Biosynthesis of flavonoids in sainfoin
(Onobrychis viciifolia)

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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, anti-inflammatory 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).

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.

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.

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 (14C)-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), leucanthocyanidin 4-reductase (LAR), phenylalanine ammonia lyase (PAL), peroxidase (POD), Polyethylene glycol (PEG), 1,1-Diphenyl-2-pircrylhydrazol (DPPH)

References

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