

Flavonoids and their formation in different sainfoin varieties (*Onobrychis viciifolia*)

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Introduction

The valuable fodder legume sainfoin (*Onobrychis viciifolia*) contains high contents of flavonoids, especially tannins, which have key physiological importance for plants. Furthermore positive effects of sainfoin on ruminants such as the well documented antibloating and antiparasitic effects occurring in combination with increased amino acid absorption and protein utilisation, can also be ascribed to its inherent tannins. Therefore a screening of different sainfoin varieties and species is performed currently to identify interesting candidates for future breeding programmes.

Methods

Young (still folded) leaves were taken from single plants of 40 different sainfoin varieties, which were grown at NIAB (UK). For the enzymatic screening, an enzyme preparation method suitable for polyphenol rich material was modified [Dellus *et al.* (1997) *Phytochemistry* 45: 1415-1418]. The assays were carried out using (¹⁴C)-labelled substrates [Halbwirth *et al.* (1997) *Plant Science* 122: 125-131]. A screening on a genetic level was done via qRT-PCR. cDNA was synthesized from mRNA using a kit based on magnetic beads which was applied on total RNA that was obtained by a method for pine trees [Chang *et al.*, PMB Reporter 1993;11: 113-116]. The different phenolic compounds were extracted with 80% aq. methanol and quantified by rp-HPLC-DAD as described by Regos *et al.* [manuscript in preparation: (Identification and quantification of phenolic compounds from the forage legume sainfoin, *Onobrychis viciifolia*)].

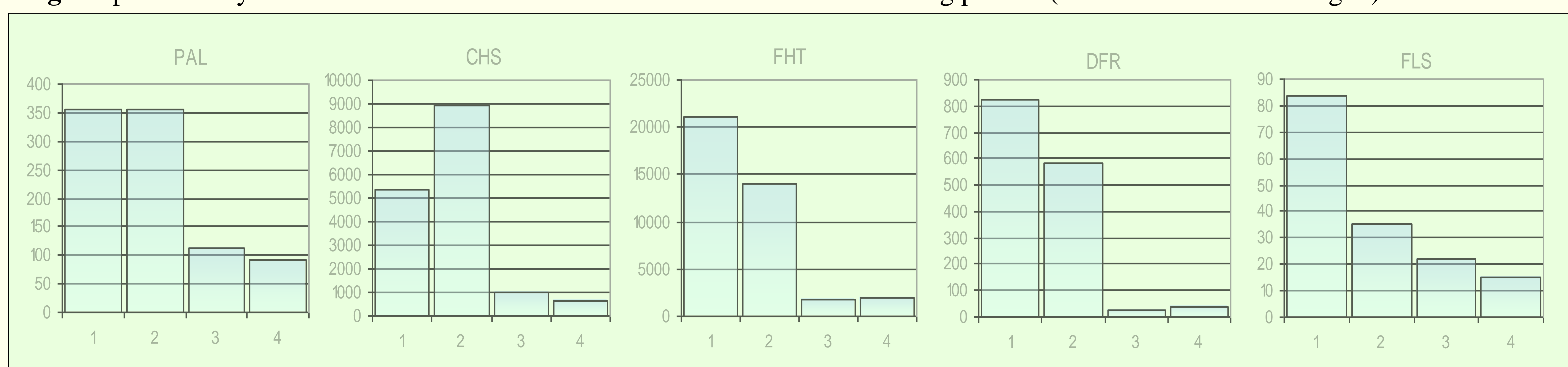
Fig. 1 Four different sainfoin varieties (f.l.t.r: 1 Bivolari, Rumania; 2 CPI63840, former Soviet Union; 3 CPI63854, Switzerland; 4 SCOP China



Results

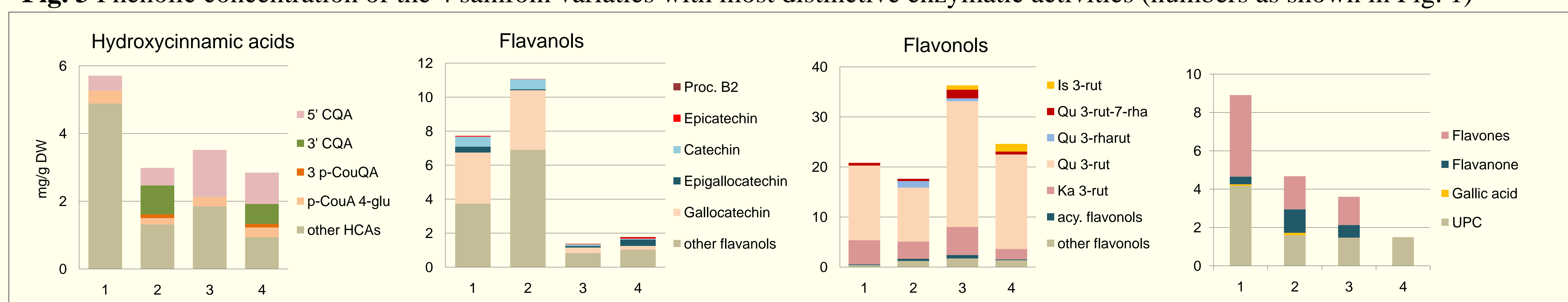
The enzymatic screening showed considerable differences in the enzymatic activities of the varieties. All enzymes could be determined in all the varieties, except the DFR which in one single variety showed no activity in the enzymatic assay. Figure 2 gives an overview on the specific enzymatic activity of varieties with the most distinctive results.

Fig. 2 Specific enzymatic activities of the 4 most distinct varieties in nmol*s⁻¹/ kg protein (numbers as shown in Fig. 1)



As expected, for the diverse sainfoin varieties significant differences in the phenolic profiles were found. Figure 3 shows the concentrations of the single phenolic compounds from the 4 sainfoin varieties.

Fig. 3 Phenolic concentration of the 4 sainfoin varieties with most distinctive enzymatic activities (numbers as shown in Fig. 1)



CQA: caffeoylquinic acid; p-CouQA: p-coumaroylquinic acid; p-CouA 4-glu: p-coumaric acid 4-O-glucoside; HCAs: hydroxycinnamic acids; UPC: unknown phenolic compounds; Is.: isorhamnetin; Qu.: quercetin; Ka.: kaempferol; acy.: acylated; ru: rutinoside; rh: rhamnoside.

Acknowledgements

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