Polymorphism and EST-SSR cross-amplification study on a germplasm collection of *Onobrychis viciifolia*

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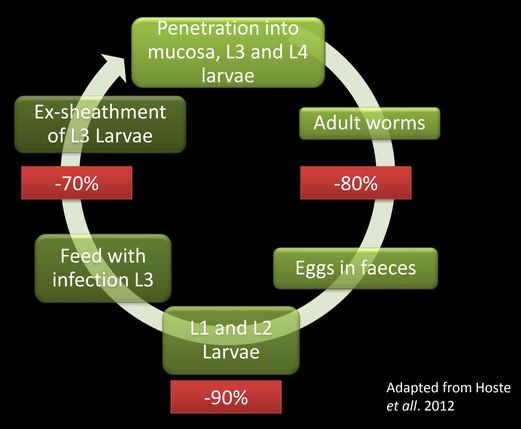
1. Abstract

Onobrychis viciifolia is an emerging leguminous forage crop, traditionally grown across Eurasia and North America, where it is used primarily for livestock feed [1]. *O. viciifolia* could re-emerge as an important forage legume due to multiple beneficial properties, however, the knowledge surrounding this species is under developed. We have embarked on an in depth genetic characterisation of *O. viciifolia* by developing molecular markers and using RNAseq.

Due to a lack of characterised molecular markers in *O. viciifolia*, we decided to screen a set of EST-SSR derived from *Medicago truncatula* tested in *O. viciifolia* [2] . From a suite of six markers we identified five which were polymorphic across the range of Sainfoin ecotypes assessed.

2. Introduction

Fig 1- Interferences in the parasite cycle driven by the presence of CT from *O. viciifolia*



Adapted from Hoste *et all*. 2012

The decline in the area of *O. viciifolia* and other temperate legumes across Europe since 1950 [3] especially in the 1970s during the Green Revolution [4]. *O. viciifolia* in particular is not favoured due to its relatively low productivity. However, the interest in this legume has re-emerged due to its many beneficial properties, including: its high quality nutritional profile; anti-parasitic properties; non-bloat characteristic and implications in the control of methane emissions. These attributes have been attributed to presence in the foliage of high concentrations of condensed tannins (CT).[3-5]

In order to inform the required agronomical improvement, we developed a genetic characterisation testing the polymorphism and EST-SSR cross amplification of different markers from *M. tuncatula*.

3. Material & Methods

Seven different accessions of Sainfoin were evaluated in this study. The lines selected were 1001, 1003, 1005, 1007, 1043, 1071 and 1209 from the *O. viciifolia* germplasm collection at NIAB.

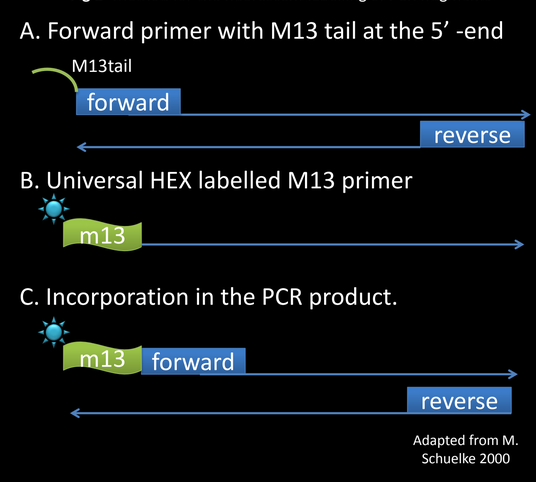
Young healthy tissues were collected with liquid nitrogen and the DNA was extracted using Phyto-pure kit from GE-Healthcare.

We tested six different primers from *M. trunculata* [2]. Using pooled DNA from selected plants per accession; amplified using gradient PCR.

Table 1.- Primers from *M. truncatula* previously amplified in *Vicia faba* (V), *Pisum sativum* (P), *Cicer arietinum* (C), *Trifolium repens* (T) [2].

Oligo Name	Sequence 5' to 3'	Oligo Name	Sequence 3' to 5'	Cross-amplification
MtBA01B04R2_F	CGATCGGAACG AGGACTTTA	MtBA01B04R2_R	CCCCGTTTTTC TTCTCTCCT	V,P,C
MtBB36F05F1_F	TCCCCTTAAGC TTCACTCTTTTC	MtBB36F05F1_R	CATTGGTGGAC GAGGTCTCT	V,P,C
MtBC47B06F1_F	CCTTTGGTTGA TTCAGTTTC	MtBC47B06F1_R	CCAATATGTCA CTCCTTGCT	V,P,C
MtBB44F02R1_F	GGTGATTGGTG TTTTCTGTC	MtBB44F02R1_R	AGCAAAACTAT CACCACCAG	V,P,C
BI74_F	TGTACCAAGCG AATGAAGTGTT	BI74_R	GGGTTGCATCT AACAACAGACA	T
AL79_F	CCCCATTGACG CATTCTTAC	AL79_R	TCCTCAACCAA CCACTTCCT	T

Fig 2- Method for the fluorescent labelling of PCR fragments



Adapted from M. Schuelke 2000

Amplification products were resolved using capillary electrophoresis. (ABI3730XL)

4. Results & Conclusions

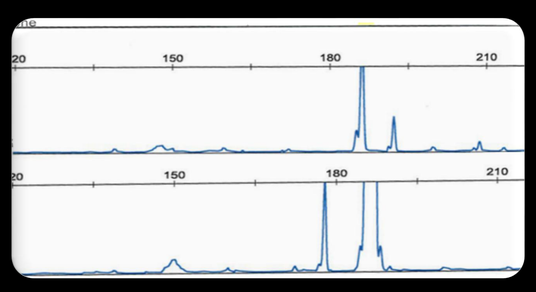
From a set of six EST-SSR from *M. truncatula* previously tested in *O. viciifolia,* we found that five of them amplified across the nine preliminary accessions that were tested. All of primer sets were polymorphic, with the majority observed to be presence/absence type polymorphisms, however, size polymorphisms were also identified. Patterns of polymorphism suggest that some ecotypes are more genetically different than others and this will be investigated further.

Continued efforts will include the screening of new EST-SSRs from different leguminous species, the study of new lines from the extensive NIAB germplasm collection and the development of a cluster distribution of this germplasm collection in order to inform ongoing breeding programs.

Table 2- Amplification obtained with PCR for the six first primers tested

Primer/ line	MtBA01B04 R2	MtBB36F05 F	MtBC47B06F 1	MtBB44F02 R1	BI74	AL79
1001	no	ambiguous	no	no	amplifies	no
1003	no	no	amplifies	amplifies	amplifies	amplifies
1005	no	amplifies	amplifies	amplifies	amplifies	no
1007	no	no	amplifies	amplifies	no	amplifies
1043	no	no	no	no	no	amplifies
1071	no	ambiguous	amplifies	amplifies	amplifies	no
1209	no	no	no	ambiguous	no	no

Fig 3- Polymorphism of the primer ALT9



6. Literature

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7. Acknowledgement

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