

CHLOROPHYLL CATABOLISM IN *Prunus serrulata* AUTUMNAL LEAVES

UDC 582.3.6 + 547.979.7 : 577.121

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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 tetrapyrrole 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 7¹ - 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 - dechelataase. A catalytic cofactor called Mg - dechelating substance (MDS) is associated with the activity of Mg - dechelataase [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.

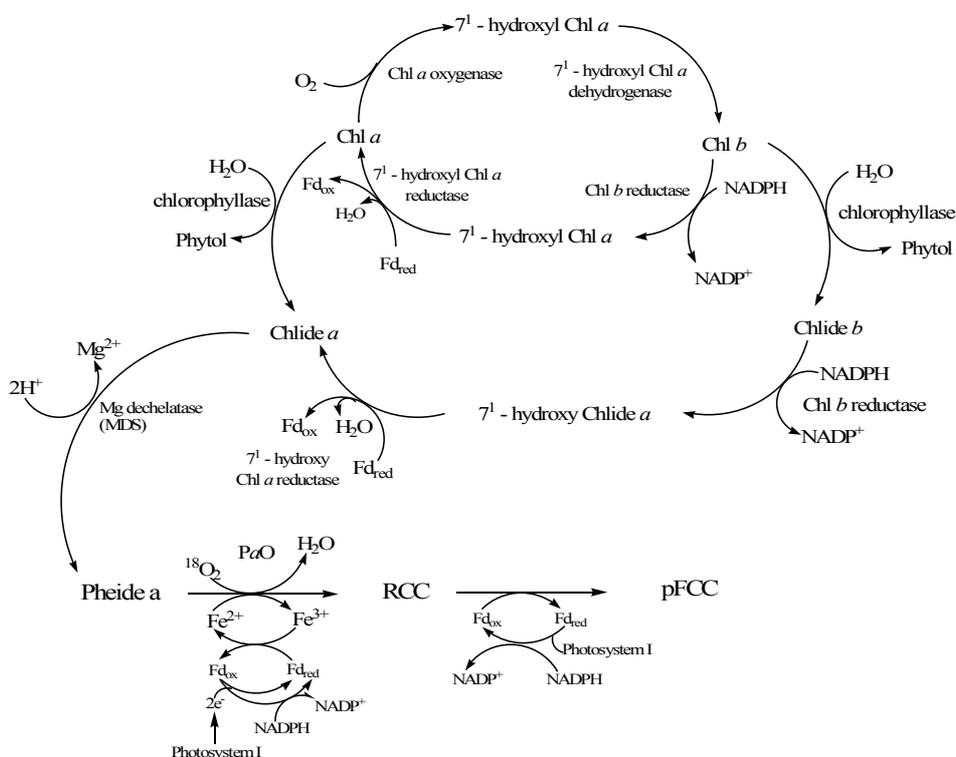


Fig. 1 The first part of chlorophyll catabolism in higher plants

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-

gioselective intermediate by cleavage of the oxirane ring. Subsequently, the oxirane ring is opened hydrolytically. Retro – Aldol reaction proceeds and the rearrangement of protons to form stereoselectively the so called RCC.

The reduction of the so called RCC proceeds via the reduction of “western” methene bridge. It is catalyzed by red chlorophyll catabolite reductase (RCCR) in a stereospecific way [5]. The RCCR is a ferredoxin dependent enzyme with no requirements of cofactors such as flavin or metals. The electrons are directly transferred to the enzyme active center. After the reduction there is a possibility of two stereoisomers formation. Mutants lacking the enzymatic stereospecific reduction form both isomers [6]. This reduction of the C20 – C1 double bond forms the so called primary fluorescent chlorophyll catabolite (pFCC) [7]. The prototropic tautomerization of pFCC appears to be a non-enzymatic reaction, proceeding in two steps according to protonation deprotonation mechanism. The π – conjugation through the double bonds is lost and the molecule is non – fluorescent therefore being named the non – fluorescent chlorophyll catabolite (NCC) [8]. In further chlorophyll catabolism side chain groups are modified. The aim of this work was to investigate the chlorophyll catabolic pathway taking place in *P. serrulata* autumnal leaves.

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 μ l 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.

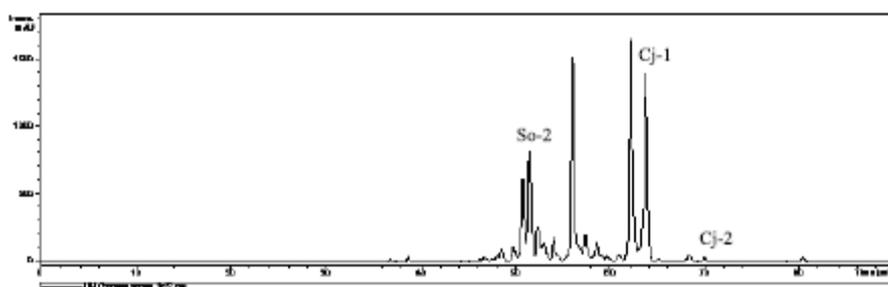


Fig. 2 UV chromatogram of *P. serrulata* autumnal leaf extract. Detection wavelength: $\lambda=312\text{nm}$

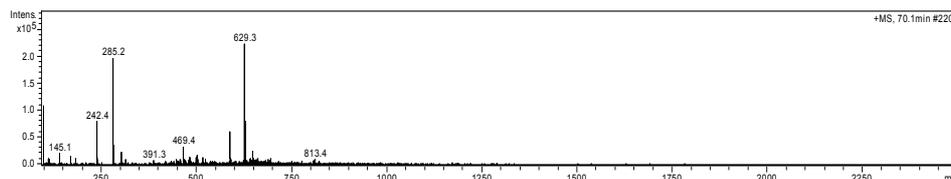


Fig. 3 Molecular ion of *Cj*-2 chlorophyll catabolite eluting at 70.1 min

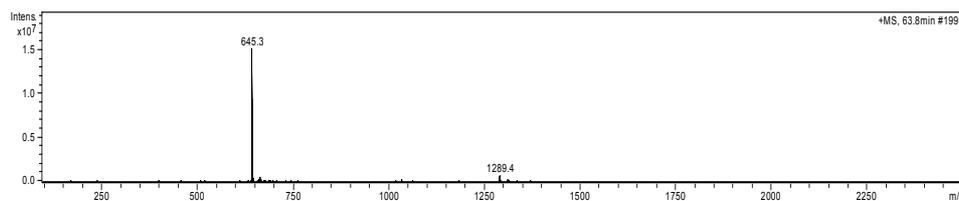


Fig. 4 Molecular ion of *Cj*-1 chlorophyll catabolite eluting at 63.8 min

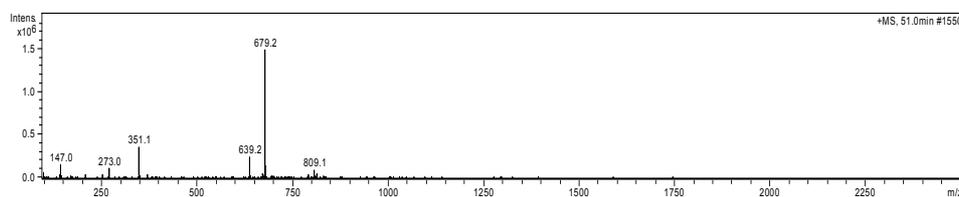


Fig. 5 One of the molecular ions of *So*-2 chlorophyll catabolite eluting at 51.0 min

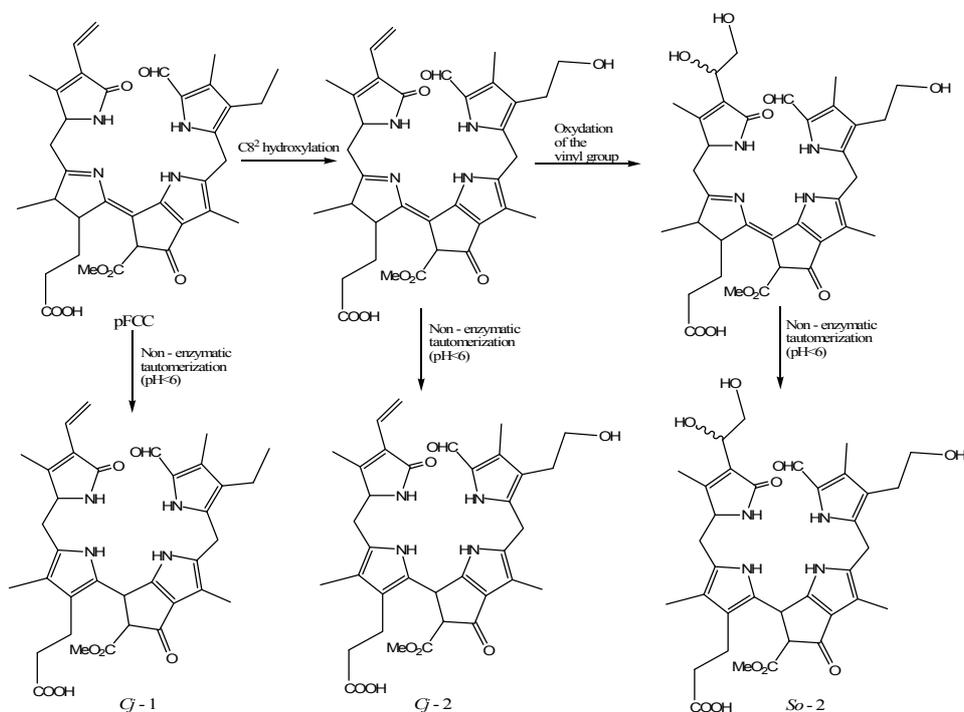


Fig. 6 The proposed chlorophyll catabolism from pFCC in *P. serrulata* autumnal leaves.

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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 ćelije, 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**