CHLOROPHYLL CATABOLISM IN *PRUNUS SERRULATA* AUTUMNAL LEAVES

N. DJAPIC*

Chlorophyll catabolism in *Prunus serrulata* autumnal leaves was investigated. The amount of chlorophyll catabolites accumulated within the same plant species varies on the time of the leaf collection, seasonal climate and developmental stage of the plant. The chlorophyll catabolites found in *Prunus 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 *Prunus serrulata* autumnal leaves. The analysis done revealed the chlorophyll catabolic pathway found in *Prunus serrulata* autumnal leaves.
Key words: chlorophyll catabolism, *Prunus serrulata*.

INTRODUCTION

The chlorophyll catabolic pathway can be divided into two groups. The first group of reactions includes the: loss of phytol, loss of magnesium and modifications on the side chain groups of the chlorophyll nucleus in which the aromatic tetrapyrrole macrocycle remains intact (Brown, 1991). The interconversion of chlorophyll *b* to chlorophyll *a* and *vice versa* occurs in oxygenic photosynthetic organisms (Willows, 2003). The chlorophyll cycle starts with the reduction of chlorophyll *b* by chlorophyll *b* reductase, an NADPH dependant enzyme, to form 7^1 – hydroxyl chlorophyll *a*. The 7^1 – hydroxyl chlorophyll *a* is a stable intermediate and it was isolated from higher plants. The next reduction step is catalyzed by ferredoxin dependant 7^1 – hydroxyl chlorophyll *a* reductase to form chlorophyll *a*. It is also suggested that chlorophyll *a* interconverts to chlorophyll *b* by an oxygen dependant enzyme chlorophyll *a* oxygenase. The enzyme oxidizes chlorophyll *a* to 7^1 – hydroxyl chlorophyll *a* which is further oxidized to chlorophyll *b* by 7^1 – hydroxyl chlorophyll *a* dehydrogenase. The bioconversion of chlorophyll *b* to chlorophyll *a* undergoes through a 7^1 – 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*.

* "Mihajlo Pupin" Technical Faculty, Djure Djakovica bb, 23000 Zrenjanin, Serbia.

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* (Ruediger, 2002). 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 (Shioi, 1996). 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.

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 (P*a*O) to yield the so-called red chlorophyll catabolite (RCC) which was, up to now, not isolated from the autumnal leaves of higher plants. The P*a*O is an iron – dependant monooxygenase. 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 regioselective intermediate by cleavage of the oxirane ring. Subsequently, the oxiran 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 (Hoertensteiner, 2000). The RCCR is a ferredoxin dependant enzyme with no requirements of cofactors such as flavin or metals. The electrons are directly transferred to the enzyme active centre. After the reduction there is a possibility of two stereoisomers formation. Mutants leaking the enzymatic stereospecific reduction form both isomers (Hoertensteiner, 2006). This reduction of the C20 – C1 double bond forms the so-called primary fluorescent chlorophyll catabolite (pFCC) (Hoertensteiner, 1998). The prototropic tautomerisation of pFCC appears to be a nonenzymatic 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) (Oberhuber, 2003). In further chlorophyll catabolism side chain groups are modified. The aim of this work was to investigate the chlorophyll catabolic pathway taking place in *Prunus serrulata* autumnal leaves.

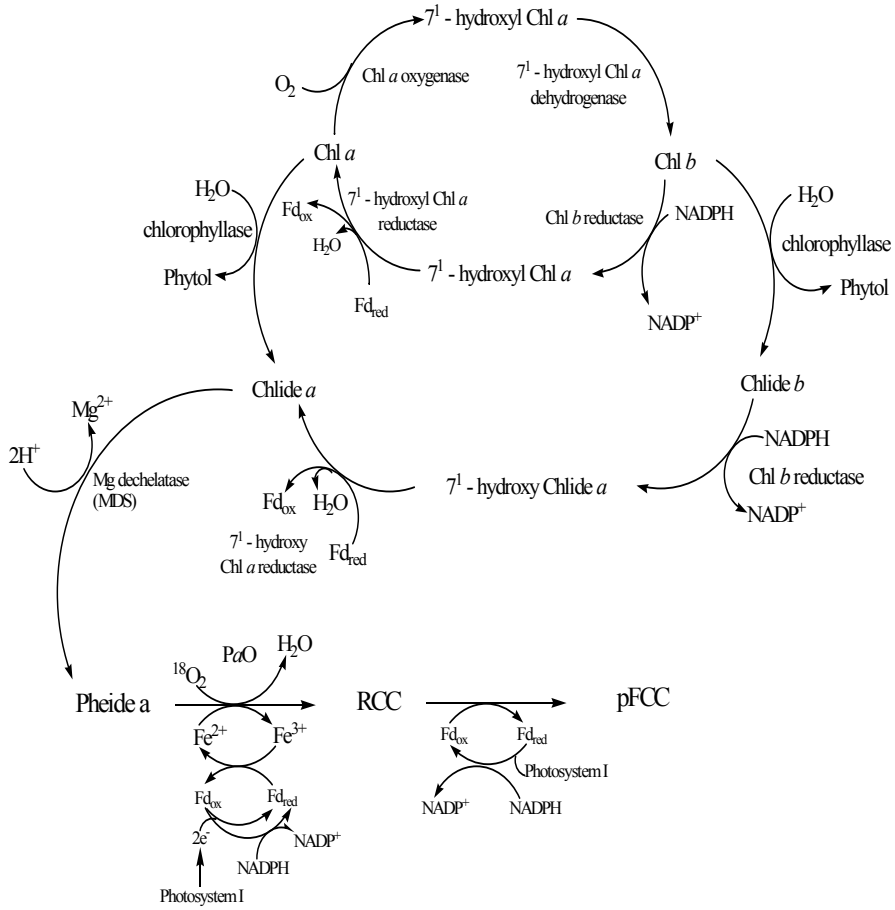

Fig. 1. The first part of chlorophyll catabolism in higher plants.

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 (Djapic, 2009). Liquid chromatography (LC) separation was carried on the reverse phase (RP) EC 250x4 mm Nucleosil 100-5 C_8 column together with RP CC 8x4 mm Nucleosil 100-5 C_8 precolumn (Macherey-Nagel, Oesingen, Switzerland). The temperature of the column oven was 22 ^oC. 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 the 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.

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 – C_8 analytical column by LC – MS. The results obtained gave insight into the chlorophyll catabolic compounds present in *Prunus serrulata* autumnal leaves.

In *Prunus 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 (Oberhuber, 2003; Berghold, 2002). In Figure 2, the UV chromatogram of the *Prunus 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 (Figs. 3–5). The chlorophyll catabolites detected permitted the construction of the chlorophyll catabolic pathway in *Prunus serrulata* autumnal leaves (Fig. 6). The hydroxylation 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 (Oberhuber, 2003) (Fig. 6).

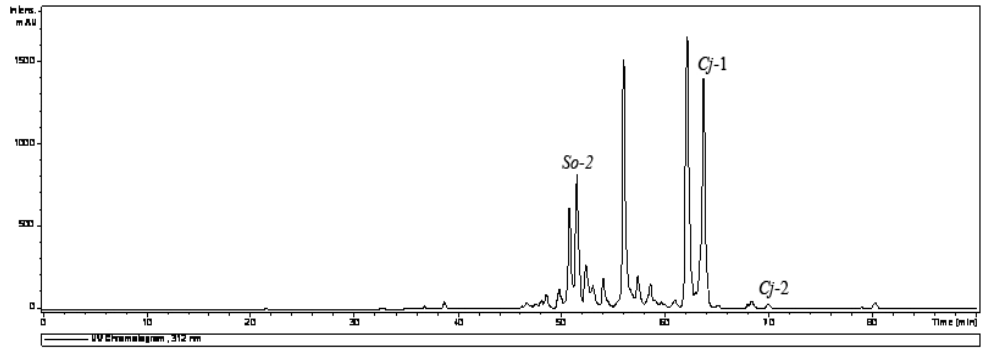

Fig. 2. UV chromatogram of *Prunus serrulata* autumnal leaf extract.

Detection wavelength: λ = 312 nm.

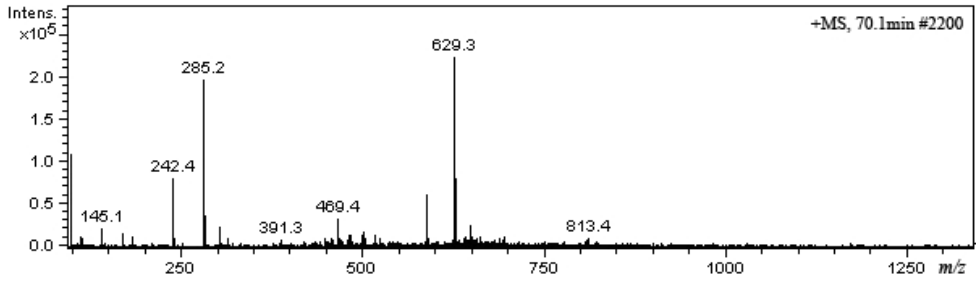

Fig. 3. Molecular ion of *Cj* – 2 chlorophyll catabolite eluting at 70.1 min.

The oxidation of the NCC vinyl group forms the dihydroxylated NCC *So* – 2. The proposed chlorophyll catabolism in *Prunus serrulata* autumnal leaves is depicted in Figure 6. The same chlorophyll catabolic pathway has been observed in *Juglans regia* and *Fagus sylvatica* var. purpurea autumnal leaves.

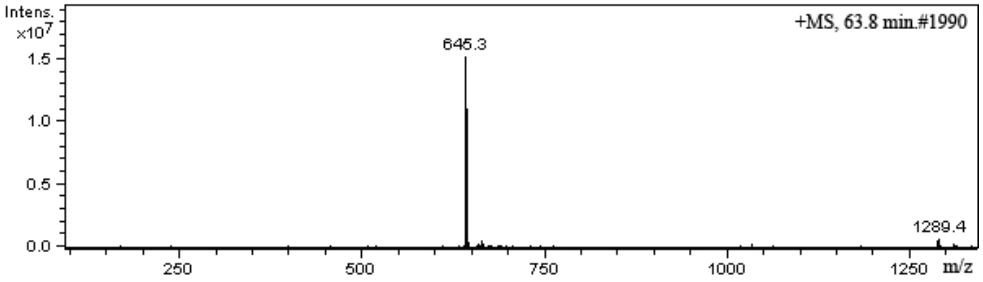

Fig. 4. Molecular ion of *Cj* – 1 chlorophyll catabolite eluting at 63.8 min.

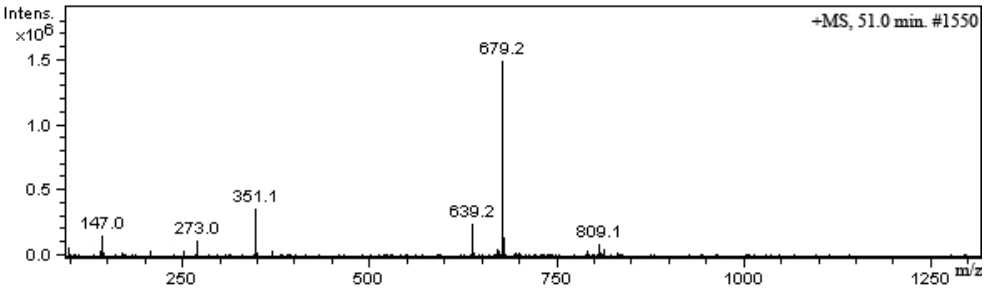

Fig. 5. One of the molecular ions of *So* – 2 chlorophyll catabolite eluting at 51.0 min.

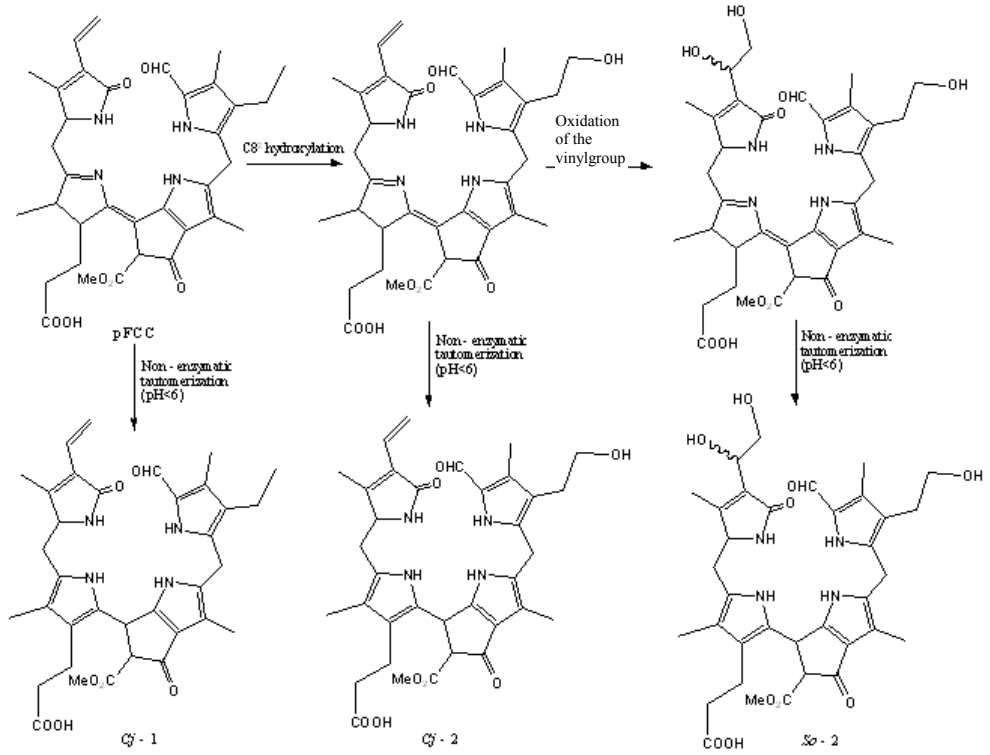

Fig. 6. The proposed chlorophyll catabolism from pFCC in *Prunus serrulata* autumnal leaves.

REFERENCES

1. Berghold J., Breuker K., Oberhuber M., Hoertensteiner S. and Kraeutler B., 2002, Chlorophyll breakdown in spinach: on the structure of five nonfluorescent chlorophyll catabolites, *Photosynthesis Research* **74**, pp. 109–119.
2. Brown S. B., Houghton J.D. and Hendry G.A.F., 1991, Chlorophyll breakdown, In: H. Scheer, Editor, *Chlorophylls*, CRC Press, Boca Raton, Florida, pp. 465–489.
3. Djapic N., Djuric A. and Pavlovic A., 2009, Chlorophyll biodegradation in *Vitis vinifera* var. Pinot noir autumnal leaves, *Research Journal of Agriculture Science*, **41**, pp. 256–260.
4. Hoertensteiner S., Wuethrich K.L., Matile P., Ongania K.-H. and Kraeutler B., 1998, The key step in chlorophyll breakdown in higher plants. Cleavage of pheophorbide *a* macrocycle by a monooxygenase, *The Journal of Biological Chemistry*, **273**, pp. 15335–15339.
5. Hoertensteiner S., Rodoni S., Schellenberg M., Vicentini F., Nandi O. I., Qui Y. L. and Matile P., 2000, Evolution of chlorophyll degradation: the significance of RCC reductase, *Plant Biol.*, **2**, pp. 63–67.
6. Hoertensteiner S., 2006, Chlorophyll Degradation During Senescence, *Annu. Rev. Plant. Biol.* **57**, pp. 55–77.

7. Oberhuber M., Berghold J., Breuker K., Hoertensteiner S. and Kraeutler B. 2003, Breakdown of chlorophyll: A nonenzymatic reaction accounts for the formation of the colorless "nonfluorescent" chlorophyll catabolites, *Proc. Natl. Acad. Sci. USA*, **100**, pp. 6910–6915.
8. Ruediger W., 2002, Biosynthesis of chlorophyll b and the chlorophyll cycle, *Photosynthesis Research* **74**, pp. 187–193.
9. Shioi Y., Watanabe K. and Takamiya K., 1996, Enzymatic conversion of pheophorbide *a* to a precursor of pyropheophorbide *a* in leaves of *Chenopodium album*, *Plant and Cell Physiol*, **37**, pp. 1143–1149.
10. Willows R. D., 2003, Biosynthesisof chlorophylls from protoporphyrin IX, *Nat. Prod. Rep.*, **20**, pp. 327–341.