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BRIEF COMMUNICATION

The effects of irradiance on the production of phenolic compounds and condensed tannins in *Larix gmelinii* needles

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Abstract

Needles of *Larix gmelinii* seedlings grown under different irradiances (100, 52, and 26 % of natural sunlight) were collected from June to August 2009. The content of phenolic compounds and condensed tannins in needles were strongly affected by different irradiances. The highest content of phenolic acids occurred under the lowest irradiance. Chlorogenic acid and syringic acid were detected only under the shade. In contrast, the needles under full irradiance showed the highest content of condensed tannins.

Additional key words: chlorogenic acid, phenolic acid, larch, syringic acid.

Plant secondary metabolites are the main components of the chemical defense system which is important to the plants resistance (Wang *et al*. 2008). They also play a very important role in response to abiotic stresses (Shelton 2000, Muetzel and Becker 2006). Numerous studies have been conducted on different horticultural plants dealing with the connection between the defense mechanisms and the content of secondary metabolites in plant tissues (Yasemin *et al*. 2005, Carlo *et al*. 2006, Petkovsek *et al*. 2011a,b). Many secondary metabolites also respond to the impact of environment. Phenolic acids are known as multipurpose bioactive compounds and are widely spread throughout the plant kingdom (Xu *et al*. 2008). Plant tannins, as a kind of plant polyphenols, are known to play a considerable role in the plant resistance to the environmental stresses (Schofield *et al*. 2001). The variations of phenolic compounds and tannins are considered to be associated with plant resistance to drought, diseases, insects, *etc.* (Ivory 1972, Ruiz-Sanchez 2000, Soner 2006, Kimberly *et al.* 2011). For various organisms, radiation is one of the most crucial environmental factors during their growth and development in nature which could also affect the biosynthesis of secondary metabolites (Miyake *et al*. 2005, Geraldo *et al*. 2007, Hou *et al*. 2010, Pollastrini *et al*. 2010) including the composition or content of phenolic compounds (Ali and Neda 2011).

Larix gmelinii Rupr. is one of the major tree species in northeastern China. It is fast growing and cold and drought resistant. *L. gmelinii* trees are grown under different irradiances and are often infested by several insects including *Lymantria dispar* and *Dendrolimus superans*. In this paper, we studied the effects of different irradiances on the content of phenolic compounds and condensed tannins in the needles of *L. gmelinii* seedlings.

The experiment was carried out during the growing season of 2009. Needle samples were taken from 4-year-old *L. gmelinii* seedlings growing at the Pingshan nursery in Harbin of Heilongjiang province, P.R. China (latitude: 45° 32’ 55’’ N, longitude: 126° 57’ 29” E). The climate is continental monsoon type with four distinct seasons, the average temperature is about -19 °C in January and about 23 °C in July. Annual sunshine is around 2 641 h, the average rainfall is 477.3 mm. Different irradiances were applied by building shade shelters covered by nylon screen (6 × 7 m): T1 - 100 % natural sunlight without shading, T2 - 52 % of T1, and T3 - 26 % of T1. Needles were collected from fifteen

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healthy seedlings for each treatment in June, July, and August. The samples were frozen in liquid nitrogen and stored at -20 °C.

The needles were freeze-dried for 24 h and then grounded to fine powder with liquid nitrogen. The total phenol content was determined in 80 % (v/v) methanol extract of the powdered needles using the Folin-Ciocalteu reagent (Singleton and Rossi 1965). The extraction of phenolic acids was conducted as described by Yan *et al*. (2010) with some modifications. The fine powder (100 mg) was extracted with 15 cm^3 of 1 M NaOH for 3 h in a cooled water bath. The extract was filtered three times and then adjusted to pH 2 with 1 M HCl. The solution was extracted three times with ether (10 cm^3) and the extracts were centrifuged at 8 000 *g* and 0 °C for 6 min. The supernatant was evaporated to dryness in vacuum with a rotary evaporator. Each extract was dissolved in methanol (2 cm^3) and then filtrated through a 0.45 μm membrane prior to injection into the HPLC system (*Waters*, Milford, MA, USA) with a diode array detector at 254 nm. The column was a *Diamonsil* C18 (250 × 4.5 mm) operated at 25 °C. The elution solvents were aqueous 2 % (m/v) glacial acetic acid and 10 % (v/v) methanol (A) and 2 % glacial acetic acid and 90 % methanol (B). Samples were eluted according to the linear gradient as described by Yan *et al.* (2010) with an injection amount of 0.01 cm^3 and a flow rate of 0.45 cm^3 min^{-1}. The amounts of phenolic compounds were calculated from the peak areas of samples and corresponding standards.

The extraction of condensed tannin was performed according to Yuan *et al.* (2009). The needles were freeze-dried at -57 °C and then grounded into powder over a 60 mesh sieve. The fine powder (200 mg) was extracted with 70 % methanol (10 cm^3) at -20 °C for 24 h. After centrifugation at 10 000 *g* and 4 °C for 10 min, the supernatant was dissolved in 10 cm^3 of 70 % methanol. The reaction solution consisted of 4 % (m/v) vaniline (3 cm^3), concentrated HC1 (1.5 cm^3) and the extract (0.5 cm^3) was injected into cuvettes wrapped with aluminum foil. After shaking, the cuvettes were placed into a water bath at 20 °C for 20 min. The absorbance at 510 nm was measured (spectrophotometer *UV-mini-1240*, *Shimadzu*, Japan), with 70 % methanol as a control. The tannin content in the needles was calculated according to the standard curve obtained in accordance with the previously described method (Yuan *et al*. 2009).

The data were analyzed by using the *SPSS v. 18.0* software. The results were tested using the one-way *ANOVA* and differences of means among the treatments were tested with the LSD test at $P \leq 0.05$.

The irradiance had a significant effect on the total content of phenolic acids during the whole season. The total content of phenolic acids was significantly higher under T2 and T3 shading than under T1 during the same month (Table 1). Higher total phenolic acid content was found in plants more resistant to diseases and insects than in more susceptible ones (Li *et al*. 2003, Martti *et al.* 2004). Thus, such a high total phenolic acid content under the shading conditions may be beneficial in response to diseases or insects in *L. gmelinii*. The total content of phenolic acids under the same irradiance decreased from June to August with the amounts in August being significantly lower than those in the previous two months. Plant secondary metabolites are derived from primary metabolites, however, many studies show that the responses of primary and secondary metabolism to environmental conditions are different (Su *et al.* 2005, Lu *et al.* 2006, Yang *et al*. 2010). The net photosynthetic rate of *L. gmelinii* needles in August was the highest during the growing season (Wang 1994) whereas the content of phenolic acids was the lowest.

Twelve phenolic acids were identified, and eight of them were found under the T1 conditions whereas ten compounds were detected under the T2 and T3 conditions. Gallic acid and caffeic acid were not detected in any of our samples. The maximum content of protocatechuic acid, *p*-coumaric acid, phenylacetic acid, chlorogenic acid, benzoic acid, and salicylic acid appeared under T2 conditions whereas the maximum content of ferulic acid, cinnamic acid, and carbolic acid was under the T3 condition. Phenylacetic acid was the most dominant component among all the phenolic compounds. Therefore it might be an important and critical defensive substance in the needles of the *L. gmelinii* seedlings against insect pest.

Chlorogenic acid, which is expected to considerably participate in production of phytoalexin (Niggeweg *et al*. 2004), was found in the needles of *L. gmelini* only under shading (T2 and T3). Syringic acid, which is closely related to the modified cell wall structures and synthesis of lignin (Tan *et al*. 2010), was only identified under the T2 condition. Ferulic acid, a precursor of lignin synthesis, is considered to have a protective role in plants against the external stress (Graf 1992, Bily *et al.* 2003, Sarma *et al.* 2003) and was found under the shading. Therefore, increased content of these phenolic acids might play a role in *L. gmelini* responses to attacks by diseases and insects. It suggests that low irradiance is favourable for production of phenolic acids which validate the previous reports (Jennifer *et al*. 2005, Ali and Neda 2011). Irradiance also had a significant effect on the content of condensed tannin in the needles of the *L. gmelinii* seedlings during the three sampling months (Table 1). The content of condensed tannins significantly decreased as irradiance decreased from T1 to T3. For each irradiance, it remained unchanged during the season.

It is commonly known that many stress factors modify the content of secondary metabolites in plants (Brignolas *et al*. 1995, Yasemin *et al*. 2005, Petkovsek *et al*. 2009a,b). Different shading might change the plant morphology and physiological characteristics, therefore, it may affect the secondary metabolites including the phenolic compounds (Kurata *et al*. 1997, Briskin *et al*. 2001, Débora *et al*. 2009, Sevgi *et al*. 2010). In our study, an increase of the total phenol content appeared in the needles of *L. gmelinii* under T2 and T3. The reduction of

Table 1. The content [mg g^{-1}(DM)] of phenolic acids and condensed tannins in the needles of *Larix gmelinii* seedlings grown under different irradiances (T1 - 100 % natural sunlight without shading, T2 - 52 % of T1, and T3 - 26 % of T1) Means ± SE, $n = 5$. Means with different letters in the same row are significantly different at $P < 0.05$.

Compound	Month	T1	T2	T3
Total content of phenolic acids	Jun.	2.175 ± 0.087 c	3.094 ± 0.016 a	2.650 ± 0.127 b
	Jul.	1.997 ± 0.194 c	2.892 ± 0.059 a	2.420 ± 0.059 b
	Aug.	0.649 ± 0.042 b	0.805 ± 0.020 ab	0.951 ± 0.003 a
Protocatechuic acid	Jun.	0.005 ± 0.000 b	0.007 ± 0.000 a	0.004 ± 0.000 b
	Jul.	0.004 ± 0.001 a	0.005 ± 0.001 a	0.005 ± 0.000 a
	Aug.	0.004 ± 0.000 a	0.005 ± 0.000 a	0.004 ± 0.001 a
p-Coumaric acid	Jun.	0.218 ± 0.003 a	0.252 ± 0.012 a	0.235 ± 0.007 a
	Jul.	0.117 ± 0.003 a	0.219 ± 0.035 b	0.176 ± 0.018 c
	Aug.	0.028 ± 0.004 a	0.054 ± 0.001 a	0.032 ± 0.001 a
Ferulic acid	Jun.	0.073 ± 0.003 b	0.088 ± 0.008 a	0.091 ± 0.005 a
	Jul.	0.040 ± 0.005 b	0.081 ± 0.003 a	0.053 ± 0.007 b
	Aug.	0.011 ± 0.002 a	0.019 ± 0.000 a	0.014 ± 0.001 a
Phenylacetic acid	Jun.	1.456 ± 0.113 c	2.298 ± 0.000 a	1.696 ± 0.059 b
	Jul.	1.008 ± 0.004 c	1.941 ± 0.107 a	1.569 ± 0.031 b
	Aug.	0.312 ± 0.066 b	0.526 ± 0.002 a	0.656 ± 0.015 a
Chlorogenic acid	Jun.	--	0.031 ± 0.003 a	0.022 ± 0.003 b
	Jul.	--	0.021 ± 0.002 a	0.019 ± 0.002 a
	Aug.	--	0.019 ± 0.000 a	0.016 ± 0.002 a
Cinnamic acid	Jun.	0.033 ± 0.002 c	0.224 ± 0.011 a	0.187 ± 0.003 b
	Jul.	0.076 ± 0.003 c	0.144 ± 0.007 a	0.116 ± 0.003 b
	Aug.	0.033 ± 0.004 c	0.163 ± 0.005 b	0.232 ± 0.016 a
Benzoic acid	Jun.	0.047 ± 0.000 a	0.054 ± 0.001 a	0.049 ± 0.001 a
	Jul.	0.034 ± 0.002 b	0.045 ± 0.003 a	0.036 ± 0.001 b
	Aug.	--	0.067 ± 0.002 a	0.052 ± 0.007 b
Salicylic acid	Jun.	0.080 ± 0.005 b	0.131 ± 0.008 a	0.118 ± 0.006 a
	Jul.	0.060 ± 0.004 b	0.100 ± 0.008 a	0.068 ± 0.000 b
	Aug.	--	0.087 ± 0.008 a	0.068 ± 0.002 b
Syringic acid	Jun.	--	0.016 ± 0.000	--
	Jul.	--	0.015 ± 0.000	--
	Aug.	--	0.010 ± 0.000	--
Carbolic acid	Jun.	0.072 ± 0.011 a	0.063 ± 0.003 a	0.065 ± 0.001 a
	Jul.	0.058 ± 0.015 b	0.083 ± 0.007 a	0.095 ± 0.003 a
	Aug.	0.074 ± 0.015 a	0.013 ± 0.002 b	--
Condensed tannins	Jun.	3.975 ± 0.056 a	2.683 ± 0.028 b	1.496 ± 0.021 c
	Jul.	3.515 ± 0.032 a	2.624 ± 0.000 b	1.359 ± 0.011 c
	Aug.	4.151 ± 0.061 a	3.258 ± 0.073 b	2.012 ± 0.000 c

irradiance also cause a notable increase in content of total phenols in sweet cherry (Goncalves *et al.* 2008).

According to the growth/differentiation balance hypothesis (Herms and Mattson 1992) and the predictions of Koricheva *et al.* (1998), phenylalanine is the common precursor in the phenylpropanoid pathways leading to the protein synthesis. When the irradiance, water, and nutrients are sufficient, growth is the priority and the bulk of phenylalanine is used for protein synthesis. However, under some limitations, the growth and protein synthesis are restricted and surplus carbon is diverted to phenylpropanoid synthesis (Goncalves *et al.* 2008). The above predictions are supported by our results showing increased content of phenolics under shading. The further investigation is surely needed to determine if the increase of phenolic acid production is due to the decrease of primary metabolite production through photosynthesis or the stress induced by decreased irradiance (Geraldo *et al*. 2007, Ali and Neda 2011).

The condensed tannin content often changes with changes in environmental conditions including irradiance (Roberts *et al*. 1993). Our results show that the shade treatments significantly reduced the condensed tannin content supporting the previous reports on positive correlations between the condensed tannin content and irradiance (Colin, 1991, Francesco *et al*. 2005, Geraldo *et al*. 2007, Moore *et al*. 2010). The decrease of the condensed tannin content together with the increase of the phenolic acid content suggest a dynamic balance between these secondary metabolites (Tong *et al*. 2010).

In summary, the content of phenolic compounds and condensed tannins in the needles of *L. gmelinii* seedlings was significantly affected by different irradiances. Low irradiance was favourable for the production of phenolic acids whereas, the reverse was true for the condensed tannin production. These results enriched our understanding the relationship between plant defense and environmental factors in the larch.

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