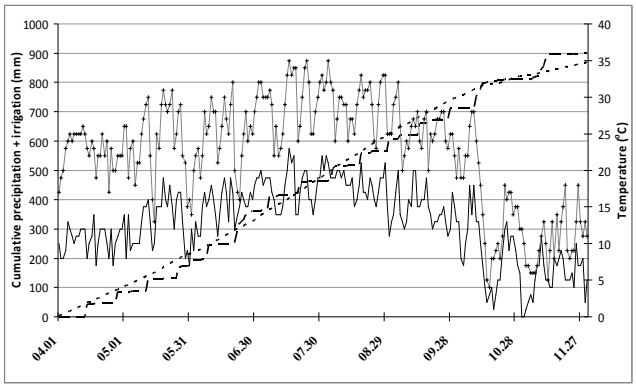

Fig. 1. Change of daily minimum, maximum air temperature (°C), cumulative precipitation plus irrigation (mm) and cumulative potential evapotranspiration (mm) during the vegetation period (April-November). Grey line (+), black line, dotted line, dashed line represents maximum temperature, minimum temperature, cumulative potential evapotranspiration, cumulative precipitation plus irrigation respectively.

Preparation of *Phyllostachys* leaf extracts

To compare the different extraction methods extracts were prepared from 65 °C air dried leaves of *Phyllostachys flexuosa*. Leaves were cut into small pieces, and 1 g of dried leaves were infused or decocted with 50 ml water (80, 90, or 100 °C; 5, 10, 20 min or 24 h or with aqueous methanol (25 °C; water/ methanol 80/ 20, v/v 72 h). For the investigation of the effect of seasonal variation, 65 °C air dried leaves of the different *Phyllostachys* taxa were also cut into small pieces, and 1 g of dried leaves were infused with 50 ml water (90 °C, 5 min or 24 h) or with aqueous methanol (25 °C; water/ methanol 80/ 20, v/v 72 h). The methanolic and aqueous extracts were stored at room temperature for 24 h. After centrifugation (13,000 rpm, 10 min) the supernatants were stored at -20 °C until the analyses.

Determination of total phenolics content

The total amount of soluble phenols (TP) was determined using Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1970) and determined spectrophotometrically at 760 nm. The content of soluble phenols was calculated from a standard curve obtained by different concentrations of gallic acid (GA) and given in μg GA/ml.

Determination of total antioxidant capacity by FRAP assay

The total antioxidant capacity (AC) related to ascorbic acid was determined spectrophotometrically using the FRAP (Ferric Reducing Antioxidant Power) method according to Benzie and Strain (1996). It is based on the reduction of the Fe^{3+}-TPTZ complex to the ferrous form at low pH. This reduction was monitored by measuring the absorption change at 593 nm. Results were expressed as μg equivalents of ascorbic acid (AA).

Statistical analysis

Results were expressed as the average plus/minus standard deviations. The data were analysed by two-factor analysis of variance (ANOVA) with repetitions and the means separated using the LSD test at p=0.05. Regression analysis was performed using Statistica 9 software.

Results and discussion

Effect of different extraction methods on total phenolic content and antioxidant capacity in *Phyllostachys flexuosa*

Fig. 2 shows that the highest TP values were obtained by aqueous infusion, compared to decoction or methanolic extraction. The highest significant value of 492.3±12.7 μg GA/ml for TP was measured in the case of infusion at 90 °C for 5 min which lead similarly the highest measured value (539.2±10.8 μg AA/ml) in the case of AC, but this latter was not significantly the highest compared to infusion at 80 °C for 5 min (531.4±19.5 μg AA/ml). The second and third highest (14 and 21% lower) TP values were measured in the case of infusion at 90 °C for 10 and 20 min (421.3±8.4 and 391.9±5.6 μg GA/ml) respectively, but the latter was not significantly higher than infusion at 80 °C for 20 min (365.6±25.7 μg GA/ml). TP value of infusion at 80 °C for 20 min (365.6±25.7 μg GA/ml) was not significantly lower than TP value of decoction at 20 min (363.4±6.7 μg GA/ml). In the case of AC there was no significant difference between the second, third and fourth highest measured values (531.3±19.3; 511.9±14.6; and 508.6±11.7 μg AA/ml) of infusion at 80 °C for 5 and 20 min and at 90 °C for 10 min respectively. AC values of infusion at 80 °C for 10 min and at 90 °C for 20 min (472.8±9.2 and 466.4±24.6 μg AA/ml) were significantly lower (by 7-12%) than the above extraction methods but were not significantly different compared to each other. Methanolic extraction for 72 h gave similarly high AC values (382.3±11.6 μg AA/ml) compared to decoction for 20 min (381.7±19.4 μg AA/ml) but these values were not significantly different from each other. AC values of methanolic extraction for 72 h (382.3±11.6 μg AA/ml) and decoction for 20 min (381.7±19.4 μg AA/ml) were 29% lower than infusion at 90 °C for 5 min (539.2±10.8 μg AA/ml). The method of extraction and the used solvent had a significant effect on extraction yield of polyphenols and on antioxidant activity in teas, but results vary depending on which solvent and method produces the highest levels. In *Camellia sinensis* tea, Wang and Helliwell (2001), found aqueous ethanol as the best solvent, Turkmen *et al.* (2006) suggested N,N-dimethylformamide, while Khokhar and Magnusdottír (2002) reported water to be the best solvent of cathecins. Park and Lim (2009) have found that in the leaves of bamboo *Sasa borealis* the total polyphenol contents of the 70% ethanol and the water crude extracts were not significantly different, while in the 70% ethanolic extract the total flavonoid contents were significantly higher than in water crude extracts. Our extraction results with aqueous infusion were similar to those of Perva-Uzunalic *et al.* (2006) who have found the highest extraction efficiency of *Camellia sinensis* tea cathecins with aqueous infusion at 80 °C for 20 min (97%) and at 95 °C for 10 min (90%). Our results were also comparable to those of Jeong *et al.* (2008) with *Sasa borealis* leaf tea, with the specification that they found the highest PT in 80% methanol extract, and also with those of Kim *et al.* (2012), although freeze-drying in their case can account for the much higher values of TP found in *P. nigra* infused with 80 °C water for 10 min. The TP values of aqueous extractions in this study were similar to those of In *et al.* (2010) in the case of maengjong-juk (*Phyllostachys pubescens*) leaf tea.

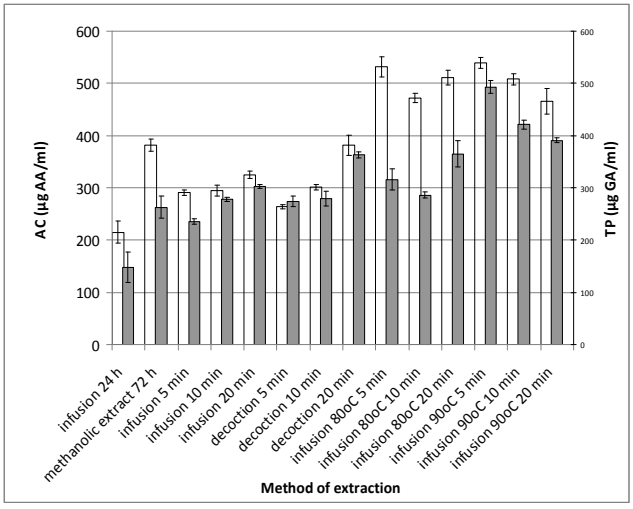

Fig. 2. Total phenolics content (TP) (μg GA/ml) and antioxidant capacity (AC) (μg AA/ml) of leaf extracts of *Phyllostachys flexuosa* using different extraction methods and times (n=4, ±SD). White and grey columns refer to antioxidant capacity and total phenolic content, respectively.

Changes in total phenolic content and antioxidant capacity throughout the vegetation period in different *Phyllostachys* taxa

Changes in total phenolics content

Tab. 1 shows the results of all three extraction methods (infusion 90 °C, 5 min or 24 h or aqueous methanol 72 h). The highest values were obtained in the case of infusion at 90 °C, for 5 min or 24 h. Only in May, August and September the values were slightly higher (11-14% higher on average) with infusion at 90 °C for 24 h compared to infusion at 90 °C for 5 min. The lowest (33-39% lower on average) values were measured for aqueous methanol for 72 h. During the course of the vegetation period the highest values of TP were measured in April and May in the case of *P. aureosulcata f. aureocaulis* and *P. humilis* with infusion at 90 °C for 24 h or with aqueous methanol for 72 h, and the difference was not significant. Only in the case of infusion at 90 °C for 5 min were the TP values of *P. humilis* significantly higher than *P. aureosulcata f. aureocaulis* during April and May. Throughout the vegetation period the highest TP values were more or less consistently measured in these two taxa. There was no significant change in TP values of *P. flexuosa* with infusion at 90 °C for 5 min or with aqueous methanol for 72 h between April and August, while values of infusion at 90 °C for 24 h showed significant slight decline (by 13%) in this species until August. Changes in TP values of *P. flexuosa, P. humilis* and *P. sulphurea var. sulphurea* from September to November were significantly different and showed the same tendency with the three extraction methods. *P. flexuosa* had the lowest values during most months, with the significantly lowest value in October

with aqueous methanolic extraction for 72 h, but the difference in other months compared especially to *P. sulphurea var. sulphurea* was not always significant. If we compared the changes in all taxa shown in Tab. 1, there was a clear trend in the changes of TP during the vegetation period, with high values in April and November, which is consistent with the findings of Zhang *et al.* (2002) who have found the highest total flavonoid level in *Phyllostachys nigra* leaves between November and April and of Ni *et al.* (2012) who have reported that total phenolics and total flavonoids in leaves of bamboo *Sasa argenteostriatus* were the highest from November to March. In addition, the same tendency was measured in leaves of bamboos *Indocalamus herklotsii*, *I. decorus* and *I. latifolius* by Su *et al.* (2011) and in *Phyllostachys heterocycla, Pleioblastus amaru, Dendrocalamus oldhami* and *Acidosasa edulis* by Lü *et al.* (2011). TP values declined during May, June and July and the change was significant for most extraction methods, months and taxa except for *P. flexuosa*, as described above. This decline is also reflected in the report of Zhang *et al.* (2002) and Ni *et al.* (2012). Our results clearly show a peak in August and September which has not been reported in the literature before, with an abrupt drop and low values in October. *P. aureosulcata f. aureocaulis* was the only taxon which had exactly the opposite tendency in October compared to the other taxa, with the highest significantly TP values (292.6±9.2; 386.0±13.2; 207.9±2.8 μg GA/ml) with all three extractions (infusion 90 °C, 5 min or 24 h or aqueous methanol 72 h. This indicated a putative effect of the environmental factors on total phenolic contents of bamboo leaves, which required further analyses.

Tab. 1. Changes in total phenolics (TP) content (μg GA/ml) and antioxidant (AC) capacity (μg AA/ml) using different extraction methods (M-methanol; A-aqueous infusion; 90- infusion at 90 °C for 5 min) *Phyllostachys* taxa between April and November. PAA-*Phyllostachys aureosulcata f. aureocaulis,* PF-*P. flexuosa,* PH-*P. humilis,*PSS-*P. sulphurea* var. *sulphurea*

Month	Taxon	TPM	ACM	TPA	ACA	TP90	AC90
April	PAA	258.7±8.0	534.5±9.2	409.5±12.0	334.9±4.0	266.1±12,1	337.8±12.8
	PF	183.9±4.8	334.8±3.2	252.9±2.0	261.3±2.5	257.9±4.4	339.2±7.2
	PH	252.9±8.0	519.7±8.4	388.4±8.1	339.5±4.1	391.3±4.3	513.5±13.2
	PSS	178.1±12.1	302.3±14.0	259.1±20.4	223.8±10.8	244.6±2.0	329.2±25.2
May	PAA	119.0±8.0	394.9±11.2	314.9±10.5	225.3±11.6	214.0±22.7	275.4±13.6
	PF	171.1±16.2	328.7±6.8	279.8±8.8	220.7±17.6	232.2±9.2	302.0±20.4
	PH	253.3±4.0	566.3±26.4	411.6±2.1	339.5±2.0	395.0±14.7	510.4±15.2
	PSS	154.1±8.7	280.6±4.0	241.7±8.0	191.5±6.7	195.0±8.8	259.9±7.6
June	PAA	112.7±4.1	399.5±8.3	278.1±1.2	193.6±7.2	240.1±16.4	355.5±4.8
	PF	148.8±28.0	339.4±4.0	262.4±4.7	157.5±23.6	240.1±18.2	346.4±19.2
	PH	205.4±4.3	442.8±30.0	334.7±24	244.1±18.4	295.0±28.0	408.5±22.7
	PSS	109.5±14.0	175.1±19.2	178.1±17.6	69.5±6.8	190.5±22.1	264.5±18.0
July	PAA	144.6±2.5	344.6±3.6	251.2±4.0	143.1±2.0	226.4±21.6	320.9±21.2
	PF	132.2±6.1	260.9±4.0	212.4±4.9	135.7±6.4	225.2±11.2	324.6±16.0
	PH	139.3±16.2	291.9±9.6	238.8±8.1	124.1±15.2	191.3±15.2	266.8±7.2
	PSS	90.5±6.0	119.0±11.2	126.4±8.0	31.9±6.8	145.5±22.6	209.5±14.1
August	PAA	178.5±14.3	383.0±4.0	290.1±8.2	187.7±8.	184.3±12.3	331.8±1.2
	PF	153.3±18.4	329.9±6.8	255.8±12.3	151.6±8.0	268.6±20.0	373.5±20.0
	PH	181.0±8.0	430.8±12.4	372.7±28.7	190.9±6.4	177.3±19.6	394.2±3.2
	PSS	190.1±2.7	272.0±2.0	256.2±18.0	163.2±6.8	272.3±3.2	446.3±15.6
September	PAA	153.7±4.8	365.8±7.6	283.9±6.4	131.2±4.8	202.5±16.0	376.7±8.8
	PF	185.1±4.8	386±16.0	311.2±8.1	175.0±7.2	254.1±4.1	406.7±18.4
	PH	196.7±24.1	437.6±2.8	335.5±14.7	194.2±22.4	268.2±28.4	378.1±7.2
	PSS	174.8±2.0	320.7±12.4	281.0±5.2	141.0±1.6	294.6±24.2	400.2±11.2
October	PAA	207.9±2.8	438.0±16.8	386.0±13.2	208.7±1.2	292.6±9.2	350.1±7.2
	PF	56.6±16.2	142.8±15.6	93.8±11.2	22.5±15.2	81.4±4.0	163.2±7.2
	PH	155.8±3.6	191.8±8.0	212.4±14.4	133.3±6.0	249.2±23.2	367.0±20.4
	PSS	73.6±8.0	139.4±19.2	126.9±18.0	33.4±4.8	103.7±2.8	149.4±11.2
November	PAA	180.2±16.8	314.9±4.8	270.7±17.6	190.6±18.0	235.1±15.6	329.8±2.0
	PF	252.1±16.7	303.1±16.0	255.8±14.0	232.0±27.6	245.9±6.0	363.2±27.6
	PH	190.1±4.0	413.3±5.6	311.6±15.2	185.9±2.0	318.6±8.1	420.2±22.4
	PSS	154.1±6.8	249.6±8.4	205.0±3.2	134.8±2.4	232.2±14.4	298.3±21.6
Month		*	***	*	***	NS	NS
Genotype		*	***	***	***	*	*
Month*Genotype		*	***	**	***	*	*

NS - Non-significant,* significant at P=0.05,** significant at P=0.01 ,*** significant at P=0.001.

Changes in total antioxidant capacity

Results for AC in the case of all three extraction methods (infusion 90 °C, 5 min or 24 h or aqueous methanol 72 h) are shown in Tab. 1. The highest AC values were obtained in the case of infusion at 90 °C, for 5 min. Somewhat lower (8-17% lower on average) values were measured with aqueous methanol for 72 h. Only in April and May, values were similar or slightly higher (12% higher on average) with aqueous methanolic extraction for 72 h compared to infusion at 90 °C for 5 min. The lowest (31-59% lower on average) values were measured for infusion at 90 °C for 24 h. Similar to the results described for TP during the examined period the highest values of AC were measured in April and May also in *P. humilis* and *P. aureosulcata f. aureocaulis* but this time in the case of aqueous methanolic extraction for 72 h and infusion at 90 °C for 5 min, but the difference was not always significant. Only in the case of infusion at 90 °C for 5 min were the AC values of *P. humilis* significantly higher than those of *P. aureosulcata f. aureocaulis* during both months of April and May. During the course of the examined period similar to the results of TP the highest AC values were more or less consistently measured in these two taxa. As the results of TP, there was no significant change in AC values of *P. flexuosa* with aqueous methanol for 72 h or with infusion at 90 °C for 5 min or between April and August, while values of infusion at 90 °C for 24 h showed a significant decline (by 50%) in this species until August. From September to November changes in AC values of *P. flexuosa, P. humilis* and *P. sulphurea var. sulphurea* were significantly different and showed the same tendency with the three extraction methods, as in the case of TP. *P. flexuosa* and *P. sulphurea f. sulphurea* had the lowest values during most months, with the lowest value for *P. flexuosa* in October with infusion at 90 °C for 24 h, but the difference was not significant compared to *P. sulphurea var. sulphurea*. When changes in all taxa were compared (see Tab. 1), there was a clear trend in the changes of AC during the vegetation period, with high values in April and November, which is similar to the findings of Zhang *et al.* (2002) who have found the highest antioxidant activity in *Phyllostachys nigra* leaves, between autumn and spring, with the strongest measured activity in February. The results of Ni *et al.* (2012) also showed that the highest antioxidant activity in bamboo *Sasa argenteostriatus* leaves occurred in December and remained generally high from October to April. AC values declined during May, June and July and the change was significant for most months, extraction methods and all taxa except for *P. flexuosa*, as described above. This decline is also reflected in the report of Zhang *et al.* (2002) and Ni *et al.* (2012). Our results clearly showed a peak in August and September which has not been reported in the literature before, then a steep decline and low values in October. *P. aureosulcata f. aureocaulis* was the only taxon which had exactly the opposite tendency in October compared to the other taxa, with significantly AC higher values with infusion at 90 °C for 24 h or aqueous methanol for 72 h. Since both Hu *et al.* (2012) and Ni *et al.* (2012) have found a positive correlation between bamboo total phenolics and antioxidant activity our results were also in agreement with those of Su *et al.* (2011) and Lü *et al.* (2011).

Correlation between environmental parameters and total phenolic content

A late summer peak in TP values has not been reported in the literature before and since *Phyllostachys* is neither native nor naturalized in Hungary, we examined the possible environmental cause of this phenomenon. Environmental parameters including daily maximum and minimum temperature (°C), cumulative precipitation plus irrigation (CPI) and cumulative potential evapotranspiration (CE) values, for the examined April to November period, are shown on Fig. 1. The temperature range was more or less the same till early May, and remained with larger variations till the end of May. From the beginning of June both daily minimum (were above +10 °C) and maximum (were above +15 °C) values started to rise and levelled off between mid-July to mid-August. From mid-August temperatures started to decline and the lowest daily minimum and maximum temperatures (at or below +5 °C) were recorded from mid-October. CPI values were similar or lower than the CE values till late June. From late June CPI values were higher than the CE values until the end of July. From early August CPI values were lower than CE values until mid-October. From mid-October, CPI values were similar or higher than CE. The above changes in CPI compared to CE can be interpreted, as there was probably water surplus during the period between late June and end of July and between mid-October and end of November. Abiotic stress factors are known to induce phenylpropanoid metabolism (Dixon and Paiva, 1995) including temperature (Janda *et al.*, 1999; Pál *et al.*, 2013) and water stress (De Abreu and Mazzafera, 2005). To find correlation between TP and water and/or temperature stress, changes of TP in the case of all taxa and extraction methods expressed as % of maximum value were compared, with stress index values derived from daily maximum and minimum temperature, CPI and CE values for the examined April to November period. A close correlation $R^2 = 0.32$ ($p=0.001$) was found between stress index values and the change in TP shown on Fig. 3. By comparing the change of TP in *P. flexuosa, P. humilis* and *P. suphurea var. sulphurea,* with stress index values the correlation is even closer $R^2 = 0.52$ ($p=0.001$). Our results were in agreement with the findings of Oh *et al.* (2009) who have found that low temperatures caused an increase in total phenolics content in lettuce. The same was reported for *Hypericum perforatum* (Zobayed *et al.*, 2005). The effect of altitudinal variation also caused an increase in total phenolic and flavanoid contents and antioxidant activity in bamboo *Indocalamus latifolius* (Ni *et al.*, 2013). Our findings are especially in conjunction with those of De Abreu and Mazzafera (2005) who have found that water or low temperature stress caused an increase of phenolic contents in *Hypericum brasiliense*. During the cold-acclimation of plants an accumulation of secondary metabolites, including TP was reported by Cansev *et al.* (2012) and Pennycooke *et al.* (2005). Our interesting results with *P. aureosulcata f. aureocaulis* in which TP values immediately rose after

exposure to low temperatures in October, while the other taxa produced high values only later in November, could be explained by the different freeze tolerance of these species, *P. aureosulcata* being the most freeze tolerant of the four taxa (Ohrnberger, 2002), and also by possible inter/intra-specific differences in responsiveness to low temperature exposure during cold-acclimation as reported for other plants (Li *et al.*, 2002; Li *et al.*, 2005; Guárdia *et al.*, 2013). Previous available studies on bamboos have not attempted to find reasons for seasonal changes in TP, only defined the period of the year optimal for harvest of bamboo leaves rich in TP. Our findings suggested that climatically applicable exposure to moderate water stress and low temperatures could enhance the phytochemical content of bamboo leaves intended for harvest as *herba* or food additive. But further specific experiments involving temperature and or water stress with regards to phytochemical content of bamboo leaves need to be carried out to underline this.

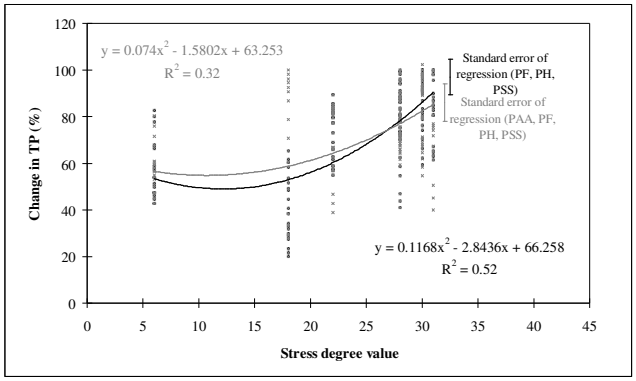

Fig. 3. Correlation between stress index and change in TP content (%) expressed as average of all four taxa PAA-*Phyllostachys aureosulcata f. aureocaulis,* PF-*P. flexuosa,* PH-*P. humilis,* PSS-*P. sulphurea var. sulphurea* or three taxa except *Phyllostachys aureosulcata f. aureocaulis*. (p=0.001). Black and grey dots and lines refer to (PF, PH, PSS) and (PAA, PF, PH, PSS) respectively.

Conclusions

The highest significant TP and AC values were obtained by aqueous infusion at 90 °C for 5 min. During the vegetation period the highest values of TP and AC were measured in April and May in the case of *PAA* and *PH*. The lowest values during most months were measured in *PF* and *PSS*. Changes of TP and AC during the vegetation period gave high values in April and November in all taxa. A peak in TP and AC detected in August-September has not been reported in the literature before. Compared to the other taxa *PAA* produced exactly the opposite tendency in October, with significantly the highest TP and AC values. A close correlation was found between stress index values and the change in TP showing that water or low temperature stress caused an increase of phenolic contents. The results of *PAA* could possibly be explained by inter/intra-specific differences during cold-acclimation in the responsiveness to low temperature exposure.

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