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REGULAR ARTICLE

DEVELOPING THE iPBS STRATEGY FOR YAKON GERMPLASM EVALUATION.

Jana Žiarovská[*1], Eloy C. Fernández[2], Milan Bežo[1]*

Address: Ing. PaedDr. Jana Žiarovská, PhD.,

[1]Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Genetic and Plant Breeding, Tr.A. Hlinku 2, 949 76 Nitra, Slovak Republic.

[2]Czech University of Life Sciences, Institute of Tropics and Subtropics, Department of Crop Sciences and Agroforestry in Tropics and Subtropics, Kamýnská 129, 165 21 Praha-Suchdol, Czech Republic.

*Corresponding author: jana.ziarovska@uniag.sk

ABSTRACT

Two yacon varieties PER05 and ECU45 were used for iPBS method developing for yacon - *Smallanthus sonchifolius*, (Poepp. et Endl.) germplasm evaluation. Because of high level of polyphenols in yacon, four DNA extraction methods were tested for the best results in the iPBS method. Using a set of universal primers that anneal to the conserved regions of retrotransposons, polymorphism of amplified fragments of DNA was analysed and for the development of iPBS protocol primers that produce PCR fragments within the whole possible range of PCR were chosen. Selected primers were subsequently used in a set of gradient PCR for finding of optimal annealing temperatures for each of them and three groups of primers according to the optimal annealing temperature were found - primers with a optimum at 53°C (1845, 1875 and 1886), at 56°C (1846) and 61 °C (1880 and 2078).

Keywords: iPBS, yacon, retrotransposon, molecular marker

INTRODUCTION

Retrotransposons in eukaryotes can be divided into two major classes, i.e. LTR retrotransposons and non-LTR retrotransposons, based on whether there is a long terminal repeat sequence, an identical repeat structure at both ends of retrotransposons, which is very important for the auto-transposition of LTR retrotransposons, because it contains the transcription related promoter and terminator. The length of LTR usually ranges from 100 bp to 5 kb and has a unique feature which begins with TG (TA in a few cases) and ends with CA (TA in a few cases) and the flanking 5 bp target site duplication as the remaining of transposition signature (**Zou *et al.*, 2009**).

Four types of LTR retrotransposons have been recognized presently in plants in which the most common types are Ty1- copia and Ty3-gypsy. They usually carry the structure of LTR, group £-specific antigen (gag), RNA £-dependent DNA polymerase (pol) and integrase (int), and can transpose autonomously because they possess of all the necessary coding sequences responsible for the retro-transposition process (**Bennetzen 1996**; **Kumar and Bennetzen 1999**). The other two types of LTR retrotransposon, LARD (Large Retrotransposon Derivatives) and TRIM (Terminal-Repeat Retrotransposons in Miniature), which are newly discovered, are composed very simply with short LTR structure and lack integrase genes necessary for the transposition. Therefore, LARD and TRIM have no autonomous transposition activity, however, both of them can be transposed with the assistance of autonomous retrotransposons (**Antonius-Klemola *et al.* 2006**; **Kwon *et al.* 2007**; **Witte *et al.* 2001**; **Yang *et al.* 2007**).

Without the structure of LTR, the transposition activity of non-LTR retrotransposons is regulated by the inner promoter. Two types of non-LTR retrotransposons are recognized presently: LINE (Long Interspersed Nuclear Elements) and SINE (Short Interspersed Nuclear Elements). Compared with LTR retrotransposon, LINEs also carry gag and pol genes, while they lack the int gene. In addition to be an endonuclease, the function of gag protein in LINEs is possibly associated with the process of integration. It was speculated that LINE was the most ancient type of retrotransposon and evolved into LTR retrotransposons after gaining LTR structure (**Xiong and Eickbush 1990**). SINEs have the simplest structure. They are very short (usually less than 500 bp) and without any transposition-related coding sequence. Therefore, SINEs cannot perform autonomous transposition. However, they can be integrated into the host genome with the help of transposition-related proteins encoded by LINE and

LTR retrotransposons (**Bennetzen 1996**; **Kumar and Bennetzen 1999**). The way that SINE completes the process of replication and integration is poorly understood (**Zou *et al.*, 2009**). The dynamism and dispersion of the various groups of TEs have led to their widespread exploitation as molecular markers (**Kalendar *et al.*, 2011**).

Kalendar *et al.*, (2010) has described a method that can both isolate LTR retrotransposons in virtually any organism as well as serve as a general marker system in its own right. It is based on the nearly universal use by both retroviruses and LTR retrotransposons of cellular tRNAs as primers for reverse transcription during their replication cycles. The tRNA binds to the primer binding site (PBS) adjacent to the 5LTR and primes synthesis of minus-strand cDNA by reverse transcriptase. The method was named iPBS and is applicable to any organism with retrotransposons containing PBS sites complimentary to tRNA. The iPBS marker technique is based on retrotransposon sequences, which are ubiquitous in plant genomes, and large portions of plant genomes are comprised of retroelement sequences (**Sabot and Schulman, 2006**).

The principle of the technique is as follows (**Kalendar *et al.*, 2010**). For iPBS, two retrotransposons must be in opposite orientation and either near enough for efficient ampliffication, as shown in the figure 1, or nested. The diagram depicts two key structural features of retrotransposons, the LTR (long terminal repeat) and PBS (primer binding site). The internal domain is shown as a thick bar, the intervening genomic DNA as thick line. The predicted product is show above, together with the orientation of the PBS ampliffication primers. The PCR product contains both LTRs and PBS sequences together with the genomic sequence between the LTRs. The sequence of a set of PBS domains, the 0–5 base spacer and the universal 5´ TG of LTRs is shown below the figure.

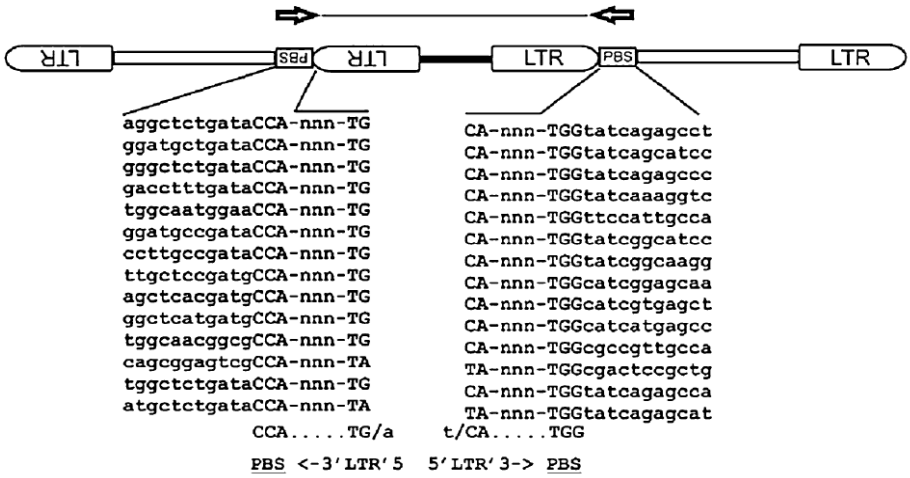

Figure 1 The iPBS scheme as was described by Kalendar et al. (2010)

In the repotred study, the development of iPBS strategy is reported for the yacon - *Smallanthus sonchifolius*, (Poepp. et Endl.). *Smallantus sonchifolius* (Poepp. and Hendl.) H. Robinson; *Asteraceae* is a perennial herb 1.5–3 m tall with the root system composed of 4–20 freshly edible tuberous storage roots weighing up to 2 kg, originally cultivated in South America. The parenchyma accumulates sugars and, in some cases, pigments typical of certain landrace groups (**Milella *et al.,* 2005**). Yacon was considered by the early Andean inhabitants as a fruit and it has a relatively low energy value despite its juiciness and sweet taste. In South America, Bolivia, Brazil and Argentina, yacon roots and leaves are commonly consumed by people suffering from diabetes or various digestive or renal disorders and this ethnobotanical use was confirmed by recent scientific research (**Aybar *et al.,* 2001**; **Simonovska *et al.,* 2003**). Recently, the interest in this crop has increased due to its good post-harvest life if managed properly (**Ohyama *et al.* 1990**), exceptional qualities for low-calorie diets thanks to its abundant content of fructooligosaccharides that humans cannot digest in the colon, the absence of starch and medicinal properties, (**Inoue *et al.,* 1995**; **Aybar *et al.,* 2001**). In spite of advancements in yacon morphological characterizations, the genetic diversity of the crop in molecular terms is still unknown (**Mansilla *et al.,* 2006**).

In this study we report the results of three different types of yacon DNA extraction methods and the protocol for iPBS based evaluation of genetic diversity in yacon germplasm.

MATERIAL AND METHODS

Two genotypes of *Smallanthus sonchifolius*, (Poepp. et Endl.) were chosen from the Institut of Tropics and Subtropics of the Czech university of Life Sciences, concretely - PER05 and ECU45 for development of the iPBS strategy and a set of ECU41-45 genotypes were used for testing of the reproducibility of selected primers. The plants were grown in field conditions on the experimental base of the Department of the Genetics and Plant Breeding. For the purpose of molecular analyses the parts of the leaves without insect or another damages were chosen.

Three total genomic DNA extraction methods were prooved, because of high level of polyphenols content in the yacon leaves. Four basic protocols – **Rogers and Bendich** (**1994**), **Saghai-Maroof *et al.*** (**1984**), **Friar** (**2005**) and one manufactured extraction kit (GeneJET™ Plant Genomic DNA Purification Mini Kit - ThermoScientific) were used. Following the

Saghai-Maroof *et al.* (**1984**), 1% CTAB (cetyltrimetyl ammonium bromide) buffer DNA extraction procedure was used. The principal of the method is in the using of CTAB with 3% mercaptethanol and ascorbic acid double precipitation of the DNA with cold chloroform:izoprophanol (24:1) mix. When modified method of **Friar** (**2005**) was followed, 2% CTAB, 1% PVP and mercaptoethanol with double ice-cold 95% ethanol precipitation of DNA was used. When extracting DNA using the kit, DNA purification from lignified and polyphenol-rich plant tissues was used according the instructions of manufacturer.

For quantity setting of the extracted DNA was used Nanodrop Nanophotometer™. Determination of DNA quality was done by agarose gel electrophoresis on 1,5 % agarose gel in 1xTBE buffer coloured by GelRed™.

Ten primers (**Kalendar *et al.*, 2010**) that show high PCR efficiency both in plants and animals were used for screening of their efficiency for yacon germplasm evaluation (table 1).

Table 1 Primers used for iPBS fingerprinting

Primer name	Nucleotide sequence (5´→ 3´)
1846	ctggcatttccattgtcgtcgatgc
1880	agaactccctggtggcatcgtgagc
2078	gcggagtcgcca
1845	agcctgaaagtgttgggttgtcg
1875	tcagtttccaagaggtcggcca
2080	cagacggcgcca
1899*	tgagttgcaggtccaggcatca
1868*	cacttcaaattttggcagcagcggatc
1886	attctcgtccgctgcgcccctaca
1833*	cttgctggaaagtgtgtgagagg

* primers where no amplification was detected

PCR reactions were performed in a 15 µl reaction mixture with MyTaq™ Mix with 20 ng of DNA and 300 nM of iPBS primers. Amplification was performed in BIO-RAD C1000™ Thermal Cycler under following conditions of gradient of the annealing temperature: 95°C 4 min (95 °C 1 min; 52 - 62 °C 1 min; 72 °C 2 min) 35x; 72 °C 10 min. PCR product segregation was performed in 1,2 % agarose gel and fingerprints were captured by GeneBox system.

RESULTS AND DISCUSSION

Smallanthus sonchifolius, (Poepp. et Endl.) has high level of polyphenols (**Viehmanová, 2009**) tha decreases the quality of extracted DNA. Because of the good up to the high quality DNA is needed for retrotransposon-based marker techniques, three protocols (**Rogers and Bendich, 1994**; **Saghai-Maroof *at al.*, 1984** ; **Friar, 2005**) and one extraction kit (GeneJET™ Plant Genomic DNA Purification Mini Kit - ThermoScientific) were tested.

When **Rogers and Bendich** (**1994**) extraction protocol was used, the high level of contamination and viscose pellet formation was observed through the extraction process and the DNA extraction was not successful.

In the case of the **Saghai-Maroof** (**1984**) the amount of DNA concentration was low - only about 5 ng/µl and the quality of the DNA was not suitable for PCRs, because contamination was detected.

Friar´s (2005) extraction protocol was comparable with the results of the GeneJET™ Plant Genomic DNA Purification Mini Kit where the amount of the DNA ranged from 10 - 200 ng/µl and no contamination was detected, so both of the protocol gives the DNA suitable for PCR analyses.

Using a set of universal primers that anneal to the conserved regions of retrotransposons, polymorphism of amplified fragment of DNA was analysed for *Smallanthus sonchifolius*, (Poepp. et Endl.). Using the same genotypes PER05 and ECU45, ten universal iPBS primers were tested (figure 2).

For the development of iPBS protocol for *Smallanthus sonchifolius*, (Poepp. et Endl.) analyses were chosen primers that produce PCR fragments within the whole possible range of PCR without using of specific polymerases.

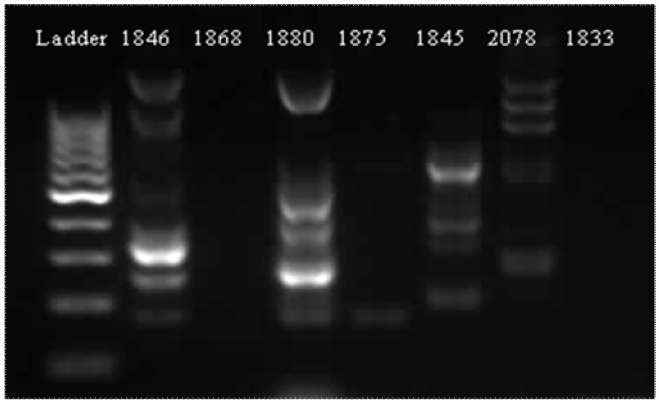

Figure 2 Profile of seven tested universal iPBS primers for PER05 accession

Selected primers were subsequently used in a set of gradient PCR for finding of optimal annealing temperatures for each of them (figures 3, 4, 5). Three groups of primers according to the optimal annealing temperature were found - primers with a optimum at 53°C (1845, 1875 and 1886), at 56°C (1846) and 61 °C (1880 and 2078).

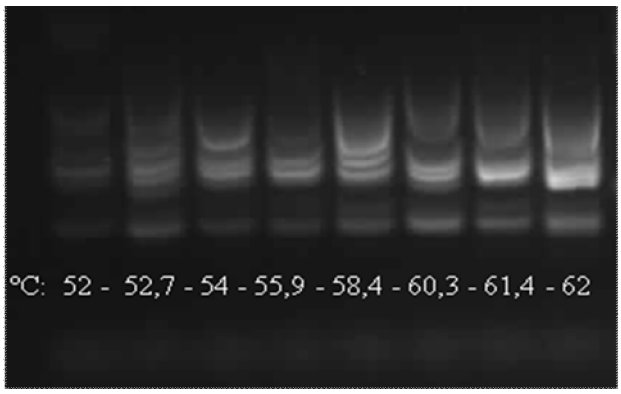

Figure 3 Effect of the increasement of the annealing temperature on the iPBS profile for the 1846 primer when PER05 accession used

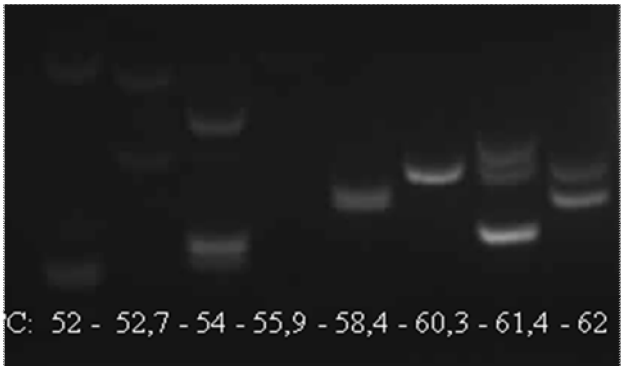

Figure 4 Effect of the increasement of the annealing temperature on the iPBS profile for the 1880 primer when ECU45 accession used

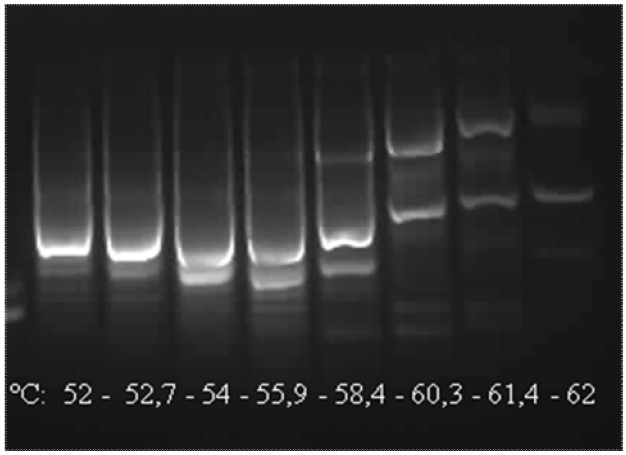

Figure 5 Effect of the increasement of the annealing temperature on the iPBS profile for the 2078 primer when ECU45 accession used

After the gradient PCR all the selected iPBS primers were used in PCRs for testing of the stability and reproducibility of the protocol when using it on a set of 5 different genotypes (figure 6). All the reactions were repeated three times an in all cases except of the primer 1875 the iPBS profiles were fully reproducible.

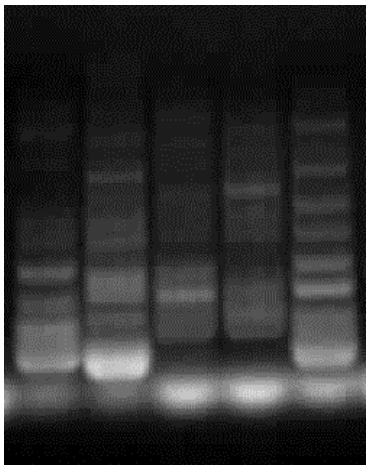

Figure 6 Control IRAP profile of 5 yacon accessions (ECU41-45) for 1846 iPBS primer

Most of the retrotransposon marker methods take advantage of two basic properties, namely that they cause large insertions by their transpositional activity and they contain conserved domains from which PCR primers can be designed. Some other methods target the small insertions and deletions found within otherwise conserved TE domains to generate fingerprints. Most of the techniques are also anonymous, producing fingerprints from multiple sites of retrotransposon insertion in the genome (**Schulman *et al.*, 2004**) by using PCR primed on conserved motifs in the element and on some widespread and conserved motif in the surrounding DNA. For LTR retrotransposons, the primers are generally designed from

the LTRs near to the insertion site, in LTR sub-domains that are conserved within retrotransposon families and differ between families. Although regions internal to the LTR containing conserved segments can be used for this purpose, generally the LTRs are chosen to minimize the size of the target to be amplified and to assay insertion site polymorphism rather than events internal to the element.

Kalendar *et al.*, (2010) has described a method that overcomes these difficulties and can both isolate LTR retrotransposons in virtually any organism as well as serve as a general marker system in its own right.

For analysis where retrotransposon markers are used, good quality DNA (high molecular weight DNA free of RNA, protein and phenol contaminants) in a concentration range from 60-100 ng/µl is required (**Kalendar *et al.*, 2010**). In the case of yacon, there is difference, if the plant material for isolation is young and grown in glasshouses or is from field conditions. When working with young yacon leaves, the **Rogers and Bendich** (**1994**) extraction procol was successfully applied for total genomic DNA isolation (**Žiarovská *et al.*, 2012**) and subsequently not only PCRs but the sequencing of yacon ITS (internal transcribes spacers) was performed. When extracting DNA from the plants from field conditions, choosing of the exctraction methods is a crucial step that affects the rest of the analyses.

Belogrudova *et al*. (**2012**) have used both of the methods tested in this study for the extraction of the DNA from *L. loeselii* for the purposes of iPBS and repoted very similar results for them. When **Saghai-Maroof** (**1984**) extraction was used, very low amounts of DNA were reported (4,2- 7,4 ng/µl) along with the phenol contamination. Using the DNA extraction by **Friar** (**2005**) method with some modifications large amounts of high quality DNA were obtained and DNA concentration has ranged from 6,0 till 187,0 ng/µl.

Unlike methods for retrotransposon isolation that rely on conserved protein coding domains (**Pearce *et al.* 1999**), the PBS primers also directly visualize polymorphisms for retrotransposon loci in the genome (**Kalendar *et al.*, 2010**). Furthermore, this is the only retrotransposon-based method to our knowledge that has been shown to visualize polymorphism throughout the plant kingdom and for animals as well. Of the sequences matching tRNA in the genome, the greatest proportion consists of retroelements. The tRNA genes themselves comprise small families for each isoacceptor. Moreover, the iPBS primers contain CCA at their 3termini, which is complementary to the 5TGG motif in PBS sites but which is not found in eukaryotic tRNA genes. In eukaryotes, 3terminal CCA is added post-transcriptionally by ATP(CTP):tRNA nucleotidyltransferase. Hence, given the diVerence in the number of tRNA genes and retrotransposons and their genomic position, the lack of tRNA

mobility, and the specificity conferred by the 3CCA of iPBS, primers, iPBS selectively displays polymorphism in retrotransposon insertion sites (**Kalendar *et al.*, 2010**).

Yacon belongs to the organisms where only a very limited information about the genome sequences are known. That is why for the reliable detection of molecular markers only universal and sequence non-specific methods like RAPD (Random Amplified Polymorphic DNA), ISSR (Inter-Simple Sequence Repeat) or AFLP (Amplified Fragment Length Polymorphism) can be used. When RAPD and ISSR methods are used, the problems about reproducibility, low level of polymorphism and inter laboratory cross analyses need to be overcome. But all of them are still used as a start point for species with no or only a few informations about the sequences. All of them were tested for yacon germplasm evaluation and present a substantial part of yacon molecular data actually available (**Mansilla *et al.*, 2006**; **Milella *et al.*, 2011**; **Svobodová *et al.*, 2011**).

The iPBS can be carried out with single primers, for which the analyses above have been given, or with combinations of two primers. The banding patterns obtained when more than one primer is used will depend on the relative abundance of differerent retrotransposon families as well as on their distribution with respect to one another. The PBS primers can also be combined with microsatellite primers as in REMAP (Retrotransposon Microsatellite Amplified Polymorphism) and with adapter primers as in SSAP for generation of additional scorable polymorphisms. In analyses of the three barley varieties, the iPBS method proved to be as informative as those obtained using IRAP (Inter Retrotransposon Amplified Polymorphism), REMAP or SSAP (Sequence-specific Amplified Polymorphism), and about an equal level of polymorphism compared to IRAP and REMAP (**Kalendar *et al.*, 2010**).

The limiting factor, however, in the development of molecular marker systems based on LTR retrotransposons for new plant species is availability of retrotransposon sequences. If extensive genome sequences are not available, LTR ends must be cloned and sequenced, then trialled for their usefulness as markers. Previous methods for doing this have relied on ampliffication with degenerate primers matching conserved domains in retrotransposon polyproteins, particularly integrase or reverse transcriptase (**Pearce *et al.* 1999**), followed by walking to the LTR ends.

CONCLUSION

Using a set of universal primers that anneal to the conserved regions of retrotransposons, polymorphism of amplified fragment of DNA was analysed for *Smallanthus*

sonchifolius, (Poepp. et Endl.) by iPBS. The method provides markers that are able to distinguish yacon accessions and are suitable for yacon germplasm evaluation when the DNA of high quality is extracted. The selected primers will be used for finding a polymorphism among the yacon germplasm.

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REFERENCES

ANTONIUS-KLEMOLA, K. – KALENDAR, R. – SCHULMAN, A.H. 2006. TRIM retrotransposons occur in apple and are polymorphic between varieties but not sports. In *Theoretical and Applied Genetics,* vol. 112, p. 999-1008

AYBAR, J. – SANCHEZ A. – GRAU, A. – SANCHEZ, S. 2001. Hypoglycaemic effect of the water extract of *Smallantus sonchifolius* (yacon) leaves in normal and diabetic rats. In *Journal of Ethnopharmacology*, vol.74, p. 125–132.

BELOGRUDOVA, I. – GRAUDA, D. – JAKOBSONE, G. – RASHAL, I. 2012. Usability of etrotransposone-based molecular marker system to assess genetic diversity of *Liparis loeselii*. In *Acta Biologica Universitatis Daugavpiliensis,* Vol. 12, p. 40-43

FRIAR E. A. 2005. Isolation of DNA from Plants with Large Amounts of Secondary Metabolites. In *Methods in Enzymology.* Vol. 395, p. 1–12

BENNETZEN, J.L. 1996. The contributions of retroelements to plant genome organization, function and evolution. In *Trends in Microbiology,* Vol.4, p.347-53

SCHULMAN, A.H. – FLAVELL, A.J. – ELLIS, T.H.N. 2004. The application of LTR retrotransposons as molecular markers in plants. In: *Methods in Molecular Biology,* vo. 260, p. 145–173

INOUE, A. – TAMOGAMI, S. – KATO, H. – NAKAZATO, Y. – AKYAMA, M. – KODAMA, O. – AKATSUKA, T. – HASHIDOKO, Y. 1995. In: Antifungal melanpolides from leaf extract of *Smallantus sonchifolius*. In *Phytochemistry*, vol. 39, p. 845–848.

KALENDAR, R. – ANTONIUS, K. – SMÝKAL, P. – SCHULMAN, A.H. 2010. iPBS: a universal method for DNA Fingerprinting and retrotransposon isolation. In *Theoretical and Applied Genetics,* Vol. 121, p. 1419–1430

KALENDAR, R. – FLAVEL, A.J. – ELLIS, THN. – SJAKSTE, T. – MOISY, C. – SCHULMAN, A.H. 2011. Analysis of plant diversity with retrotransposon-based molecular markers. In *Heredity,* vol. 106, p. 520–530

KUMAR, A. – BENNETZEN, J.L. 1999. Plant retrotransposons. In *Annu. Rev. Genet.* 33: 479-532

KWON, S.J. – KIM, D.H. – LIM, M.H. – LONG, Y. – MENG, J.L. – LIM, K.B. – KIM, J.A. – KIM, J.S. – JIN, M. – KIM, H.I. – AHN, S.N. – WESSLER, S.R. – YANG, T.J. – PARK, B.S. 2007. Terminal repeat retrotransposon in miniature (TRIM) as DNA markers in Brassica relatives. In *Molecular Genetics and Genomics,* Vol. 278, p.361-70

MANSILLA, R.C. – LÓPEZ, C. – BLAS, R. – CHIA, W. J. – BAUDOIN, J. 2006. Molecular variability analysis of a peruvian *Smallanthus sonchifolius* (Poepp & Endl) H. Robinson "YACON" Collection. In *Ecología Aplicada*, vol. 5, p. 75-80

MILELLA, L. – SALAVA, J. – MARTELLI, G. – GRECO, I. – CUSIMAMANI, E.F. – VIEHMANNOVÁ, I. 2005. Genetic Diversity between Yacon Landraces from Different Countries Based on Random Amplified Polymorphic DNAs. In *Czech Journal of Genetics and Plant Breeding*, vol. 41, p. 73–78

MILELLA, L. – MARTELLI, G. – SALAVA, J. – FERNANDEZ, E. – OVESNÁ, J. – GRECO, I. 2011. Total phenolic content, RAPDs, AFLPs and morphological traits for the analysis of variability in Smalanthus sonchifolius. In *Genetic Resources and Crop Evolution*, vol 58, 2011, p. 545–551.

OHYAMA, T. – ITO, O. – YASUYOSHI, S. – IKARASHI, T. – MINAMISAVA, K. – KUBOTA, M. – TSUKIHASHI, T. – ASAMI, T. 1990. Composition of storage carbohydrate in tubers of yacon (*Polimnia sonchifolia*). In *Soil Science and Plant Nutrition*, vol. 36, p. 167–171

PEARCE, S.R. – STUART-ROGERS, C. – KNOX, M.R. – KUMAR, A. - ELLIS, T.H.N. – FLAVELL, A.J. 1999. Rapid isolation of plant Ty1-copia group retrotransposon LTR sequences for molecular marker studies. In *Plant Journal,* vol. 19, p.711–717

ROGERS, S.O. – BENDICH, A.J. 1994. Extraction of total cellular DNA from plants, algae and fungi, In: *Plant Molecular Biology Manual D1*, Kluwer Academic Publishers, Belgium, pp. 1-8. ISBN 0-7923-2868-2

SABOT, F. – SCHULMAN, A.H. 2006. Parasitism and the retrotransposon life cycle in plants: A hitchhiker's guide to the genome. In *Heredity*, vol. 97, p. 381–388.

SIMONOVSKA, B. – VOVK, I. – ANDREȘEK, S. – VALENTOVÁ, K. – ULRICHOVÁ, J. (2003): Investigation of phenolic acids in yacon (*Smallantus sonchifolius*) leaves and tubers. In *Journal of Chromatography A*, vo. 1015, p. 89–98

SVOBODOVÁ, E. – HLÁSNÁ ČEPKOVÁ, P. – DVOŘÁKOVÁ,Z. First preliminary screening of the genetic diversity of selected Peruvian accessions of Yacon (*Smallanthus sonchifolius*) using Inter-Simple Sequence Repeats molecular markers., 2011, *5th Scientific conference of Institute of Tropics and Subtropics*, 2011. Sustainable Use of Natural resources in Tropics and Subtropics. Prague. ISBN 978-80-213-2233-2.

YANG, T.J. – KWON, S.J. – CHOI, B.S. – KIM, J.S. – JIN, M. – LIM, K.B. – PARK, J.Y. – KIM, J.A. – LIM, M.H. – KIM, H.I. – LEE, H.J. – LIM, Y.P. – PATERSON, A.H. – PARK, B.S. 2007. Characterization of terminal-repeat retrotransposon in miniature (TRIM) in Brassica relatives. In *Theoretical and Applie Genetics,* Vol. 114, p.627-36

VIEHMANOVÁ, I. 2009 Indukovaná polyploidizace in vitro a protoplastové kultury u jakonu. PhD. Thesis. 111 p.

WITTE, C.P. – LE, Q.H. – BUREAU, T. – KUMAR, A. 2001. Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. In *Proceedings of the National Academy of Sciences,* USA. Vol. 98, p. 13778-83

XIONG, Y. – EICKBUSH, T.H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. In *EMBO Journal*, vol. 9, p.3353-62

ZOU, J. – GONG, H. – YANG, T.J. – MENG, J. 2009. Retrotransposons - a Major Driving Force in Plant Genome Evolution and a Useful Tool for Genome Analysis. In *Journal of Crop Science and Biotechnology,* Vol. 2009, p. 1-8

ŽIAROVSKÁ, J - FERNÁNDEZ, E.C. - RUSSO, D. - ŠTEFÚNOVÁ, V. - BEŽO, M. 2012. ITS úseky v molekulárnom hodnotení genetických zdrojov jakonu. In *Úroda,* no. 9. vědecká příloha, p. 145-149