# The effect of drought on the extractability of proanthocyanidins in sainfoin (*Onobrychis viciifolia*)

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## Abstract

Proanthocyanidins (PAs) in forage legumes may be present in either extractable or non-extractable form. Despite potential benefits of the non-extractable fractions in ruminant nutrition, few studies have analysed the composition of PA fractions in forages and a potential genotype × environment interactions has generally been disregarded, thus far. Consequently, this study examined the impact of drought on the composition of PA fractions across five sainfoin (*Onobrychis viciifolia*) accessions and based on their ontogenetic stage. Generally, drought stressed plants showed a 44% higher ($P$<0.01) extractable PA concentration than rainfed plants. However, the increase was dependent on the ontogenetic stage, with generative plants showing a 27% increment ($P$<0.05), compared to 59% ($P$<0.001) in vegetative plants. Protein- and fibre-bound PAs were unaffected by drought independent of the ontogenetic stage and only accounted 7-13 and 2-4% of PAs. As the effectiveness of non-extractable PAs has not yet been established, no conclusions can be drawn with regards to the effect of these minor fractions on environmental and animal health effects. However, the low concentration combined with the absence of changes under drought indicates both a low effect size as well as a high predictability, both of which are beneficial for future analyses.

**Keywords:** *Onobrychis viciifolia*, non-extractable proanthocyanidins, condensed tannins, water stress

## Introduction

Proanthocyanidins (PA, syn. condensed tannins) are oligomeric or polymeric plant secondary metabolites that have been shown to improve animal health and environmental performances in ruminant agriculture by acting anthelmintic and reducing methane emissions (Mueller-Harvey *et al.*, 2019).

In previous determinations of PA concentrations, the analysis was limited mainly to the acetone/water-soluble PA fraction. In addition to the fraction that is soluble in organic solvents, there are also insoluble PA fractions which are bound either to protein or fibre. There are, however, indications that these bonds can dissipate after the rumen, as a higher antiparasitic effect was detected in the abomasum compared to the rumen due to higher PA concentrations (Desrues *et al.*, 2017). Therefore, we have assessed not only the soluble PA fraction but also the proportions of protein- and fibre-bound fractions of the PA-containing fodder plants of sainfoin in order to estimate their bioactive value. Additionally, the effect of drought on the plants was determined to detect how environmental impacts might result in shifts among the PA fractions.

## Materials and methods

Thirty sainfoin accessions were cultivated in a field experiment located in the North of Zurich, Switzerland (47°44' N 8°53' E, 482 m a.s.l). The experimental setup was described in Malisch *et al.* (2016). Briefly, of the thirty accessions, a subset of five accessions was used for PA analysis. Each treatment was replicated twice and contained nine individual plants per replicate, with a 0.25×0.5 m distance to each other (within and in between rows). Drought was induced using rainout shelters. These were designed to maintain environmental conditions (temperature, irradiance) which resembled the unsheltered control. Drought lasted for 127 days, from 12 June to 17 October 2013 and reached a soil water potential of below -1.5 MPa. Half of the plants were cut at week 7 of the drought period, shifting their ontogenetic stage back to vegetative, while the uncut plants continued generative growth. The experiment was carried out as a split-plot design with the four treatment combinations being the main plot and the accessions randomly distributed within, as subplots. The effect of the treatments and their interactions on the dependent variables were determined by using a linear mixed regression model, where the treatments and accessions were fixed factors, while the variances due to the block, the main plot, the subplot and the plant were assigned as random variable. Except mentioned otherwise, all results presented here are the mean values taken from two sampling events at peak drought, sampled at weeks 10 and 14 of the drought period. PA fractions were determined according to Terrill *et al.* (1992) using the HCl-Butanol assay.

## Results and discussion

In the first 6 weeks, drought stress had no effect on the concentration of soluble, protein-bound or fibre-bound PAs (results not shown). Across week 10 and 14, the soluble PA (S-PA) concentration was significantly ($P<0.05$) higher in the drought stressed plants in the vegetative stage, while protein (P-PA) and fibre bound PA (F-PA) were not affected by environmental conditions (Table 1). Soluble PA generally accounted for 83-90% of the total PA and thus was by far the largest fraction, with P-PA ranging from 7 to 13% and F-PA from 2-4%.

The PA compositions averaged across environments were uniform across accessions with S-PA generally accounting for 83-87%, while P-PA and F-PA were in the range of 9-12 and 3-5%, respectively (Table 1). Also. PA compositions among accessions were stable across environments with no significant accession × treatment ($P$=0.1) or accession × treatment × sampling event ($P$=0.22) interaction.

The general PA composition for sainfoin without drought impact is in line with previous findings (Girard *et al.*, 2018). Regarding the impact of drought, the effect of increasing S-PA concentrations under drought has been discussed before (Malisch *et al*, 2016).The lack of differences in the protein and fibre bound PAs in sainfoin across environments and plant age was surprising, however, as plant age increases cell wall contents, whereas protein and cell content decrease, thus an increase in F-PA in generative plants was anticipated. At the same time, drought has been shown to reduce neutral and acid detergent fibre

Table 1. Concentration of soluble (S-PA), protein-bound (P-PA), fibre-bound (F-PA) and total PAs dependent on treatments averaged over harvests after 10 and 14 weeks, for plants with (cut+) or without (cut-) additional cutting, and with (drt) or without (ctr) rain exclusion (top), or averaged across all treatments for each of the five accessions (bottom).

| | | PA (mg $g^{-1}$ DM) | | | |
|---|---|---|---|---|---|
| | | S-PA | P-PA | F-PA | Total |
| Treatment | ctr/cut- | 13.13 a | 2.07 a | 0.69 a | 15.89 a |
| | ctr/cut+ | 15.18 a | 1.76 a | 0.54 a | 17.48 a |
| | drt/cut- | 16.74 a | 1.34 a | 0.63 a | 18.71 a |
| | drt/cut+ | 24.14 b | 1.97 a | 0.61 a | 26.72 b |
| | SE | 0.73 | 0.09 | 0.03 | 0.75 |
| Accession | CPI 63750 | 15.38 a | 1.67 a | 0.57 a | 17.65 a |
| | Esparsette | 15.54 a | 1.67 a | 0.62 ab | 17.83 a |
| | Perly | 11.57 b | 1.67 a | 0.67 b | 13.92 b |
| | Taja | 15.76 a | 1.84 a | 0.62 ab | 18.28 a |
| | Visnovsky | 12.64 b | 1.85 a | 0.56 a | 15.07 b |
| | SE | 0.60 | 0.06 | 0.03 | 0.60 |

(NDF and ADF), as well as lignin in forages, hence control treatments should generally have higher F-PA concentrations (Peterson *et al.*, 1992). Generally, the P-PA and F-PA shares can be considered low in comparison with other plant species, as for example in birdsfoot trefoil (*Lotus corniculatus*) cv. Polom, S-PAs accounted for 38% and F-PA for 11% (Girard *et al.*, 2018).

## Conclusions

The results of this study show that the proportions of bound proanthocyanidins in sainfoin are likely to be of lesser significance compared to soluble PAs due to their much lower concentrations. This increases the likelihood that the analysis of S-PA is most important in sainfoin. Together with the fact that only S-PA were affected by the environment, and that this behaviour was uniform across all accessions, this can be considered very promising as this facilitates the predictability of future studies with sainfoin.

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