Biosynthesis of flavonoids in sainfoin (*Onobrychis viciifolia*)

Asma F. Ahmad[1], Jana Thill[1], Ionela Regos[2], Christine Hayot[3], Heidi Halbwirth[1], Lydia Smith[3], Dieter Treutter[2], Karl Stich[1]*

[1]Technische Universität Wien, Institut für Verfahrenstechnik, Umwelttechnik und Technische Biowissenschaften, Getreidemarkt 9, 1060 Wien, Austria

[2]Technische Universität München, Unit Fruit Science, Center of Life and Food Sciences Weihenstephan, Dürnast 2, 85354 Freising, Germany

[3]National Institute of Agriculture Botany, Huntingdon Road, CB3 OLE, Cambridge, The United Kingdom

* Corresponding author: kstich@mail.zserv.tuwien.ac.at

Introduction

Sainfoin is a traditional fodder legume with high contents of flavonoids, especially tannins which have a documented beneficial effect on human and animal health [1,2]. Flavonoids play an important role as antioxidant, antimicrobial, antihelmintics and antiparasitic agent. Use of sainfoin has been reported to increase soil fertility by its ability of nitrogen fixation, as well as to facilitate the build up of soil organic matter, through a shift from urinary to faecal nitrogen. Furthermore the enzymes polyphenol oxidase and peroxidase are studied, since they are involved in the formation of quinoid structures, which could therefore influence the interaction between polyphenols and peroxidase.

Results and Discussion

All lines were tested for the enzymatic activity of PAL, CHS/CHI, FHT, DFR and FLS as well as for the quantitative gene expression of PAL, CHS, FHT, DFR, FLS, ANS, ANR, LAR and IFS. Considerable differences were observed among the lines regarding both, enzyme activities and expression of the corresponding genes of the flavonoid biosynthesis.

Despite the variation in the gene expression, the profile of the genes expressed in relation to the housekeeping gene G3PDH was similar in most of the lines (Fig. 1).

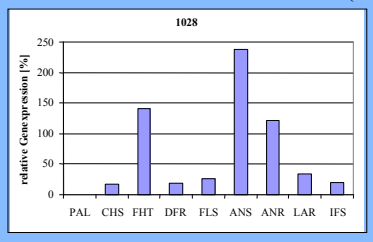
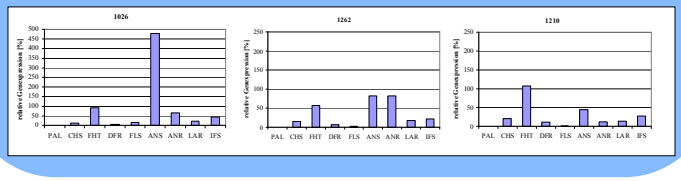


Fig. 1: Typical profile of the gene expression (line 1028, „Simpro“, France) and atypical profiles (line 1026, „Buciansky“, Slovakia; line 1262, „Cotswold Common“, UK; line 1210, „Premier“, Switzerland)



Comparison of enzyme activity and gene expression showed, that the correlation is not given in all the samples (Fig. 2). The missing correlation can be ascribed to posttranslational modification and the existence of isoenzymes.

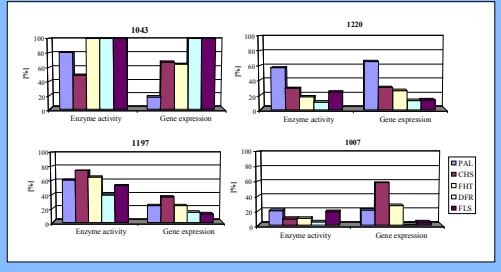


Fig. 2: comparison of enzyme activity and gene expression, correlating (line 1043, „Bivolari“, Romania; line 1220, „247“, Marocco) and non-correlating (line 1197, „CPI 63838“, Norway; line 1007, China)

When compared with the data of the polyphenol content enzyme activity/ gene expression showed a good correlation with the flavan 3-ol content and thus could serve as alternative markers for the selection of suitable sainfoin lines for breeding.

All selected sainfoin lines were tested for antioxidant activity by using DPPH assay. Mostly all the lines showed good antioxidant activity and therefore, could be considered as good source of antioxidants.

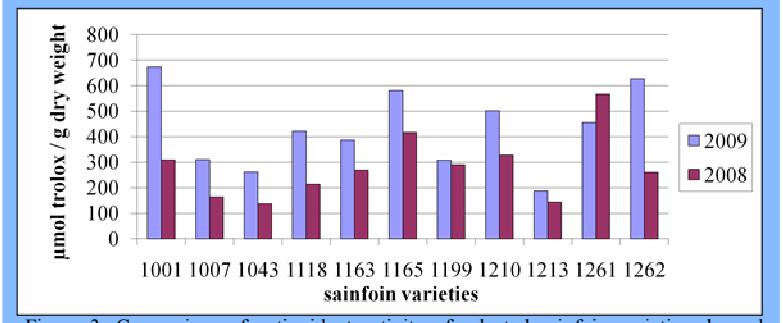


Figure 3: Comparison of antioxidant activity of selected sainfoin varieties showed similar tendency of activity in two harvesting years.

In contrast to all other flavonoid enzymes in sainfoin, peroxidase is quite stable. It showed decreased tendency of activity in different sainfoin tissues incubated in sheep rumen received/not received PEG (Fig. 4).

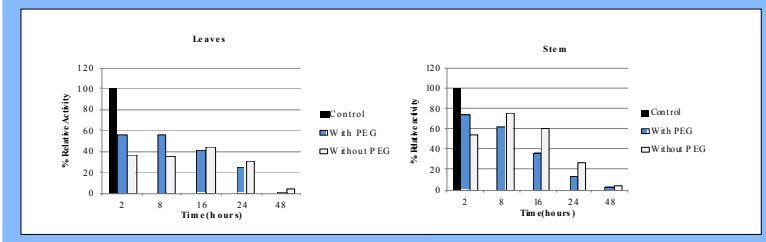


Fig. 4: POD activity in sainfoin cv. Perly after 48 hours incubation in the sheep rumen in the absence or presence of PEG compared to the fresh forage (Control). Relative activities were calculated in comparison with the activities measured before incubation.

Methods

Young (still folded) leaves were taken from 40 different lines, grown at the National Institute of Agricultural Botany in Cambridge. For the preparation of enzymes from the polyphenol rich tissues of sainfoin suitable protocols were adapted [3]. The enzymatic assays were carried out using (^{14}C)-labelled substrates [4]. POD activity was determined as described [5] with *o*-dianisidine as artificial substrate by measuring the changes in the light extinction at 460 nm. For the determination of the gene expression, real-time PCR was carried out. The different phenolic compounds were identified and quantified as described by Regos et al. [6].

Abbreviations: anthocyanidin reductase (ANR), anthocyanidin synthase (ANS), chalcone synthase/ isomerase (CHS/CHI), dihydroflavonol 4-reductase (DFR), flavanone 3-hydroxylase (FHT), flavonol synthase (FLS), glyceraldehyde 3-phosphate dehydrogenase (G3PDH), isoflavone synthase (IFS), leucoanthocyanidin 4-reductase (LAR), phenylalanine ammonia lyase (PAL), peroxidase (POD), Polyethylene glycol (PEG), 1,1-Diphenyl-2-picrylhydrazil (DPPH)

References

[1] Mueller-Harvey (1999) *In* Caygill J.C. & Mueller-Harvey I. (Eds), Nottingham University Press, Nottingham

[2] Xie et al. (2003) *Science* 5605: 396-399

[3] Dellus et al. (1997) *Phytochem* 45: 1415-1418

[4] Halbwirth et al. (1997) *Plant Science* 122: 125-131

[5] Goessinger *et al.* (2009) *Journal of Food Engineering*. 90: 171-178.

[6] Regos et al. (2009) *J Agric Food Chem* 57: 5843-52

Acknowledgements

These investigations were supported by the European Commission (Project MRTN-CT-2006-035805).