



Molecular Markers for Phylogenetics of *Onobrychis* Species



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INTRODUCTION

This project forms part of an EU funded project entitled "Healthy Hay"; the re-invention of sainfoin: an example of a novel resource for sustainable agriculture. A new sainfoin breeding programme has been initiated. NIAB is evaluating the agronomic and morphological characters of a germplasm collection and the genetic characterisation as been initiated. Partners are currently assessing our plant material for biological, environmental impact and tannins characterisation and quantification.



Onobrychis sp. seeds

ONOBRYCHIS VICIIFOLIA

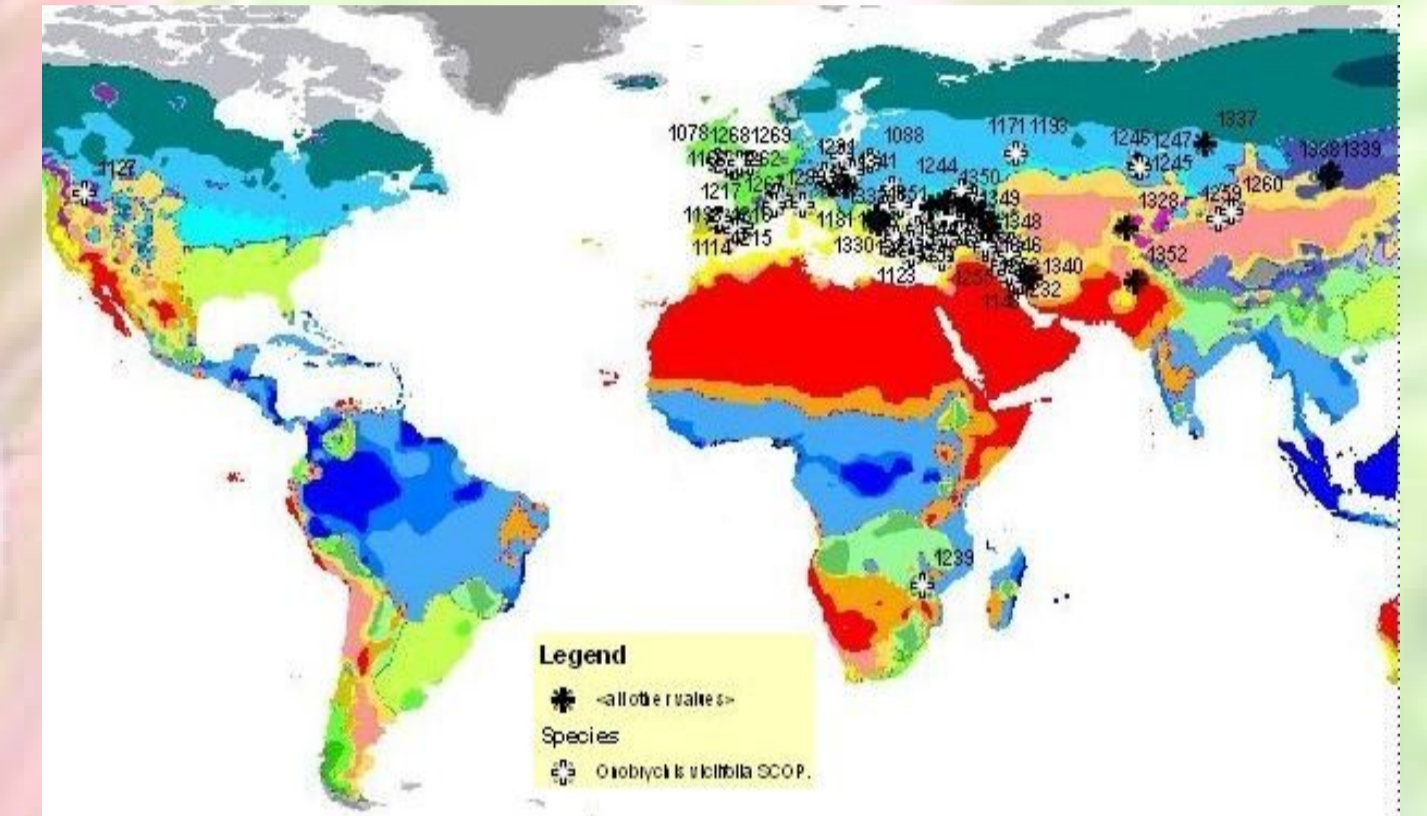
- A fodder legume with a long cultivation history
- Agronomic potential: low-input crop, soil improving crop
- Biological potential: High nutritional value, high palatability, non bloating forage, anthelmintic properties, reducing methane outputs from ruminants
- Ornamental flowers attracting wide range of insects. Allow production of high quality honey



O. viciifolia flower pollinated by bumblebee

GERMPLASM COLLECTED

- 355 accessions of *Onobrychis* sp.
- Most are *O. viciifolia*: wild and cultivated, giant and common types



Provenance of accessions on Köppen climate map

PHENOTYPIC CHARACTERISATION : a great diversity within and between accessions observed

- Field evaluation on replicated 1.5 m² plot on agronomic (yield, re-growth, flowering time, survival, diseases and pests) and morphological characteristics (inflorescence, leaf, stems, habit and homogeneity)



Differences in inflorescence colour

Differences in leaf colour and shape

Erect plant green stems vs prostrate plant red stems

Sainfoin with *Fusarium* sp. show desiccated leaves, then senescence

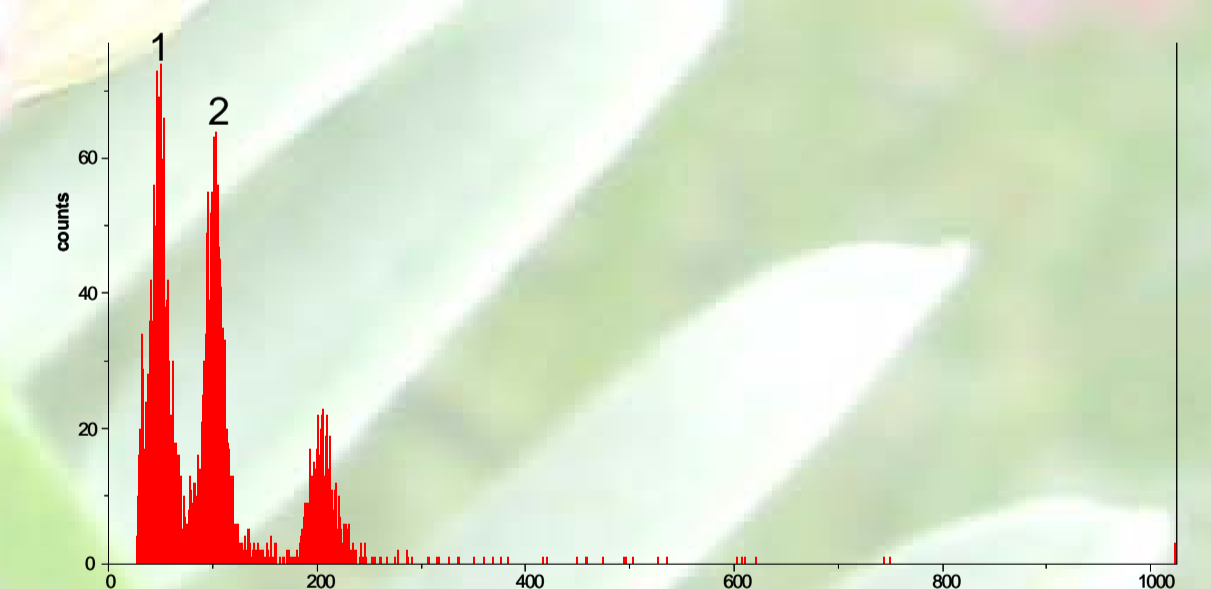
Mostly accessions of *O. viciifolia* are tetraploid (whether giant or common) but a few of the wild types are diploid

KARYOTYPING STUDIES

Ploidy of other *Onobrychis* sp. also investigated

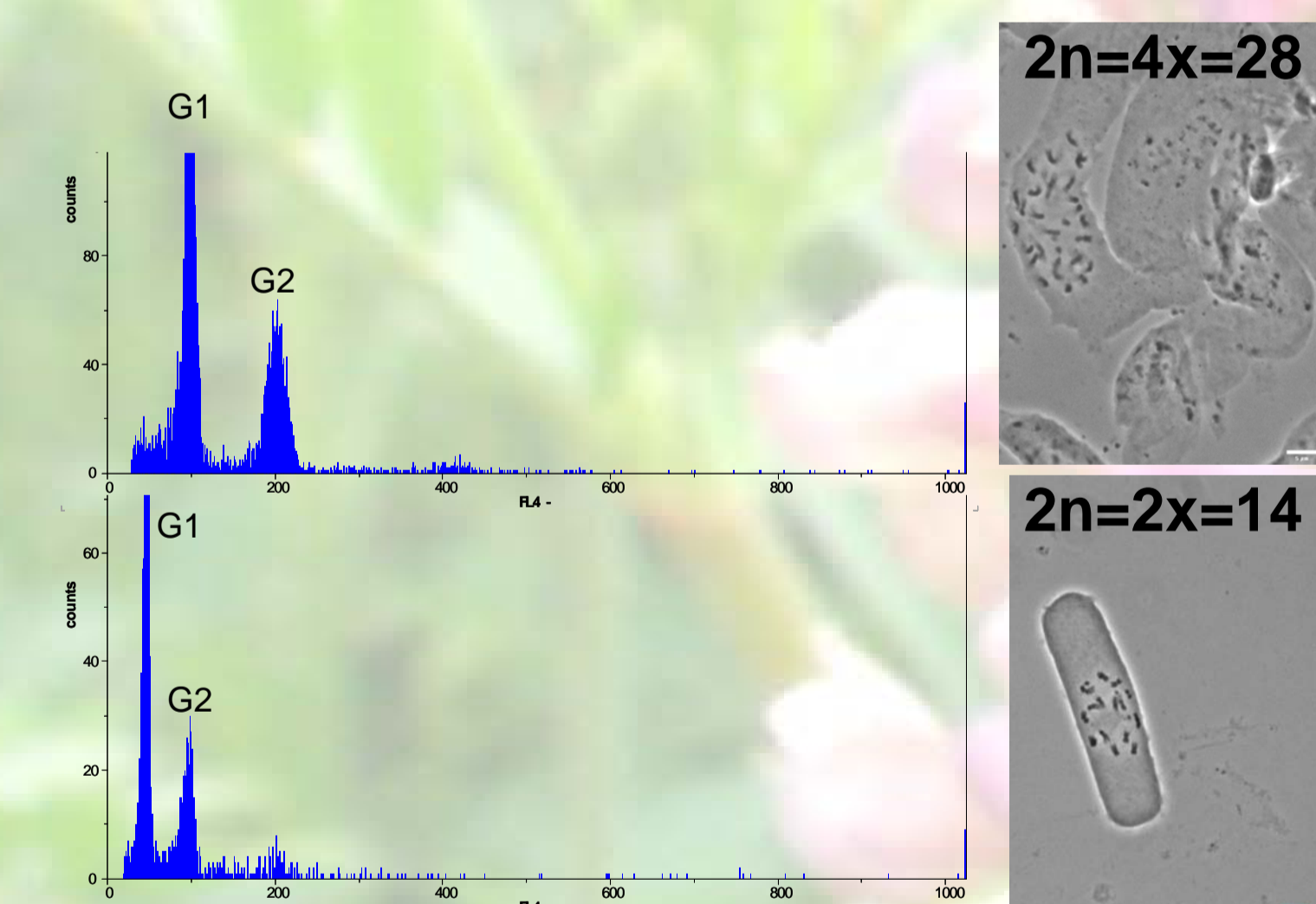
Species	Section	Ploidy observed	Chromosomes
<i>O. aequidentata</i>	Lophobrychis	Diploid	16
<i>O. alba</i>	Lophobrychis	Diploid/Tetraploid	14/28
<i>O. altissima</i>	Onobrychis	Tetraploid	28
<i>O. antasiatica</i>	Onobrychis	Tetraploid	28
<i>O. arenaria</i>	Onobrychis	Diploid/Tetraploid	14/28
<i>O. biebersteinii</i>	Onobrychis	Tetraploid	28
<i>O. bungei</i>	Onobrychis	Tetraploid	28
<i>O. crista-galli</i>	Lophobrychis	Diploid	16
<i>O. cyri</i>	Onobrychis	Tetraploid	28
<i>O. gracilis</i>	Onobrychis	Tetraploid	28
<i>O. iberica</i>	Onobrychis	Tetraploid	28
<i>O. montana</i>	Onobrychis	Tetraploid	28
<i>O. petrea</i>	Onobrychis	Diploid	14
<i>O. radiata</i>	Hymenobrychis	Diploid	14
<i>O. subacaulis</i>	Heliobrychis	Diploid	14
<i>O. transcaucasica</i>	Onobrychis	Tetraploid	28
<i>O. viciifolia</i>	Onobrychis	Diploid/Tetraploid	14/28

O. viciifolia genome size of about 2.5pg



Determination of genome size using *Zea mays* as a reference and PI as staining agent

$$\text{Sample 2C value} = \frac{\text{Reference 2C value} \times \text{Sample G1 mean peak position (1)}}{\text{Reference G1 mean peak position (2)}}$$



Tetraploid vs diploid *O. viciifolia* revealed by flow cytometry confirmed by microscopy

FINGERPRINTING

- Capillary electrophoresis based AFLP fingerprinting work is ongoing
 - Polymorphic regions observed
 - Specificity and discriminatory power of the system needs improvement
- The taxonomy of the *Onobrychis* genus is being investigated, many of the species appear to be sub-species
- Phylogeny will allow to gain knowledge on cultivation history of *O. viciifolia*



Example of 7 polymorphic regions between 3 samples with one similar primer combination Eco+3 Mse+3



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