CHLOROPHYLL CATABOLISM IN Prunus serrulata AUTUMNAL LEAVES

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Abstract. Chlorophyll catabolism in Prunus serrulata autumnal leaves was investigated. The amount of chlorophyll catabolites accumulated within the same plant species varies with the time of the leaf collection, seasonal climate and developmental stage of the plant. The chlorophyll catabolites found in P. serrulata autumnal leaves presented the tendency of the organism to decrease the level of photodynamically active chlorophyll before the programmed cell death. In the methanol extract several chlorophyll catabolites were identified. The results obtained by liquid – chromatography/mass spectrometry permitted the identification of the chlorophyll catabolites found in P. serrulata autumnal leaves. The analysis done revealed the chlorophyll catabolic pathway found in P. serrulata autumnal leaves.

Key words: chlorophyll catabolism, Prunus serrulata

1. INTRODUCTION

The chlorophyll catabolic pathway can be divided into two groups. The first group of reactions includes the loss of phytol, the loss of magnesium and modifications on the side chain groups of the chlorophyll nucleus in which the aromatic tetrapyrrrole macrocycle remains intact [1]. The interconversion of chlorophyll b to chlorophyll a and vice versa occurs in oxygenic photosynthetic organisms [2]. The chlorophyll cycle starts with the reduction of chlorophyll b by chlorophyll b reductase, an NADPH dependent enzyme, to form 71 – hydroxyl chlorophyll a. The 71 – hydroxyl chlorophyll a is a stable intermediate and it was isolated from higher plants. The next reduction step is catalyzed by ferredoxin dependent 71 – hydroxyl chlorophyll a reductase to form chlorophyll a. It is also suggested that chlorophyll a interconverts to chlorophyll b by an oxygen dependent enzyme chlorophyll a oxygenase. The enzyme oxidizes chlorophyll a to 71 – hydroxyl chlorophyll a which is further oxidized to chlorophyll b by 71 – hydroxyl chlorophyll a dehydrogenase. The bioconversion of chlorophyll b to chlorophyll a undergoes through a 71 – hydroxyl intermediate. The chlorophyll interconversion cycle can be expanded to the
catabolic steps toward the chlorophyllide \(a\). Chlorophyll \(a\) is enzymatically hydrolyzed to chlorophyllide \(a\). Chlorophyll \(b\) is enzymatically hydrolyzed to chlorophyllide \(b\) and undergoes through the formation of \(7^{1}\) – hydroxyl chlorophyllide \(a\) which is enzymatically reduced to chlorophyllide \(a\) \[3\]. The chlorophyll catabolism continues with dechelating chlorophyllide \(a\) molecule. The enzyme involved in demetallation is Mg – dechelatase. A catalytic cofactor called Mg – dechelating substance (MDS) is associated with the activity of Mg – dechelatase \[4\]. When magnesium is expelled from the core of chlorophyllide \(a\), the remaining structure is called pheophorbide \(a\) (Pheide \(a\)). The first part of chlorophyll catabolism is depicted in Figure 1.

**Fig. 1** The first part of chlorophyll catabolism in higher plants

The second group of reactions involves the cleavage of the macrocyclic aromatic ring system yielding open chain tetrapyrrolic molecule in which the side chains are further modified.

The chlorophyll catabolism continues with enzymatic oxidation of Pheide \(a\) methene bridge at the C4 – C5 position by pheophorbide \(a\) oxygenase (PaO) to yield the so called red chlorophyll catabolite (RCC) which was, up to now, not isolated from the autumnal leaves of higher plants. The PaO is an iron-dependent mono-oxygenase. The electrons required for the redox cycle are supplied from reduced ferredoxin. The proposed mechanism is based on the single oxygen attachment to the double bond C4–C5 forming re-
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2. MATERIALS AND METHODS

The extraction of chlorophyll catabolites from Prunus serrulata autumnal leaves was the same as for the Vitis vinifera var. Pinot noir autumnal leaves [9]. Liquid chromatography (LC) separation was carried on the reverse phase (RP) EC 250 × 4 mm Nucleosil 100-5 C8 column together with RP CC 8 × 4 mm Nucleosil 100-5 C8 precolumn (Macherey-Nagel, Oesingen, Switzerland). The temperature of the column oven was 22 °C. The injection volume was 10 µl via autosampler injection. Mobile phase consisted of 0.1 % TFA (modifier) in water and methanol. The proportion of methanol was increased linearly from 10% to 100% in 70 minutes and in next 20 minutes elution was continued with methanol. The flow rate of 0.2 ml/min. was used for the liquid chromatography – mass spectrometry (LC/MS) method. After each separation the column was reequilibrated linearly from 100% methanol to 90% water (0.1% TFA):10% methanol in 10 minutes and additional 5 minutes at 90% water (0.1% TFA):10% methanol.

3. RESULTS AND DISCUSSION

The autonomous induction of leaf senescence occurs in autumn. The major consequence of leaf senescence is chlorophyll catabolism. The crude extract of Prunus serrulata autumnal leaves was analyzed on RP – C8 analytical column by LC – MS. The results obtained gave insight in the chlorophyll catabolic compounds present in P. serrulata autumnal leaves.

In P. serrulata crude leaf extract three chlorophyll catabolites were detected: the Cj-1 (m/z 645) and Cj-2 (m/z 629) were present along with the isomers of m/z 679 or the So – 2 chlorophyll catabolite [8, 10]. In Figure 2 the UV chromatogram of the P. serrulata autumnal leaves is shown. The elution of the Cj-2 was at the 70.1 minutes, Cj-1 eluted at 63.8 minutes and one of the So – 2 chlorophyll catabolites was detected at 51.0 minutes (Figure 3, 4 and 5). The chlorophyll catabolites detected permitted the construction of the chlorophyll catabolic pathway in P. serrulata autumnal leaves (Figure 6). The hydroxy-
lation of the chlorophyll catabolite $Cj$-$2$ ethyl side chain forms the $Cj$-$1$ chlorophyll catabolite and is performed at different stages of the chlorophyll catabolism. It surely occurs after the formation of the pFCC. It can proceed before or after the oxidation of the vinyl group or tautomerization. The enzyme that catalyses the hydroxylation is still unknown [8] (Figure 6).

The oxidation of the NCC vinyl group forms the dihydroxylated NCC $So$-$2$.

The proposed chlorophyll catabolism in *P. serrulata* autumnal leaves is depicted in Figure 6. The same chlorophyll catabolic pathway has been observed in *Juglans regia* and *Fagus silvatica* var. *purpurea* autumnal leaves.

![Fig. 2 UV chromatogram of *P. serrulata* autumnal leaf extract. Detection wavelength: $\lambda=312$nm](image)

**Fig. 2** UV chromatogram of *P. serrulata* autumnal leaf extract. Detection wavelength: $\lambda=312$nm

![Fig. 3 Molecular ion of $Cj$-$2$ chlorophyll catabolite eluting at 70.1 min](image)

**Fig. 3** Molecular ion of $Cj$-$2$ chlorophyll catabolite eluting at 70.1 min

![Fig. 4 Molecular ion of $Cj$-$1$ chlorophyll catabolite eluting at 63.8 min](image)

**Fig. 4** Molecular ion of $Cj$-$1$ chlorophyll catabolite eluting at 63.8 min

![Fig. 5 One of the molecular ions of $So$-$2$ chlorophyll catabolite eluting at 51.0 min](image)

**Fig. 5** One of the molecular ions of $So$-$2$ chlorophyll catabolite eluting at 51.0 min
Fig. 6 The proposed chlorophyll catabolism from pFCC in *P. serrulata* autumnal leaves.

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KATABOLIZAM HLOROFILA KOD
Prunus serrulata JESENJEG LIŠĆA

Nina Đapić

Ispitivan je katabolizam hlorofila jesenjeg lišća biljne vrste Prunus serrulata. Količina katabolita hlorofila u jesenjem lišću, koji zapravo predstavljaju tendenciju organizma da smanji nivo fotodinamički aktivnog hlorofila pre programirane smrti čelije, zavisi od vremena sakupljanja lišća, klime i razvojnog doba biljke. Analizom hemijskog sastava metanolnog ekstrakta jesenjeg lišća vrste P. serrulata metodom tečne hromatografije / masene spektrometrije identifikovano je nekoliko katabolita hlorofila. Na osnovu dobijenih rezultata predložen je katabolizam hlorofila koji se odvija u jesenjem lišću vrste P. serrulata.

Ključne reči: katabolizam hlorofila, Prunus serrulata