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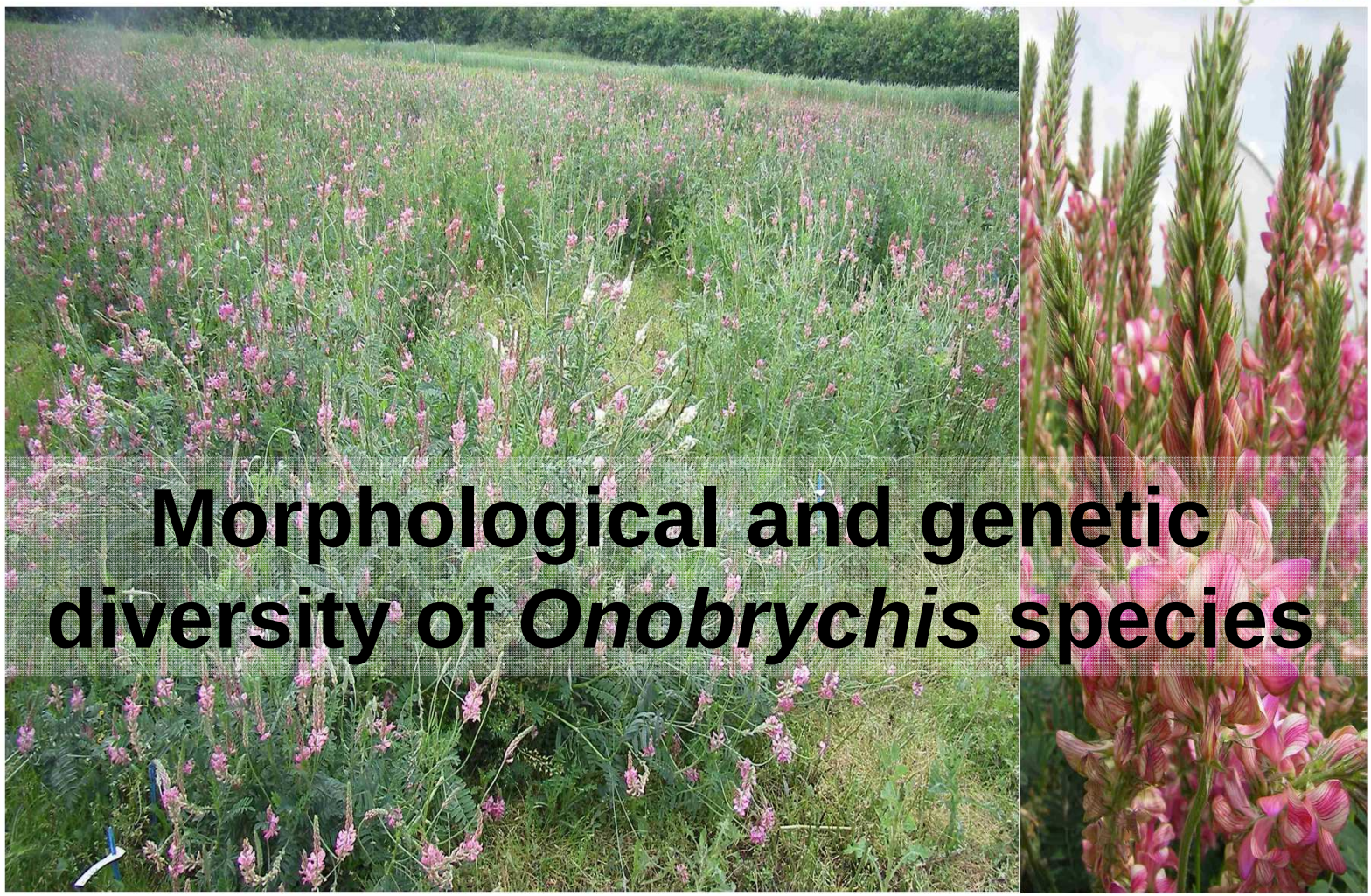


Morphological and genetic diversity of *Onobrychis* species

HEALTHYHAY
Marie Curie Research Training Network

Christine Hayot

9 September 2009,
BES annual meeting

Sainfoin (*Onobrychis viciifolia*)

- Perennial forage legume from *Hedysareae* tribe
- It was traditionnally used until early 20th century
- Declined due to low yield and high inputs encouraged

Sainfoin a great potential for sustainable farming system

- Agronomic potential: low-input crop, soil improving crop

- Biological potential: High nutritional value, high palatability, non bloating forage, anthelmintic properties, reducing methane emissions from ruminants

- Ornamental flowers attracting wide range of insects. Allow production of high quality honey

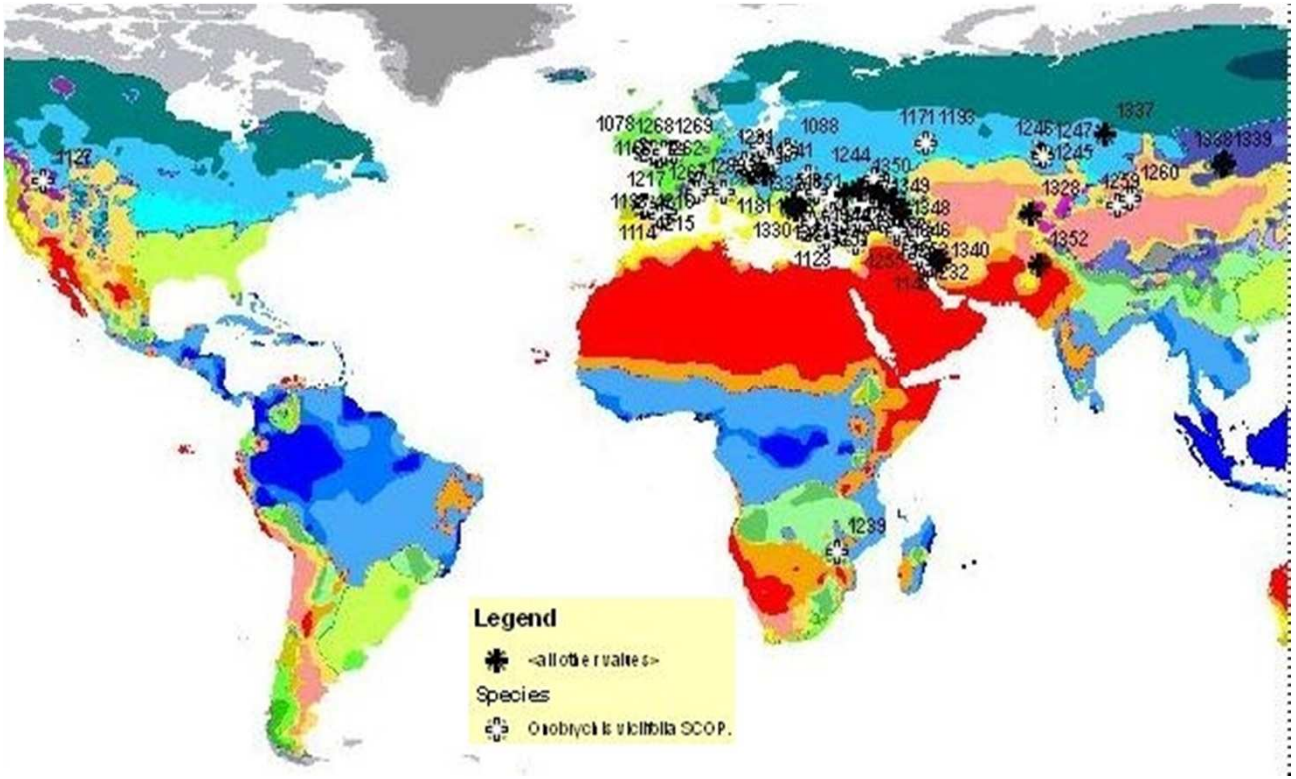
‘Healthy Hay’ project: sainfoin reinvention

- Thirteen European and one Armenian partner
- Properties evaluation (nutritional, environmental, anthelmintic, tannins…)
- NIAB role : prebreeding (germplasm collection, seed production, morphological, agronomic and genetic characterisation of lines)

Germplasm collection

- *Onobrychis viciifolia* but also other *O.* sp.
- 355 accessions collected

Cultivar or landraces with high agricultural value
Wild types well adapted to adverse environmental conditions



- 170 accessions (1 to 3 replicates) growing in field condition

Seed production

Covered with insect-proof polyethylene tunnels

Bumblebee minihives to pollinate

Seed production

- Number of seeds obtained average 3500 (17 to 18,000)

- Viability of seeds produced assessed with tetrazolium test 30 seeds average 72.5% (13 to 100%)

Sampling for network needs

- Harvest of all the accessions
- Samples sorted and freeze dried then sent to other partners for chemical/biological analyses

Morphological and agronomic evaluation

Wide diversity confirmed in 2008 and 2009:

- Flowering time
- Yield (factor of 25)
- Habit
- Stem thickness (5mm to 2cm) and colour
- Inflorescence length and colour
- Leaf colour, shape and length
- Diseases and pest
- Regrowth

Habit, stem colour

Inflorescence colour

From white to purple including salmon and pink

Leaf colour, shape and length

Wide range of leaf colour, number and shape of leaflets

Diseases

•*Phoma sp*

summer

numerous plants affected

•*Stemphyllium sp*

all year round

few plants affected

•*Erysiphe trifolii*

end summer/ autumn

few isolated plants

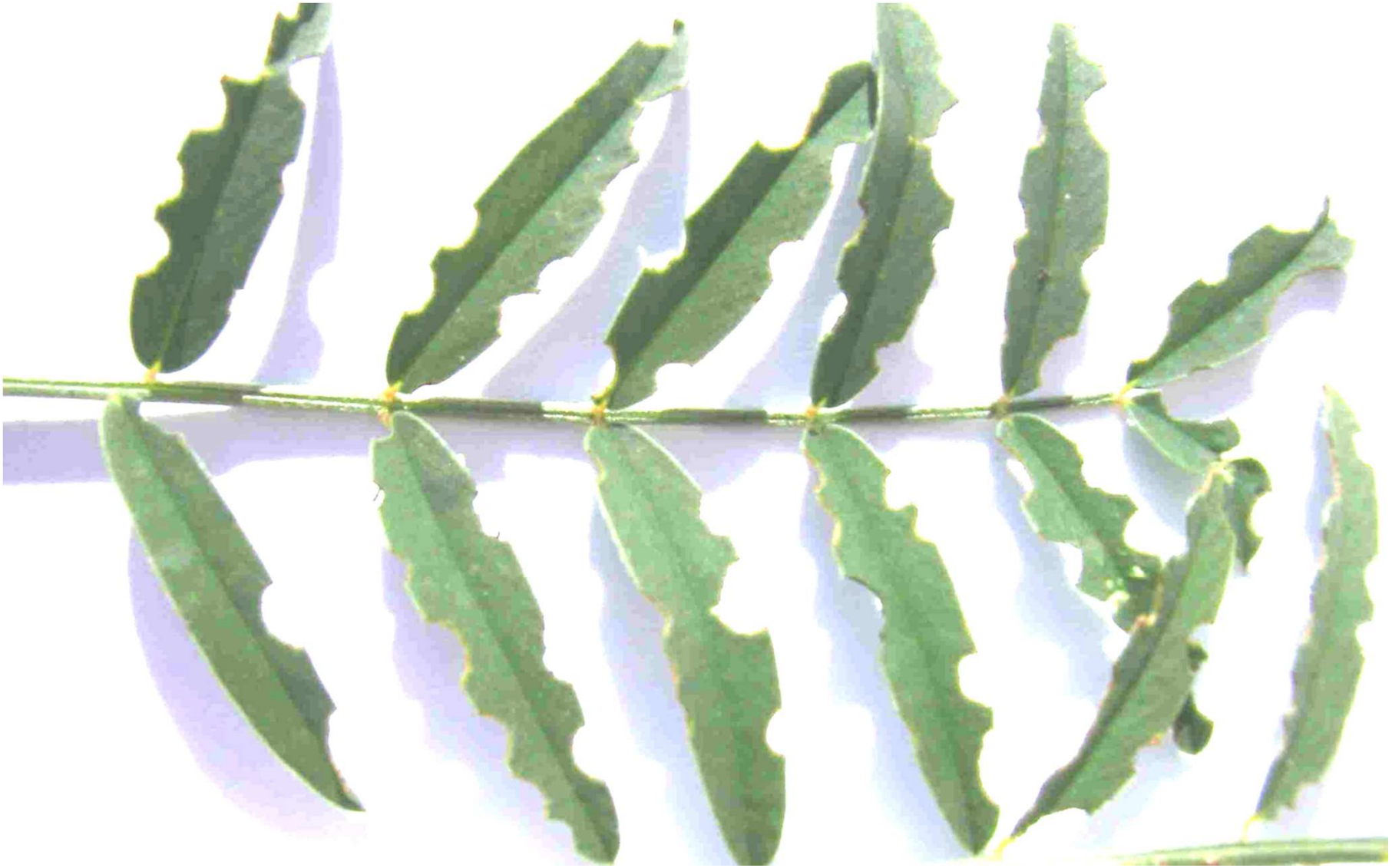
•*Fusarium sp*

all year round

few plants affected

Pest

Insect pest: *Sitona* weevil (autumn, numerous plants affected)



Morphological and agronomic work perspective

- Select best performing accessions depending on needs (grazing, cutting)

- Find groups/climatic regions showing similarities in their characteristics to facilitate diversity conservation and sainfoin future breeding

- Check the cross fertility and the potential use in breeding of close related species by crosses trials

Need for karyotyping work

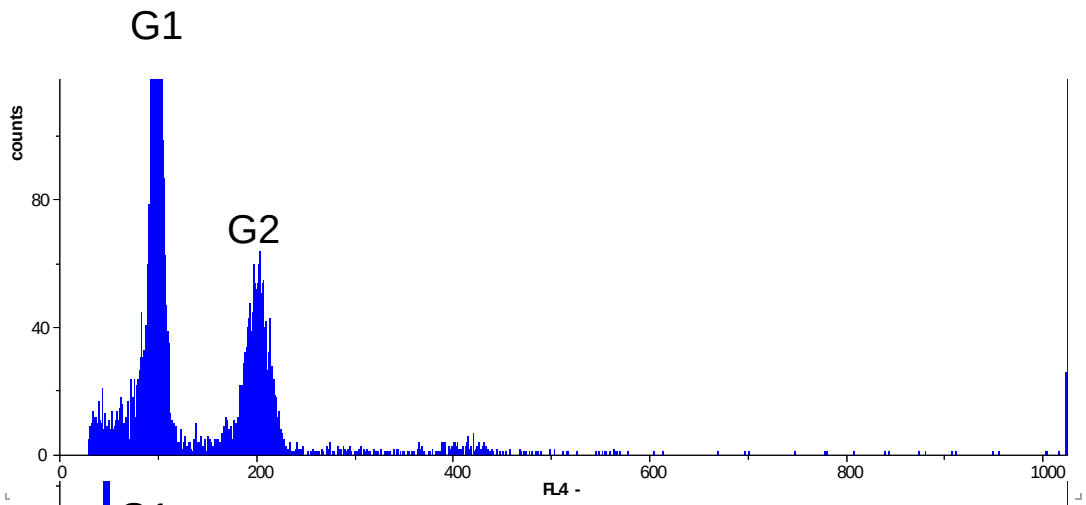
- Giant and common types: difference in persistence, flowering cycles but no known link between morphology, agronomy and genetics
- Different species of *Onobrychis* but similar morphology, crosses possible…
- Scarce and contradictive data on ploidy (diploid and/or tetraploid sainfoin)
- No information on sainfoin genome size

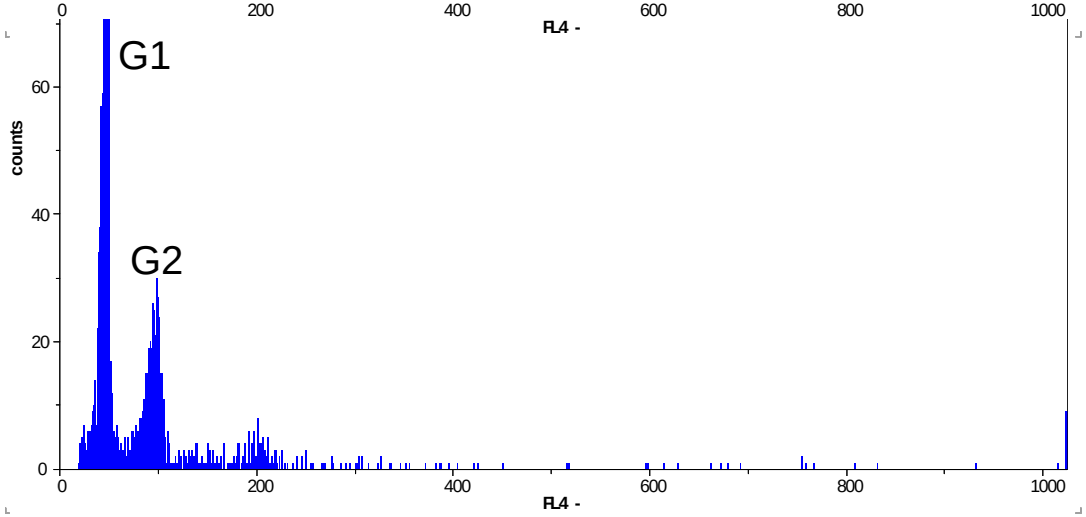
➢Use of flow cytometry and microscopy

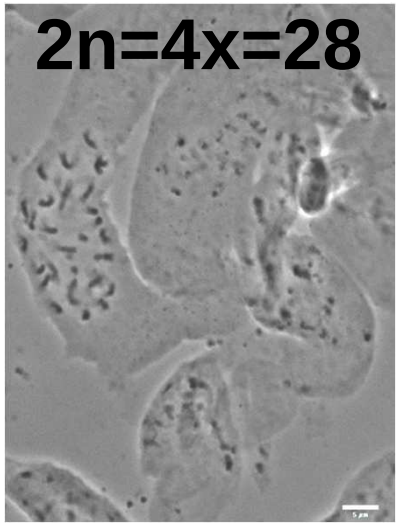
Determination of sainfoin ploidy

Several reference of known ploidy (tetraploid)

Selected accessions of sainfoin mostly tetraploid but 3 wild sainfoin accessions diploid







Other *Onobrychis sp.* karyotypes

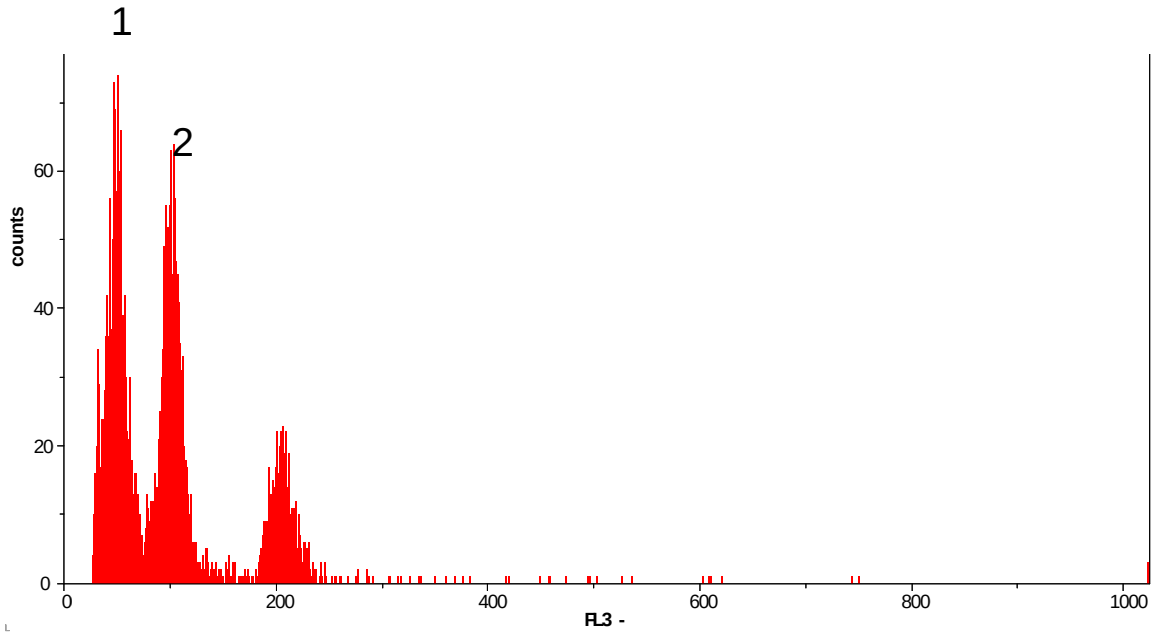
Interest for breeding purposes (adapted to extreme climate/ interesting tannins)

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

Estimation of nuclear DNA content in absolute units (genome size)

2C-value is the nuclear DNA amount

Try with several known standards to find close one (genome size calculated with difference less than 2X) maize used for sainfoin

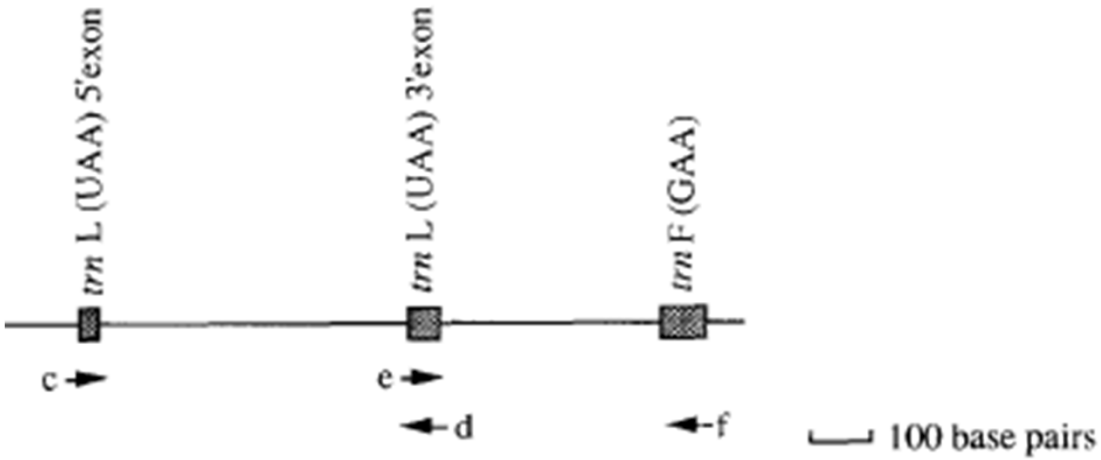


$$\text{Sample 2C value} = \frac{\text{Reference 2C value} * \text{Sample G1 mean peak position}}{\text{Reference G1 mean peak position}}$$

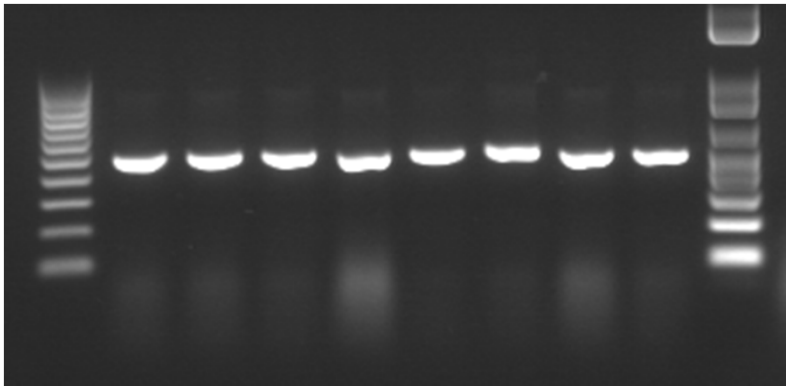
Onobrychis viciifolia 2C value = 2.5pg (with Propidium Iodide)

Initial development to evaluate genetic diversity

Chloroplast DNA highly conserved, non-coding region good for phylogenetic study

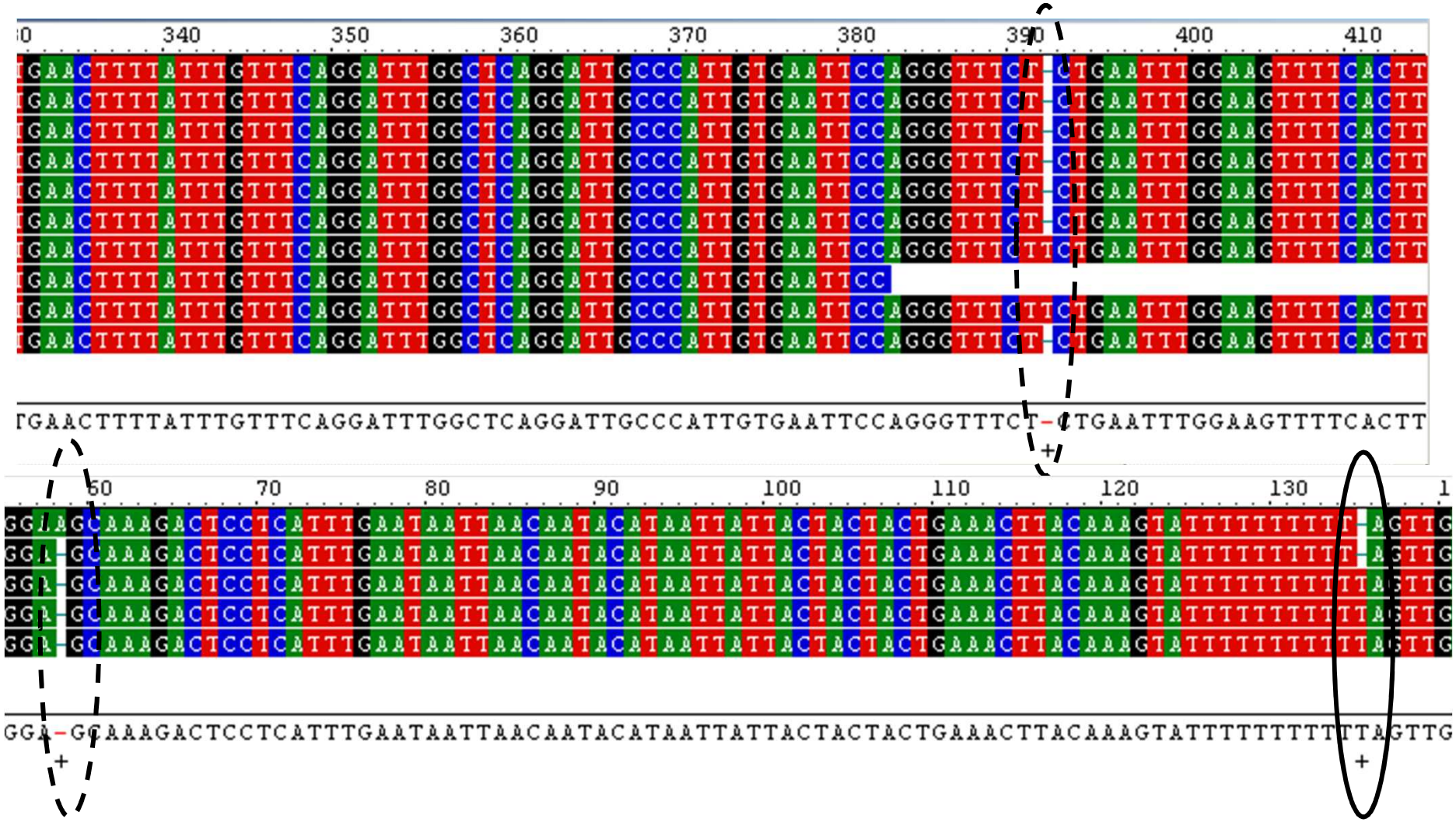


No inhibition of DNA confirmed by PCR



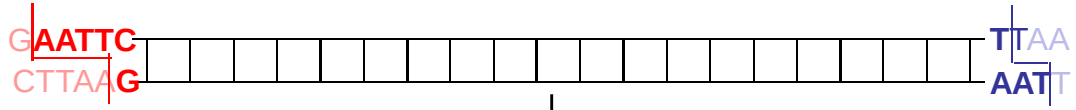
Screening for markers within chloroplast non-coding regions

Sequencing of amplicons (2 primer combinations), selection of 16 accessions considered

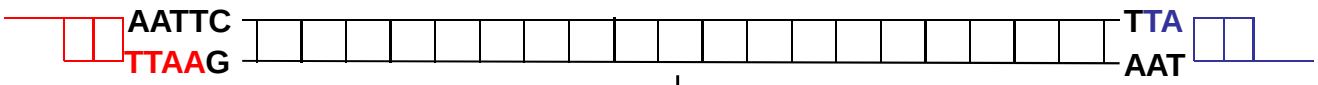


AFLP principle (Amplified fragment Length Polymorphism)

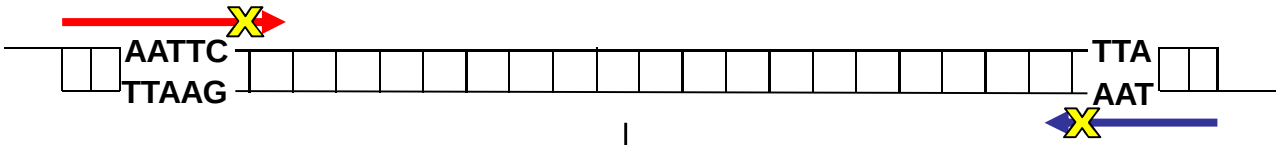
Restriction digest of DNA using EcoR1 and Mse1



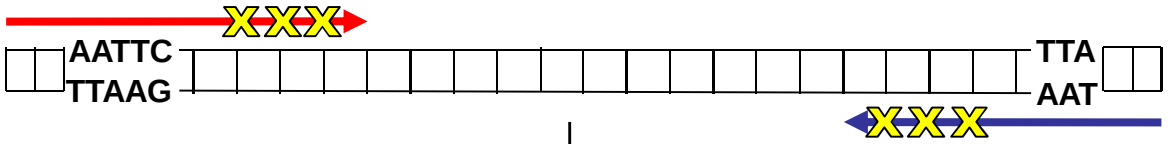
Adapters ligations using EcoR1 and Mse1 adapters



PCR1 using selective primers 1 in 16 fragments amplified



PCR2 using selective primers 1 in 256 fragments amplified

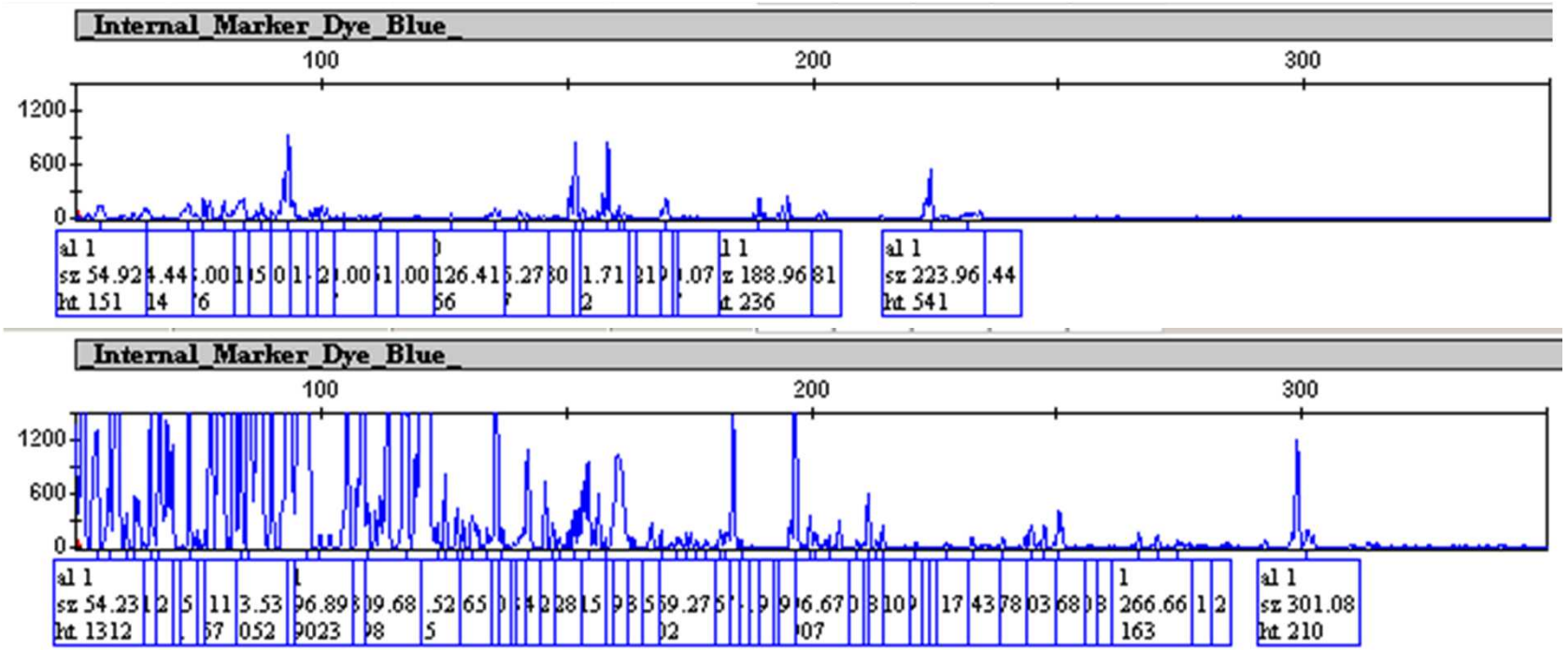


Li-Cor sequencer band analysis or **ABI sequencer** peak analysis

AFLP work ongoing

Improvement of the protocol achieved

Pattern observed improved (size fragments, height peaks)



Examples of polymorphic regions observed

AFLP work plan: phylogeny and taxonomy clarification

- Best combinations to choose (highest polymorphism)

Sainfoin 2n=4x=28 so 28 to 280 distinct polymorphic fragments

- 20 accessions, 10 plants per accessions

Decision on number, bulk

- Assessment of minimum of selected accessions and other species of *Onobrychis*

Acknowledgement

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