CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Crop Sciences and Agroforestry in Tropics and Subtropics

In vitro propagation of *Arracacia xanthorrhiza* and assessment of genetic stability in regenerants using molecular markers

Author: Bc. Hana Mrhalová

Supervisor: Ing. Iva Viehmannová, Ph.D.

Diploma thesis

Prague 2013

Declaration:

I confirm, that this Diploma thesis "*In vitro* propagation of *Arracacia xanthorrhiza* and assessment of genetic stability in regenerants using molecular markers" is original result of my own work and that I have used no other resources than referenced. I agree this work to be placed of CULS Prague and was accessible to study purposes.

Prague April 2013

Hana Mrhalová

Acknowledgement

Primarily, I would like to express my gratitude to my supervisor Ing. Iva Viehmannová, Ph.D. for goodwill and supporting me in every problem, which occured during writing this thesis or during the work on practical part of the thesis. Also I am very grateful for her help in improving my knowledge in these topics.

My thanks also belongs to Ing. Petra Hlásná Čepková, Ph.D. and Ing. Zuzana Bortlová, for goodwill and help with molecular markers.

Also I would like to thank to Faculty of Tropical AgriSciences for support by letting me work in the Laboratory of plant tissue cultures and Laboratory of molecular biology of Faculty of Tropical AgriSciences at Czech University of Life Sciences Prague (CULS).

This research was financially supported by research projects IGA (Project No. IGA 51110/1312/3115) and CIGA (Project No. 51110/1313/3105), Czech University of Life Sciences Prague.

Abstract

The main goal of this thesis was the development of a protocol for efficient propagation via indirect morphogenesis in arracacha (*Arracacia xanthorrhiza* Bancr.), an important Andean root crop belonging to family *Apiaceae*. Various concentrations of BAP (6-benzylaminopurine) and 2,4-D (2,4-dichlorophenoxyacetic acid) were tested for efficiency to induce callus from the arracacha petioles and to regenerate plants from the callus. Calli from all the treatments, except medium supplemented with BAP alone, provided satisactory regeneration capacity, when transferred on the medium without plant growth regulators. Genetic fidelity in regenerated plants was assessed using ISSR (inter simple sequence repeat) markers. Nine ISSR primers generated 418 amplified bands of which 6 (1.4%) were polymorphic. Polymorphism was revealed in all types of regenerants regardless the type of media used for induction of callus. Genetic stability was assessed also in plant material multiplicated via direct organogenesis. These plants were used for the establishment of the callogenesis experiment. ISSR analysis in these plants revealed, that they were genetically identical to the control plant, i.e. the amplification products were monomorphic. Thus, it can be concluded that in arracacha, propagation via intermediate callus phase induces the somaclonal variation, while direct organogenesis provides true-to-type plants. Somaclonal variation can be used in plant breeding to obtain new genotypes with valuable qualitative or quantitative traits. This phenomenon is especially important in root and tuberous crops, where classical breeding methods are often limited due to barriers in reproduction.

Keywords: arracacha, indirect morphogenesis, ISSR markers, plant growth regulators, somaclonal variability

Abstrakt

Cílem této práce byla optimalizace protokolu pro získání regenerantů druhu *Arracacia xanthorrhiza* (Bancr.), významné andské okopaniny z čeledi *Apiaceae*, nepřímou morfogenezí a následné zhodnocení genetické variablility takto získaných jedinců s využitím molekulárních ISSR (inter simple sequence repeat) markerů. Efektivita tvorby kalusu a následná regenerace rostlin byla testována na 8 iniciačních mediích obohacených různými koncentracemi BAP (6-benzylaminopurin) a 2,4-D (2,4-dichlorfenoxyoctová kyselina). Rostliny regenerovaly dostatečně z kalusů kultivovaných na všech iniciačních mediích kromě media obohaceného pouze cytokininem BAP. Variabilita regenerantů získaných nepřímou morfogenezí byla testována pomocí ISSR markerů. Devět použitých primerů generovalo 418 bandů, z nichž 6 (1,4%) bylo polymorfních. Naopak u rostlin získaných přímou organogenezí byla potvrzena genetická stabilita získanými monomorfními DNA spektry. Z toho lze vyvodit, že regenerace rostlin z kalusu indukuje somaklonální variabilitu, zatímco přímá organogeneze vede k produkci geneticky uniformních jedinců. Somaklonální variabilita může být využita ve šlechtění rostlin k získání nových hodnotných genotypů. Tento postup je vhodný především u okopanin, jejichž reprodukční schopnost bývá často nízká a neumožňuje využití klasických šlechtitelských metod.

Klíčová slova: arakača, ISSR markery, rostlinné regulátory růstu, somaklonální variabilita

List of tables:

List of figures:

Abbreviation list

2,4-D - 2,4-Dichlorophenoxyacetic acid

AC - activated charcoal

AFLP - amplified fragment lenght polymorphism

ARTC - Andean root and tuber crops

BAP - 6-Benzylaminopurine

BSA - bovine serum albumin

CIP - Centro Internacional de la Papas (International Potato Center)

cpSSR - chloroplast simple sequence repeat

CULS - Czech University of Life Sciences Prague

DENAREF - Departamento Nacional de Recursos Fitogenéticos y Biotecnología (National Department of Plant Genetic Resources and Biotechnology)

DNA - deoxyribonucleic acid

GA_3 - gibberellic acid

IAA - Indole-3-acetic acid

ISSR - inter-single sequence repeat

KIN - kinetin

MAS - marker-assisted selection

MS - Murashige and Skoog (1962) medium

MSAP - methylation-sensitive amplified polymorphism

NAA - α -Naphthaleneacetic acid

PCR - polymerase chain reaction

PGR - plant growth regulator

RAPD - random amplified polymorphic deoxyribonucleic acid

RFLP - restriction fragment lenght polymorphism

rpm - revolutions per minute

SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis

SNP - single-nucleotide polymorphism

SRAP - sequence related amplified polymorphism

SSRs - simple sequence repeats

TBE - Tris/Borate/EDTA buffer

UBC - University of British Columbia

UPGMA - unweighted pair group method with arithmetic mean

Table of Contents:

1. Introduction

Arracacha (*Arracacia xanthorrhiza* Bancr.) species belonging to the family *Apiaceae* is one of the most valuable vegetatively propagated root crop originating from the Andes. It is valued for its tuberous crunchy roots free from undesirable substances that limit the uses of some other Andean root and tuber crops.

Breeding of this root crop is limited due to its lower sexual reproductive capacity. This limitation in breeding could be overcame using *in vitro* technologies. To date, some studies regarding *in vitro* introduction, micropropagation and conservation of arracacha germplasm are available. Nevertheless, genomic stability or variability of *in vitro* maintained arracacha have not been assessed yet. Genetic stability of micropropagated plants is desirable to obtain true-to-type plants. However potential somaclonal variability obtained via *in vitro* cultivation can serve as an important tool during breeding efforts and via selection of obtained variable somaclones some valuable traits such as resistance to pests and diseases or improvement of yield can be acquired.

This thesis is therefore focused on propagation of arracacha plants via indirect morphogenesis and assessment of genetic stability/variability in regenerants using molecular ISSR markers.

2. Literature Review

2.1. Andean root and tuber crops

From the region of the Andes, which is in these days recognized as one of the most important center of crop origin and diversity on the world (National Research Council 1989), many cultivars of edible plants originate. They have been domesticatated by autonomous tribes several millenia ago, even long time before expansion of Incas (Barrera *et al.,* 2004). A significant group of these species is formed by Andean root and tuber crops (ARTC), which are mainly but not solely grown by indigenous people for their edible underground organs. These nine lesser known species, apart from the seven species of cultivated potatoes, of which only Irish potato (*Solanum tuberosum*), is known almost in all parts of the world, are used by native people as a cash crop or for subsistence (Barrera *et al.,* 2004; Hermann, 1997).

The genetic diversity and potential of these species is remarkable (Flores *et al.,* 2003). These crops differ significantly in their boundaries of adaptation and tolerance to environmental conditions (Arbizu *et al.,* 1997; CGIAR, 1996), they are classified in different taxonomic families and they also differ in their propagation, storage behaviour, economic potential, use and underground edible part (Arbizu *et al.,* 1997) and therefore they can be classified as extensively heterogeneous group (Hermann, 1997). The ARTC include no less than nine taxonomical families: *Umbelliferae*, *Brassicaceae*, *Basellaceae*, *Leguminosae*, *Solanaceae*, *Oxalidaceae*, *Nyctaginaceae*, *Trapaeolaceae*, *Asteraceae* (Flores *et al.,* 2003). According to different agro-ecological conditions of the Andes, the ARTC can be divided to three categories. The five species producing edible roots or rhizomes grow in the warm Andean valleys: arracacha (*Arracacia xanthorrhiza* Bancr.), achira (*Canna edulis* Ker Gawl.), yacon [*Smallanthus sonchifolius* (Poeppig & Endlicher) H. Robinson], mauka [*Mirabilis expansa* (Ruiz & Pav.) Standl.] and ahipa [*Pachyrhizus ahipa* (Wedd.) Parodi]. In the second agro-ecological area - the temperate Andean valleys - the tuber crops such as oca (*Oxalis tuberosa* Molina) mashua (*Tropaeolum tuberosum* Ruiz & Pav.) and ulluco (*Ullucus tuberosus* Caldas), are cultivated associated with Andean potatoes. The third group represented by only one root-hypocotyl frost tolerant crop named maca (*Lepidium meyenii* Walp.), occurs on the Andean highlands (Arbizu *et al.,* 1997; National Research Council, 1989).

Root and tuber crops, from which the most famous are the potato (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz) are considerably widespread in the diet of humans throughout the world (Libreros, 2001). Root crops are the second important source of food for

people after cereals. The ARTC possessing diferent types of underground organs such as corms, tubers, rhizomes and roots (National Research Council, 1989) represent staples for an estimated 25 millions people in the highlands of Andes, and are at least occational food source for another hundreds million people in Peru, Chile, Argentina, Ecuador, Bolivia, and Colombia (Flores *et al.,* 2003). Although these species have a nutritive value and even in other characteristics they seem to be very promising, they have not been fully utilized. It is probably caused by their restriction in cultivation and their local utilization (Libreros, 2001). The tradition in terms of Andean agriculture is confronted to many challenges and insecurity. For these reasons it is necessary to promote and preserve ARTC by the studies improving pest management and crop productivity to promote popularity of these crops among farmers (Flores *et al.,* 2003). Some of these ARTC at least could become a new sources of food not only for the Andean region, but also for the countries, where these crops are for this moment unknown (National Research Council, 1989).

2.2. Arracacha

2.2.1. Taxonomy and relative species

Arracacha (*Arracacia xanthorrhiza* Bancrof*t*), species botanically related to celery and carotts, belongs to family *Apiaceae* (syn. *Umbelliferae*) (National research council, 1989), a family, which includes about 300 genera and 2,500 to 3,000 of species (Hermann, 1997). *Apiaceae* family is known mainly due to many species used as essential oil producing plants (Degtjareva *et al.,* 2012; Evergetis *et al.,* 2013) (i.e. coriander, cumin), vegetables (carrots, parsley, celery, fennel), and drug plants (dill, ferule) (Degtjareva *et al.,* 2012). The apioid species are widespread throughout the world, but in lower latitudes can be rare. In the Americas a little less than one-third of apiod species occurs (Hermann, 1997).

Mathias and Constance (1962) reported that *Arracacia* genus contains 25, whereas Hermann (1997) estimated the number of species in the genus about 30. The problem to define *Arracacia* species correctly was probably caused by the lack of cytological and field studies as well as deficiency of appropriate herbarium material (Hermann, 1997).

According to Constance and Affolter (1995) *Arracacia* represents one of the largest and most important Umbelliferous genera in the New World, because it served an important role as a food plant for the Incas. Moreover these authors supposed this genus as central to a severity of other Mesoamerican genera (i.e. *Neonelsonia*, *Coaxana*, *Coulterophytum*, *Myrrhidendron*, *Tauschia*). South American species belonging to genus *Arracacia* Bancroft are listed in Table 1.

Table 1: List of South American Arracacia species according to Hermann (1997)

no	species	author	reference
1	*A. colombiana*	Constance & Affolter	Brittonia 47: 322-323, 1995
2	*A. tillettii*	Constance & Affolter	Brittonia 47: 324-327, 1995
3	*A. moschata*	(Kunth) DC.	Prodr. 4: 244, 1830
4	*A. elata*	Wolff	Bot. Jahrb. 40: 304, 1908
5	*A. xanthorrhiza*	Bancroft	Trans. Agr. Hort. Soc. Jamaica 1825: 5, 1825
6	*A. andina*	Britton	Bull. Torrey Bot. Club 18: 37, 1908
7	*A. equatorialis*	Constance	Bull. Torrey Bot. Club 76: 46, 1949
8	*A. incisa*	Wolff	Bot. Jahrb. 40: 305, 1908
9	*A. peruviana*	(Wolff) Constance	Bull. Torrey Club 76: 45, 1949

2.2.2. Vernacular names

The most common name used for *Arracacia xanthorrhiza* is arracacha. Cultivars of arracacha can refer in Spanish language to colour of root (i.e.,'arracacha amarilla' - yellow arracacha) or indicates supposed origin ('Salamineña' - from Salamina) (Hermann, 1997). List of *Arracacia xanthorrhiza* vernacular names can be seen below.

List of vernacular names according to language:

Ayomán (extint language native in Venezuela): aricachi

Muzo (extinct language native in Colombia): arocueche (Hermann, 1997)

Quechua (Ecuador): laqachu, rakkacha, huiasampilla

Aymara (from northern Bolivia to central Peru): lakachu, lecachu (Barrera *et al.,* 2004; Hermann, 1997; National research council, 1989)

Spanish: arracacha, racacha, virraca (Peru) (Barrera *et al.,* 2004; Hermann, 1997; National research council, 1989), zanahoria blanca (Ecuador), apio criollo (Venezuela), arrecate (Latin America) (Barrera *et al.,* 2004; National research council, 1989), racacha (National research council, 1989), ricacha (Hermann, 1997)

Portuguese: mandioquinha-salsa, batata baroa (Barrera *et al.,* 2004; Hermann, 1997; National research council, 1989), batata cenoura, batata salsa (Barrera *et al.,* 2004; National research council, 1989), mandoquinha (National research council, 1989)

English: arracacha, Peruvian carrot, Peruvian parsnip (Barrera *et al.,* 2004; Hermann, 1997; National research council, 1989)*, racacha, white carrot*

French: arracacha, paném̀e, pomme de terre céléri (Barrera *et al.,* 2004)

2.2.3. Origin and geographical distribution

The probable place of arracacha domestication is situated on the Andean South America (Hermann, 1997; National research council, 1989). Although its wild ancestor has not been found yet (National research council, 1989; Oliveros *et al.,* 2006), the greatest germplasm diversity is in Ecuador and neighbouring regions of Peru (National research council, 1989) and Colombia (Standley and Williams, 1966; National research council, 1989). According to some studies, Peru regions as Cajamarca, La Libertad and Cuzco, are considered as main centres of arracacha diversity (Oliveros *et al.,* 2006).

Nowadays, the countries considered as the biggest producers of the crop are Brazil, Colombia (Hermann, 1997; Oliveros *et al.,* 2006), Ecuador and Venezuela (Hermann, 1997). Beyond these countries where the occurence was recorded by more authors (Hedrick, 1919; Hermann, 1997; National research council, 1989; Tene *et al.,* 2007; Rolfs, 1916; Santacruz *et al.,* 2002; Santacruz *et al.,* 2003), arracacha growing within Andean region occurs in addition in Peru (Hermann, 1997; Mathias and Constance, 1962; Oliveros *et al.,* 2006), Bolivia and Chile (Hermann, 1997). For the arracacha occurence in Southern America see Figure 1.

Occurences of arracacha in Central America and Carribean were also recorded. It was found in Costa Rica (Hermann, 1997), Cuba (National research council, 1989), Haiti, Dominican Republic (National research council, 1989), Puerto Rico (Hermann, 1997; National research council, 1989; Rolfs, 1916), Jamaica (Coulter and Rose, 1900) and Guatemala (Standley and Williams, 1966).

Arracacha cultivation was also recorded in countries such as Mexico (Hedrick, 1919; Patiño *et al.,* 2012) and USA in North America. In United States, attempts to introduce arracacha near New York and in Baltimore appeared to be worthless (Hedrick,1919).

Some authors have mentioned cultivation attempts of arracacha in the Old world - in Europe (England, France, Switzerland), India (Hedrick, 1919) and Central Africa (L'Heureux and Bastin, 1936). Beyond India, where the arracacha was successfully introduced (Hedrick, 1919), attempts to cultivate arracacha in Europe and in Africa probably failed (Hedrick, 1919; Hermann, 1997). Nevertheless, information about successfull introduction to India can not be taken seriously into consideration because of age of reference source (Hedrick, 1919).

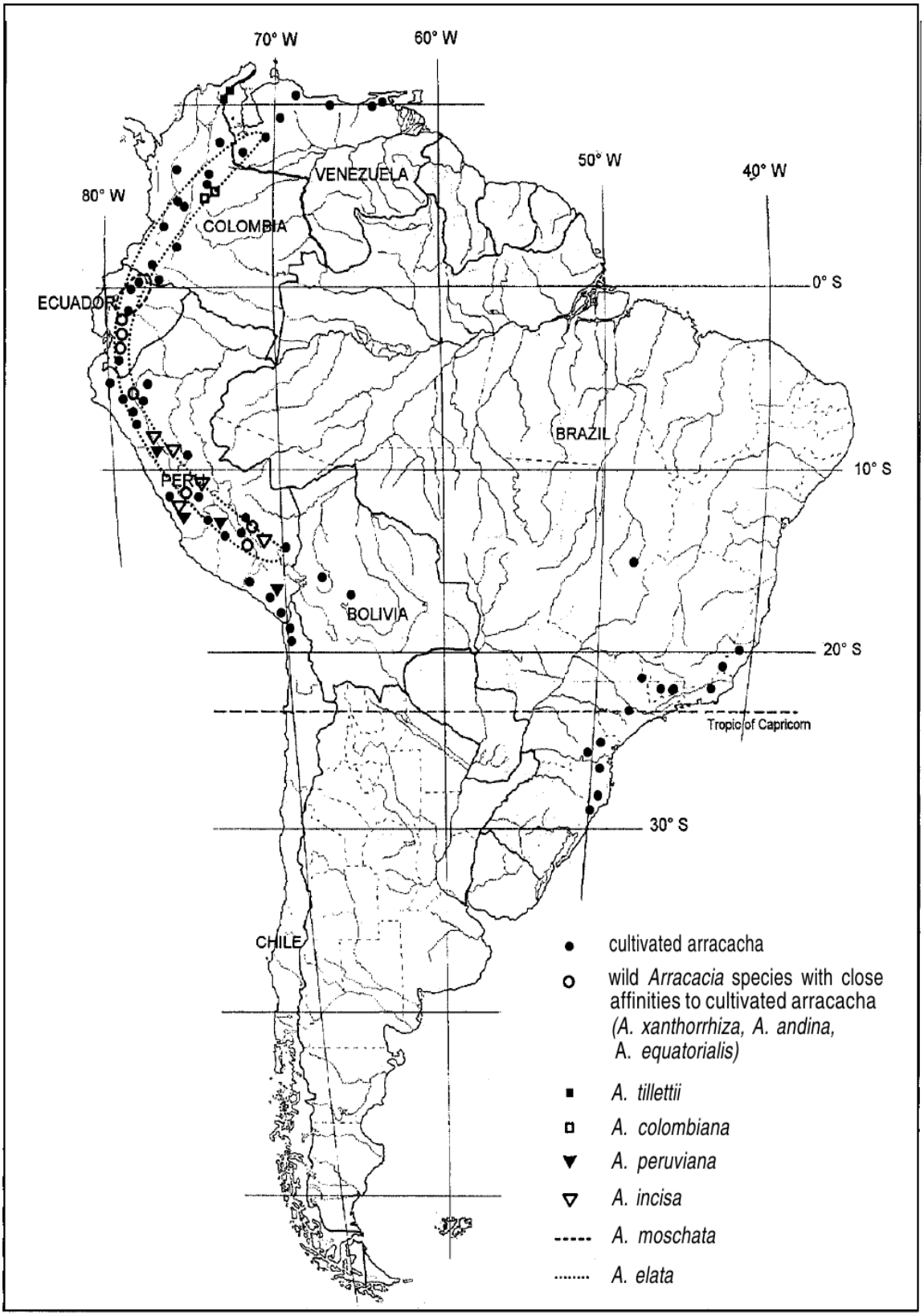

Figure 1: **Distribution of cultivated Arracacia xanthorrhiza and wild arracacha species within South America (source: Hermann, 1997)**

2.2.4. Variability

Arracacha variability can be divided according to its level on morphological, chromosomal and molecular variation (Hermann, 1997).

Previous selections according to morphological characters were based mainly on the colour of the arracacha storage root flesh. Thoughout the Andes, three principal horticultural varieties are known: amarilla (yellow), blanca (white) and morada (purple) (Hermann, 1997, Hodge, 1954, National research council, 1989). For the tradicional horticultural arracacha forms look at Figure 2. Seminario and Valderrama (2004a) identified 4 horticultural forms of arracacha during their study of germoplasm in Peru - "marilla"(yellow), "amarilla pigmentada" (yellow pigmented), "blanca" (white) and "blanca pigmentada" (white pigmented). The strains can be also various in texture, flavor and length of vegetation period (National research council, 1989). The variability in shape of root is moderate when compared with shape of other root and tuber crops. Variation of arracacha leaf is much greater in wild arracacha species than that found in cultivars within species. Arracacha leaf shape can be various as much within one accession as between accessions of a collection. The allocation of dry matter to the rootstock, which seems to be variable and variation of generative parts of plants possess a potential for the future germplasm evaluations (Hermann, 1997). Blas *et al.* (2006) in their study, which is thoroughly concerned with the topic of morphological and molecular vatiation of arracacha in Peru, mentioned the interesting fact, that internal color of storage root does not correlate with the external color of the foliage. Vásques *et al.* (2004) during their study of characterization of arracacha germoplasm according to 28 qualitative and 13 quantitative morphological destriptors found out variablity between 37% and 86% in accessions growing in similar altitudes and 100% of qualitative characteristics was variable. Germplasm in Peru according to Seminario and Valderrama (2004b) involved 76 arracacha morphotypes evaluated on the basis of 17 qualitative descriptors. Blas *et al.* (2006) characterized 66 cultivated arracacha accessions from 11 departments in Peru according to 28 morphological characters (2 of plant, 15 of flower, 4 of cormels and 7 of lateral tuberous roots) and 67 moleculars markers (RAPDs) to identify morphotypes and duplicates in the genebank maintained in the International Potato Center.

Mitotic chromosomal number of cultivated arracacha have still counted on 44 (Blas *et al.,* 1997; Slíva, 2009). Results from the study of Blas *et al.* (1997) suggest, that the basic number of arracacha chromosomes could be x = 11 and arracacha cultivated tetraploid would be 2n = 4x = 44 chromosomes. Blas and Arbizu (1995) mentioned the same number of chromosomes for two “wild *Arracacia xanthorrhiza*” accessions originating from Peru.

According to Darlington and Wilie (1955) even all genera from family *Apiaceae* have in most cases haploid series of 11 chromosomes. Therefore, these results affirm the assumption that arracacha is tetraploid. For molecular variability see chapter 2.4.2..

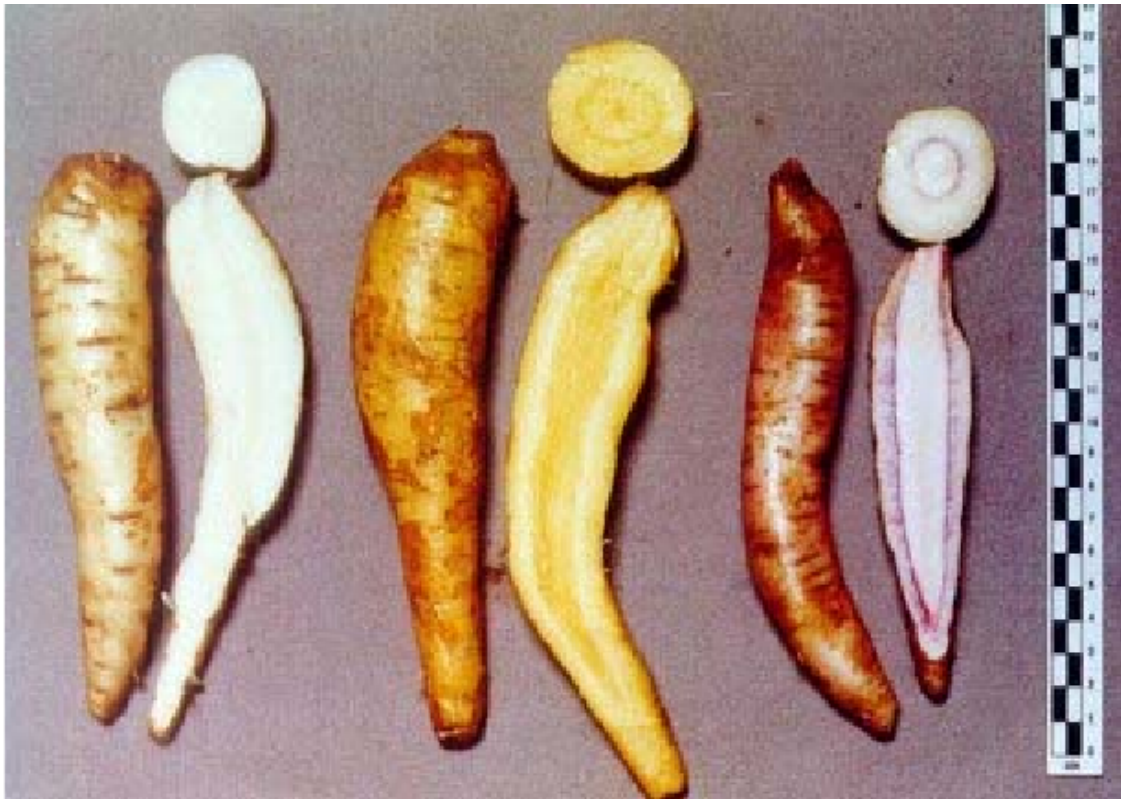

Figure 2: Traditional horticultural types of arracacha storage roots - from the left: "blanca", "amarilla"and "morada" (source: Blas *et al.*, 2006)

2.2.5. Morphology and reproductive biology

Arracacia xanthorrhiza is a perennial, stout, semi-caulescent or caulescent, glabrous herb and one of the largest of the cultivated apioids, somehow resembling celery in form in which coarse stems and leaves usually reach a height of about a meter (Hodge, 1954; Mathias and Constance, 1962; National research council, 1989).

The inflorescences with length from 3 to 10 centimeters in which peduncles arise in whorls or singly, are branching and typical of the family. They are squamulose or scaberulous at apex, with wanting involucres and comprising from 5 to 12 slender, spreading-ascending, 1.5-4 cm long rays and 3-8 slender, spreading-ascending, 2-4 mm long pedicels. Involucel is compound from 5-8 linear, entire, unequal, herbaceous and 2-5 mm long bractlets, which are shorter than flowers and fruit. Flowers are purple of yellow with petals oval, stylopodium depressed and slender ascending styles (Mathias and Constance, 1962). For the schema of

arracacha inflorescence see Figure 3. Arracacha is rarely flowering plant. This fact is probably influenced by climatic conditions and cultivation practices. After the periods of dry weather, increased flowering of arracacha was reported in some regions of Ecuador, therefore the dehydratation can be supposed as a main factor inducing the flowering (Hermann, 1997).

Carpophore supporting 2-3 mm broad and 10 mm long, glabrous fruits is 2-parted to base. Immature fruit with the face deeply sulcate is oblong, acute and having 2 rather large vittae for the commissure (Mathias and Constance, 1962). A seed is an achene or mericarp, a dry one-seeded fruit, which results from a schizocarp. The seed is orthodox, which means, that it can be dried to have a low content of moisture and therefore it can be stored at temperatures below the zero (Hermann, 1997).

Broadly ovate, biternate or bipinnate leaves, which are from 10 up to 35 cm long and broad comprise an acuminate, cuneate to rounded at base leaflets, having an ovate-lanceolate to triangular-ovate shape. Leaflets are from 4 up to 12 cm long and from 1.5 to 6.5 cm broad, coarsely simply or doubly mucronate-serrate and incised or lobed and squamulose or scaberulous on rachises having veins and margins with flattened oblong or linear scales. The lower surface is pale and reticulate with squamulose tuft on the upper side of the sulcate rachis at the base of the larger leaflets. The lower leaflets are distinct and often short-petiolulate, the upper are sessile and confluent. Petioles of leaves are from 8 to 45 cm long, sheathing only at base. The cauline leaves, which are mostly ternate or 3-parted, with lanceolate, acuminate divisions are reduced upward. The lower are alternate and petiolate, the upper are often opposite and wholly sheathing with narrow, scarcely inflated sheaths (Mathias and Constance, 1962).

The vegetative part of plant can be divided on four characteristic parts - on the storage (edible) roots, the central rootstock and the aerial stems and leaves (Figure 4). The storage roots which are of conical to cylindrical shape may be about about 1 kg of weight. This part of underground portion of plant - storage roots - does not regenerate shoots and therefore, it can not be used as a propagule. More likely apical parts with basal sections of petioles of the aerial stems or offshoots, very unique structures of arracacha which were by Hermann (1997) named as "cormels", can serve as propagules. Between these two the central rootstock, a highly compressed and swollen stem structure can be found (Hermann, 1997).

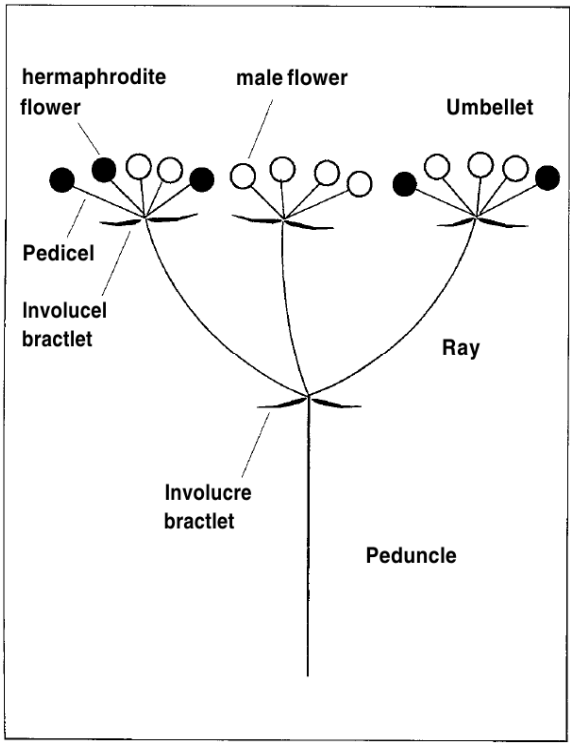

Figure 3: Schematic drawing of arracacha inflorescence structure (source: Hermann, 1997)

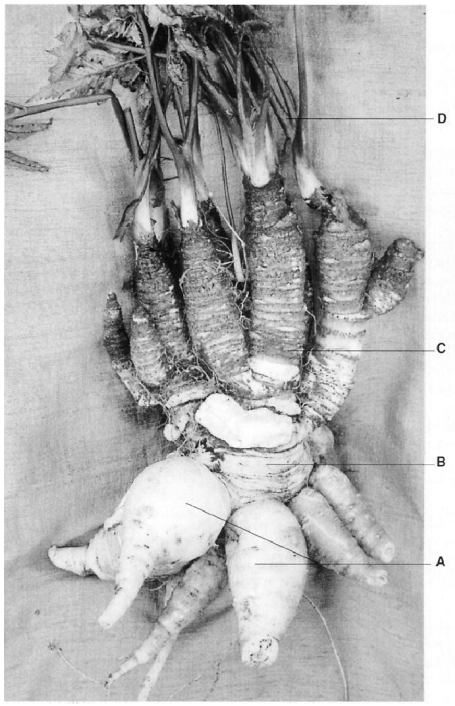

Figure 4: Main parts of arracacha plant: A - storage roots, B - rootstock, C - stems of cormels, D - leaves (source: Hermann, 1997)

2.2.6. Crop husbandry

2.2.6.1. Propagation

The seed of arracacha is rarely formed and its use significantly prolongs the time to produce crop (Hodge, 1954) thus the arracacha is as many other root crops propagated mainly vegetatively by offsets of shoots, which are produced on the crown of the main rootstock (cormels) (Hermann, 1997; Hodge, 1954; National research council, 1989; Oliveros *et al.,* 2006). Root productivity depends considerably on the preparation of the propagule (Hermann, 1997). Basal portion of shoot, which possess a bud with leaves is reduced to a two or three cm long fragment, from which all leaves are cut off few centimetrs above their attachments to the stem (Figure 5). The basal end of offset is then detached to insure that the young secondary roots will be well laterally distributed on the new primary rootstock. Without this intervention these laterals could grow vertically, which would result in crowding together with poor spacing and slowing down of growth. A cross-like mark is often cut on the cut-side end of the offset to get better spacing of the edible lateral roots. Thereafter the offsets are placed aside for two or three days the cut surfaces to dry. Propagules prepared like that can be then planted (Hermann, 1997;

Hodge, 1954; National research council, 1989). In plant of arracacha propagules can be taken at any stage of its development (Hermann, 1997).

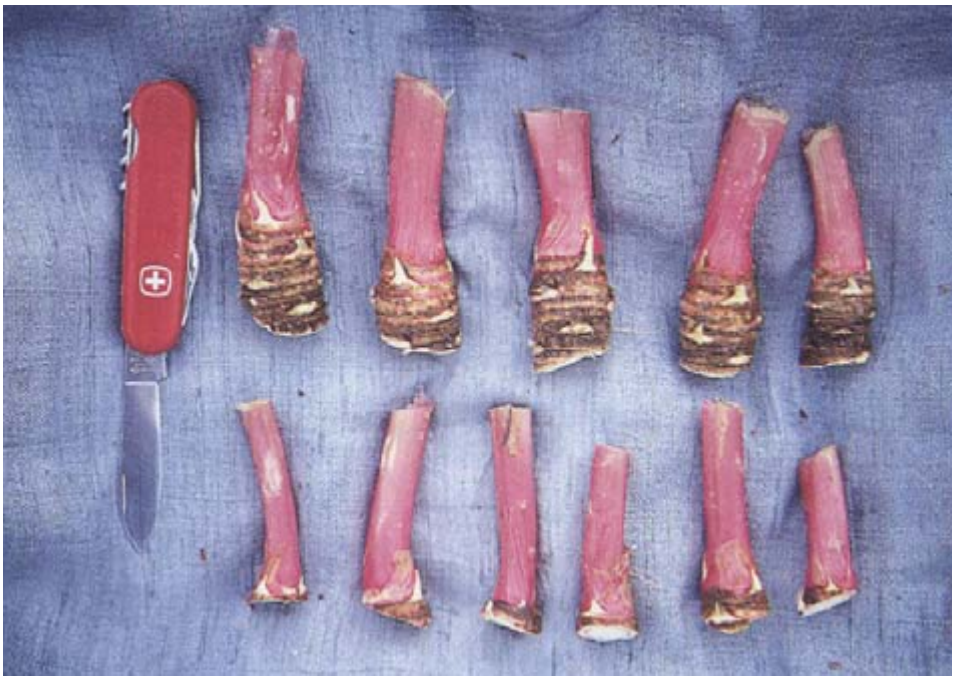

Figure 5: Propagules of arracacha (source: Oliveros *et al.*, 2006)

For rapid *in vitro* multiplication a protocol is available. Hermann (1997) mentioned gaining of 6 plantlets during 8-week cycle by using an MS (Murashige and Skoog, 1962) media supplemented with sucrose (30 $g.l^{-1}$), 6-benzylaminopurine - BAP (56 $mg.l^{-1}$) and naphthaleneacetic acid - NAA (0.05 $mg.l^{-1}$). Slíva *et al.* (2010) reached the best results (4.2 ± 0.73 new shoots per plant per 4 weeks) on MS media supplemented by NAA and BAP in concentration 0.1 and 1 $mg.l^{-1}$, respectively. This type of propagation (vegetative propagation by cloning) as well as in case of seeds is used primarily for the breeding purposes. Stability of *in vitro* regenerants obtained by *in vitro* multiplication has not been evaluated yet.

2.2.6.2. Cultivation

Arracacha is as a crop cultivated very similarly to potatoes with which it is often rotated (Hodge, 1954). It is possible to interplant this crop also with maize because of slow initial plant growth (Hermann, 1997; Hodge, 1954). It can be intercropped even with other crops but the crop has to be chosen properly because the growth of arracacha can be retarded in shading intensity exceeding 50% in the initial stage of cultivation or 18% during the whole growing cycle

(Barrella *et al.*, 2011). Propagule without root is planted at the onset of the rainy season (Hermann, 1997; National research council, 1989). Between some farmers technique of planting already rooted propagules is adopted. Pre-rooting of planting material takes up to 50 days and it is accomplished on small plots with improved soil subtrate. In comparison with unrooted propagules, crop from rooted material can be harvested 6-7 months after planting instead of 8-10 months (Hermann, 1997). On the one hectare of land approximately 20,000 plants can be planted about 60 cm apart along the furrows. Before the offsets are vertically planted to a holes (one to each), natural or artificial (or both) fertilizer is applied to each hole. After planting the rows are covered by mulch consisting often from previous harvest trash as from corn stalks, weedy bracken fern (*Pteridium*), grasses etc. Plants are kept hilled up with aim to provide ample loose soil in which the tuberous roots can easily develop. Two hand weedings are usually necessary to carry out during cultivation. They have to be done properly and carefully because of danger of harming the developing roots (Hodge, 1954).

2.2.6.3. Harvest

Harvesting time begins depending on the variety or type of propagule (rooted/unrooted) planted from 10 to 14 months after planting (Granate *et al.*, 2009; Hermann, 1997; Hodge, 1954; National research council, 1989). Maturity of plant and time of harvesting is recognized according to a sign, that the plant aboveground organs start to yellow and dry and new shoots cease to appear. Only the grower with certain level of skills can tell that his root crop is ready for harvesting by snapping his finger on one of the young lateral roots. Harvesting is done by pulling up the whole plants with the roots and all parts of plants are utilized (Hermann, 1997; Hodge, 1954; National research council, 1989). After the delayeing of the harvesting roots tend to become fibrous and tough with strong unpleasant flavor (National research council, 1989). Edible portion is used for consumption, offsets (cormels with parts of leaves) are collected for the next planting and the rest is used either for forage or for future mulching (Hodge, 1954). Root yield is in general below 20 $t.ha^{-1}$ (Granate *et al.*, 2009; Hermann, 1997; National research council, 1989) and this reflects, that plants grow under residual nutrient availability. Yield can reach more than 20 $t.ha^{-1}$ under the better conditions of cultivation provided through the fertilization, irrigation and use of suitable propagules .

Arracacha roots are highly perishable and during harvesting large roots are often cracked, thus they should be handled as carefully as possible (Hermann, 1997).

2.2.6.4. Pests and diseases

In their homeland arracachas have been considered as a non-demanding crops quite free from diseases and pests, especially when compared to other crops (Henz, 2002; Hodge, 1954). Over the world three genera of bacteria, five species of viruses, 27 genera of fungi and nine species of nematodes have been recorded in arracacha. Among the most significant diseases - root knot, caused by *Meloidogyne* spp. and postharvest soft rot caused by *Erwinia* spp. (Henz, 2002; Henz *et al.*, 2006) and *Pectobacterium chrysanthemi* (Henz *et al.*, 2006) can be considered. Leaf spots, which are caused by *Septoria* spp, *Cercospora* spp. (Henz, 2002; Mesquini *et al.*, 2009) and *Xanthomonas campestris* pv. *arracaciae*, as well as plant rots caused by *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* (Henz, 2002; Reis and Nascimento, 2011) belong also among the most serious leaf diseases that affect arracacha. Since arracacha is vegetatively propagated, viruses could become of big significance (Henz, 2002). Orílio *et al.* (2009) recorded occurence of one potyvirus species (arracacha mottle virus) infecting arracacha in Brazil, which results in reduced root production. According to National research council (1989) arracacha is also considerably susceptible to spider mites and nematodes in some regions. Ide *et al.* (2011) informed about occurences of *Semiaphis dauci,* amphid species causing even death mainly of smaller arracacha plants.

2.2.7. Ecology

Growth of arracacha is restricted to frost-free, relatively cool, tropical environments (Hermann, 1997) and it is nowadays cultivated in the highlands of the Andes, from Venezuela to Bolivia. Beyond lowlands, it gives some sort of yield at any altitude (Hodge, 1954), nevertheless it is mostly cultivated at elevations between 600 and 3,300 m a.s.l. (Hermann, 1997; Hodge, 1954; Jimenez and Faviola, 2005; National Research Council, 1989; Oliveros *et al.*, 2006).

It seems to be important to cultivate the arracacha in areas with rainfall evenly distributed thorought the year. Ideal annual amount of precipitation is about 1,000 mm and it should not be less than 600 mm (Jimenez and Faviola, 2005; National Research Council, 1989). Because of its storage tissues, arracacha is somehow resistant to temporary drought, nevertheless its growth is better, when water in soil is available during the whole growing period (Hermann, 1997).

Arracacha occurs on sites where is the mean monthly temperature between 15 and 20ºC and where the temperature rarely exceed 20ºC (Hermann, 1997; Hodge, 1954; Oliveros *et al.*, 2006). Temperatures below 14ºC delay maturity and therefore it cannot be harvested before winter. Arracacha does not tolerate frost (National Research Council, 1989).

Plant requires deep, well-drained rather sandy soils with pH of 5 or 6 (Jimenez and Faviola, 2005; National Research Council, 1989).

For good production of roots it is believed that arracacha needs the short days (National Research Council, 1989), nevertheless the effect of daylength on formation of arracacha roots is not known (Hermann, 1997).

2.2.8. Properties and uses

Dry matter is represented in arracacha roots by cca 24% (Leterme *et al.*, 2006) and starch, which is easily digested and is suitable even for diet of children and invalids (Hermann, 1997; Hodge, 1954; National Research Council, 1989), is in this matter contained by 10-25% (National Research Council, 1989). Calcium is of high level contained in all parts of plant (Jimenez and Faviola, 2005; Leterme *et al.*, 2006; National Research Council, 1989) and yellow varieties are rich on carotene (Jimenez and Faviola, 2005; National Research Council, 1989; Santos and Hermann, 1994) and ascorbic acid. On the contrary, arracacha is a poor source for protein contained by 4% in dry matter (Hermann, 1997; Jimenez and Faviola, 2005). Because of hydrolyzation of starch to sugar, arracacha roots are sweeter as much as long they are stored (National Research Council, 1989; Riberio *et al.*, 2007). Also the levels of nutrients and vitamins change during storage. Increasing amount of β-carotene is in contrast with decrease of ascrobic acid during storage (Alves *et al.*, 2010)

Young tender arracacha roots have many purposes for use. Arracacha is usually somehow prepared (boiled, fried) before it is eaten because of cooking give to it softer texture and gelatinize the starch and therefore it is more digestible (Hermann, 1997). Before any other preparation the thin outer skin should be scraped off (Hodge, 1954). Young tender roots can be eaten boiled, fried or baked or can be added to stews and soups. The flavor combines different tastes such of roasted chestnut, celery and cabbage and during cooking a fragrant aroma is emitted by the roots (Hodge, 1954; Hedrick, 1919; Hermann, 1997; National Research Council, 1989; Noguera and Pacheco-Delahaye, 2000; Rolfs, 1916). Moreover, they can be used for making flour for the instant soups (García *et al.*, 2007) baby food and school meals (Santos and Hermann, 1994). Because of good quality of their starches, they can be utilized in frozen products (Takeiti *et al.*, 2007) and production of pastry. Also they can be fried as chips, dehydrated to flakes and fermented to alcoholic beverage (Hermann, 1997). Beyond the main economic parts of arracacha - storage lateral roots - all parts of plant can be utilized. Rootstock together with aboveground plant parts are used mainly as fodder for animals (Hermann, 1997).

Young stems can be prepared as salad or can be used as a cooked vegetable (Hodge, 1954; National Research Council, 1989). Plant posses moreover medicinal properties to treat stomach ache (Tene *et al.*, 2007) or to reduce the pain in swollen breasts of recent mothers (Hermann, 1997).

Storage life of arracacha is extremly short (National Research Council, 1989). Within few days after harvest on the roots brown spots develop (Hermann, 1997). Storing under cold conditions seems to be the best to prolong arracacha storage life (Menolli *et al.*, 2011; Nunes *et al.*, 2010).

2.3. Use of *in vitro* technologies

2.3.1. Andean root and tuber crops

In biotechnology over the past three decades, many tools with considerable impact on work with genetic resources have been developed. Among these we can involve: *in vitro* propagation (Pence *et al.*, 2002), slow-growth storage (short- to medium-term storage) (Taylor *et al.*, 2010) and cryopreservation (long-term storage) of germplasm (Pence *et al.*, 2002; Taylor *et al.*, 2010), production of synthetic seeds, detection and elimination of diseases in germplasm through which the speed and safety of germplasm exchange is improved and finally an identification of useful genes (Pence *et al.*, 2002).

According to Golmirzaie and Salazar (1995) *in vitro* tissue culture method is biotechnological technique enabling maintanance and assessment of germplasm, which moreover enables modification and enhances manipulation of plant material during breeding processes.

Techniques using *in vitro* storage have many beneficial features over the field collections. These involve the ability to exchange pathogen-tested material (Arbizu, 2009; Taylor *et al.*, 2010), the maintenance away from climatic extremes and pest and diseases outbreaks and ease of distribution (Taylor *et al.*, 2010).

In vitro conservation methods are applicable and suitable for species in which *ex situ* storage of seed is not possible to use because of their nonviability or inappropriateness for application. This includes plants with recalcitrant, intermediate or highly heterozygous seeds, vegetatively-propagated plants (Ashmore, 1998; Taylor *et al.*, 2010), or in situations, when is needed to maintain the plants with elite genotypes. In addition, these techniques are usable in germplasm propagation, collection and distribution (Ashmore, 1998). *In vitro* techniques used for propagation of plant material are beyond maintenance of the uniformity among offsprings,

beneficial also in preserving of health status of plant material because of reduction of its exposure to diseases (Sharma *et al.*, 2007).

The conservation of plant genetic resources of crops and related species on which the *in vitro* conservation tools are mainly aimed, is important to ensure future access to valuable genes for plant improvement programs (germplasm conservation). Germplasm can be stored *in situ* in nature reserves or on-farm collections, or *ex situ* in seed banks, field genebanks and *in vitro* gene banks (Ashmore, 1998). Main actor in the field of germplasm conservation of Andean root and tuber crops is the International Potato Center (CIP) of which *in vitro* genebank currently stores *Solanum tuberosum* - potato (5,551 accessions), sweet potato - *Ipomoea batatas* (5,454 accessions), *Oxalis tuberosa* - oca (491 accessions), *Ullucus tuberosus* - ulluco (418 accessions), *Tropaeolum tuberosum* - mashua (47 accessions), *Smallanthus sonchifolius* - yacon (29 accessions), *Canna edulis* - achira (11 accessions) and *Arracacia xanthorrhiza* - arracacha (2 accessions) germplasm (Panta *et al.*, 2009).

During the project DENAREF (Departamento Nacional de Recursos Fitogenéticos y Biotecnología) protocols for introduction, micropropagation and conservation of some ARTC were established. Group of representative samples of Andean tubers oca and ulluco was maintained in room with temperature about 8°C and with 16/8 hours in light/dark conditions. They were kept on full strength MS (Murashige and Skoog, 1962) medium with addition of sorbitol (20 $g.l^{-1}$), sucrose (20 $g.l^{-1}$) and agar (7.5 $g.l^{-1}$). In the mashua case full strength MS medium with addition of mannitol (40 $g.l^{-1}$), sucrose (30 $g.l^{-1}$) and agar (7.5 $g.l^{-1}$) was used for conservation. Cultivation medium for short-term storage of *Mirabilis expansa* (mauka) contains full MS salts, gibberellic acid in concentration of 0.25 $g.l^{-1}$, putrescine (10 $mg.l^{-1}$), sucrose in concentration of 20 $g.l^{-1}$ and agar (7 $g.l^{-1}$). Yacon can be cultivated on full strength MS media containing gibberellic acid (2 $mg.l^{-1}$), calcium pantothenate (2 $mg.l^{-1}$), NAA (0.5 $mg.l^{-1}$), sucrose (30 $g.l^{-1}$) and agar (7 $g.l^{-1}$) (Tapia *et al.*, 2004). Skálová *et al.* (2012) established protocol for storage of yacon under slow-growth conditions. As a result of their work, medium with decreased nutrient concentration (½MS) or media containing lower concentrations of osmotic agents (mannitol and sorbitol in concentrations of 10 and 20 $g.l^{-1}$, respectively), seem to be the best for slow-growth of yacon plantlets while high survival rate and low plant height after 60 days of cultivation is maintained.

Advanced *in vitro* regeneration works are reported in potato, cassava (*Manihot esculenta*) and sweet potato in Argentina, aroids in Costa Rica, in ulluco, manihot, arracacha, oca, mashua, mauka (*Mirabilis expansa*) and achira in Ecuador and in tropical fruits, roots and tubers, cassava, potato and *Solanum nigrum* in Venezuela (FAO, 2005). For various application of *in vitro*

technologies in selected under-utilized ARTC see Table 2.

Table 2: Various *in vitro* technologies used in selected ARTC: yacon (*Smallanthus sonchifolius*), oca (*Oxalis tuberosa*) and ulluco (*Ullucus tuberosus*)

	yacon	**oca**	**ulluco**
Micropropagation	Fernández *et al.*, 2007; Górecka *et al.*, 2009; Matsubara *et al.*, 1990	Granados and Escalante, 1997	Jordan *et al.*, 2002; Granados and Escalante, 1997
Callus culture	Matsubara *et al.*, 1990; Niwa *et al.*, 2002		
Organogenesis	Handro *et al.*, 1997		
Embryogenesis	Corrêa *et al.*, 2009; Bortlová, 2012		
Polyploidization	Viehmannová *et al.*, 2009	Emshwiller, 2002	Viehmannová *et al.*, 2012
Cryopreservation		González-Benito *et al.*, 2007; Sánchez *et al.*, 2011	Sánchez *et al.*, 2011; Zámečníková *et al.*, 2011
Transgenic plants		Bais *et al.*, 2003	
Virus elimination		Fletcher and Fletcher, 2001	Fletcher and Fletcher, 2001
Microtuberisation			Jordan *et al.*, 2002

2.3.2. *Arracacia xanthorrhiza*

Slíva *et al.* (2010) established protocol for micropropagation of peruvian carrot. Primarily the plant material (roots and leaves from the leaf rosette) was sterilized and introduced into sterile *in vitro* culture (MS media supplemented with 30 $g.l^{-1}$ of sucrose and 8 $g.l^{-1}$ of agar). The best results for multiplication of explants were obtained on MS medium with addition of NAA and BAP in concentration of 0,1 $mg.l^{-1}$ and 1 $mg.l^{-1}$, respectively (4.2 ± 0.73 new shoots per plan/4-week cycle). The callogenesis was induced by cultivation of petiole segments on MS medium supplemented with combination of 2,4-D and kinetin - KIN (0.1 $mg.l^{-1}$ + 0.2 $mg.l^{-1}$) or dichlorophenoxyacetic acid - 2,4-D alone (0,1 or 1 $mg.l^{-1}$). The best regeneration of calli on explants was observed on medium with addition of 1 $mg.l^{-1}$ 2.4-D. Calli started to appear 14 days after placement of petioles on media. For indirect morphogenesis, callus was placed on media without plant growth regulators (PGRs). The best results to regenerate shoots from callus were obtained on medium supplemented with 2,4-D in concentration 0,1 $mg.l^{-1}$. The best ability of calli to regenerate roots was obtained in dark conditions on media supplemented with 1 $mg.l^{-1}$ 2,4-D. Roots in plants cultivated on MS medium without PGR and with activated charcoal (AC) added, occured rarely and spontaneously. Any other atempts to induce rooting on media

supplemented by PGR NAA, 2,4-D, AC and IAA in various concentrations, failed.

Hermann (1997) mentioned regeneration up to 8 arracacha plants within a 8-week period on the MS medium supplemented with 30 $g.l^{-1}$ of sucrose, 56 $mg.l^{-1}$ of BAP and 0.05 $mg.l^{-1}$ of NAA.

During the project DENAREF protocol for introduction, micropropagation and conservation of arracacha was established. Isolated arracacha meristems were placed on the medium consisting of 4.3 $g.l^{-1}$ MS salts, 0.25 $mg.l^{-1}$ gibberellic acid, 30 $g.l^{-1}$ sucrose and 6 $mg.l^{-1}$ agar. Plants were subsequently incubated on MS or Gamborg medium (Gamborg *et al.*, 1968) supplemented with sucrose (30 $g.l^{-1}$), agar (6 $g.l^{-1}$), BAP (5.6 $mg.l^{-1}$) and NAA (0.05 $mg.l^{-1}$) at temperature of 18 ± 2°C with light intensity of 2,000 lux and a relative humidity of 70%. Micropropagation of arracacha was tested on two cultivation media. Medium 1 contained standard amount of MS salts, sucrose (30 $g.l^{-1}$), agar (6 $g.l^{-1}$) and growth regulators BAP and NAA in concentrations of 6 and 0.05 $mg.l^{-1}$, respectively. Medium 2 was supplemented with Gamborg B5 salts, sucrose, agar and phytohormones NAA and BAP in concentrations of 0.1 and 0.2 $mg.l^{-1}$, respecitvely. Media pH was adjusted at 5.5 (Tapia *et al.*, 2004).

International Potato Center (CIP) of which *in vitro* genebank currently stores 2 accessions of arracacha germplasm have the goal to secure the in-trust genetic resources collections to perpetuity and to promote their use through the development and application of efficient practices and conservation methods. This genebank maintain the arracacha on MS media adjusted on pH 5.6 containing sucrose (25 $g.l^{-1}$), agar (7 $g.l^{-1}$), BAP (4 $mg.l^{-1}$), glycine-HCl (2 $mg.l^{-1}$), *myo*-inositol (100 $mg.l^{-1}$), nicotinic acid (0.5 $mg.l^{-1}$), pyridoxine-HCl (0.5 $mg.l^{-1}$) and thiamine-HCl (0.1 $mg.l^{-1}$) (Panta *et al.*, 2009).

Madeira *et al.* (2005) evaluated the influence of different BAP and GA_3 concentrations on the *in vitro* development of arracacha. Growth of arracacha shoot tips were evaluated on testing media supplemented with 0.0, 0.2, 0.4 $mg.l^{-1}$ of BAP and 0.0, 0.125, 0.25 $mg.l^{-1}$ of GA_3 . The increase in the concentration of BAP reduced the callus formation, as well as the shoot development. The good development of shoot with a mean of 5.5 shoots and height of 43 mm was observed on medium supplemented with 0.3 $mg.l^{-1}$ of BAP. With the increase of GA_3 concentration in the media, improvement in the mean and maximum height of the shoots and in callus size was promoted.

Pessoa *et al.* (1994) induced callus from petiole segments on MS medium supplemented with various concentrations of 2,4-D and BAP or with 2,4-D alone. The growth of calli was negatively affected by an increase of sucrose concentration.

Lopes (2009) evaluated *in vitro* germination of arracacha seeds and the induction of

direct and indirect organogenesis of three arracacha cultivars. He found out high degree of contamination on arracacha seeds. Without seed coat ascertained contamination rate was lower. Germination was irregular, and seeds in which radicle occured formed aggregates of non-organized cells. For the callus induction sterilized root sections were used. Regeneration of roots from callus was induced by using MS media supplemented with 0, 2, 4 and 6 $mg.l^{-1}$ of cytokinins (kinetin or BAP). Direct organogenesis was obtained via cultivation of sterilized apical and lateral shoots on media supplemented with BAP and NAA.

Accessions of arracacha imported to New Zealand were found to be infected with viruses, thus the plants were treated to obtain virus-free cultures. Shoot tips of arracacha were removed from the plant and established into *in vitro* conditions onto growth MS medium with pH adjusted at 6.0 supplemented with $NaH_2PO_4 \cdot 2H_2O$ (221 $mg.l^{-1}$), thiamine-HCl (0.4 $mg.l^{-1}$), *myo*-inositol (100 $mg.l^{-1}$), sucrose (30 $g.l^{-1}$) and agar (7.5 $g.l^{-1}$) and with 50 $mg.l^{-1}$ of ribavirin. The explants were introduced to the growth chamber in conditions designed to eliminate viruses (alternating periods of 4 hrs light at 35°C and 4 hrs dark at 31°C). When the explants reached the height of 1 cm, they were transferred on ribavirin-free medium and grown under following conditions: 24°C under fluorescent lights with 16 h photoperiod and 8 h dark. After successfull testing for viruses, plants were transferred to *ex vitro* conditions (Fletcher and Fletcher, 2001).

2.4. Use of molecular markers

2.4.1. Andean root and tuber crops

Molecular marker methods including RFLP (restriction fragment lenght polymorphism), RAPD (random amplified polymorphic deoxyribonucleic acid), AFLP (amplified fragment lenght polymorphism), SSR (simple sequence repeat), SNP (single-nucleotide polymorphism), etc., represent rapidly developing research tools, that can characterize and manage the genetic polymorphism in plant breeding programmes and programmes, which deal with germplasm characterization (FAO, 2005). In plant breeding programmes, molecular markers are most commonly used for marker-assisted selection (MAS) (FAO, 2005; Henry, 1998). Other applications inlude germplasm characterization (FAO, 2005), analysis the genetic constitution of somatic plant hybrids and their identification (Chawla, 2002), commercial variety protection, assessment of seed purity, verification of labelling and identity of plants in production and marketing (Henry, 1998). They can serve as highly useful tools in population genetics, molecular taxonomy, screening for somatic mutations (Zietkiewicz *et al.*, 1994), genome mapping (Reddy *et al.*, 2002; Zietkiewicz *et al.*, 1994) evolutionary biology, phylogeny (Reddy *et al.*, 2002) and

in genetic diversity studies (FAO, 2005; Reddy *et al.*, 2002). DNA-based genetic markers are often considered to be more reliable to assess genetic diversity, when compared to morphological and protein markers (FAO, 2005), due to their highly polymorphic and heritable pattern, which means that their expression is not affected by environmental variability (FAO, 2005; Chawla, 2002). The genetic diversity evaluation of ARTC using molecular markers is in tropical America of an interesting development (FAO, 2005). Arbizu (2009) mentioned redundancy of accessions of most crops maintained by Andean gene banks, thus the molecular characterization methods are needed to reduce the size of the collections by elimination of duplicated accessions.

Molecular marker methods tend to be more cheap and simple in term of application during the years. Therefore their uzilization increases routine applications in tropical and subtropical species, for which often very limited research resources are available (Henry, 1998).

From ARTC genetic germplasm diversity characterisation with help of molecular markers was assessed in arracacha - *Arracacia xanthorrhiza* (Biondi *et al.*, 2009a; Blas *et al.*, 2008), yacon - *Smallanthus sonchifolius* (Soto *et al.*, 2009; Mansilla *et al.*, 2006), oca - *Oxalis tuberosa* (Biondi *et al.*, 2009b; Pissard *et al.*, 2006, 2008a, 2008b), potato - *Solanum tuberosum* (Favoretto *et al.*, 2009), mauka - *Mirabilis expansa* (Chia *et al.*, 2006), maca - *Lepidium meyenii* (Toledo *et al.*, 1998), ulluco - *Ullucus tuberosus* (Malice *et al.*, 2009; Parra-Quijano *et al.*, 2012) and mashua - *Trapaeolum tuberosum* (Ortega *et al.*, 2007).

Soto *et al.* (2009) evaluated 359 accessions of yacon from 6 Peruvian genebanks using 309 polymorphic AFLP markers obtained by 6 primer combinations. Only 7 accessions were identified as duplicates in comparison with 352 accessions with unique genotypes. Mansila *et al.* (2006) used RAPD method for evaluation of 30 cultivated yacon accessions from northern, southern and central region of Peru. By using 34 decameric primers generating 166 bands, of which 30.7% were polymorphic, the greatest diversity was found in the central region of Peru and no duplicated individuals were found.

Biondi *et al.* (2009b) assessed germplasm diversity of 585 oca accessions maintained in the International Potato Center genebank using 175 polymorphic AFLP markers generated by 7 primer combinations. From 4 Andean countries, Peru was found to be the country with the greatest diversity of oca accessions maintained in the genebank. The analyses used in the study supports the assumption, that Peru is the centre of oca origin. Pissard *et al.* (2006) used ISSR (inter-single sequence repeat) method to assess diversity of 32 oca accessions originating from South America. Analysis of 90 used markers (generated by 9 selected primers) revealed that Peruvian oca accessions form a distinct genetic group and that genetic diversity of oca is relatively low probably due to reproduction strategy mostly used - vegetative propagation.

Favoretto *et al.* (2009) evaluated 38 potato accessions from 2 separate collections of commercial cultivars with aim to identify duplicated individiuals and possible parents for breeding programmes. They found out high levels of polymorphism in *Solanum tuberosum* species, which considers the SSR marker as useful tool for detection of genetic differences between cultivars, which can be utilized in further potato breeding programmes.

Chia *et al.* (2006) used 9 selected RAPD primers to evaluate 37 accessions of mauka from northern Peru. They obtained 60 polymorphic markers. Portion 16.2% of accessions was assessed as possible duplicates in the collection.

Toledo *et al.* (1998) evaluated 29 cultivated accessions of maca from Ecuador, Peru and Bolivia using RAPD markers. A sub-sample of 14 cultivated accessions was assessed by RFLP markers. They found out very little polymorphism revealed among accessions of maca with the RFLP markers as well as with RAPD markers.

Malice *et al.* (2009) revealed a high number of different ulluco genotypes maintained *ex-situ* in Peru and North Boliva using ISSR markers together with morphological evaluation. Molecular markers in comparison with morphological characterisation of accessions disclosed existence of intra-morphotype heterogeneity. Parra-Quijano *et al.* (2012) assessed ulluco genetic diversity in Colombia. They examined 36 cultivated ulluco accessions preserved in the Colombian Gene Bank using following molecular markers: total proteins, isozymes and RAPDs. They found out two ulluco gene pools in Colombia suggesting the fact, that ulluco was introduced to Colombia at least two times.

Ortega *et al.* (2007) studied the genetic diversity of cultivated and non-cultivated mashua in the Cusco region in Peru by using SRAP (a sequence related amplified polymorphism) markers, which revealed, that mashua is genetically highly variable crop with a range of similarity from 65 to 99%.

Beyond evaluation of germplasm diversity characterization, molecular markers can be used also in molecular taxonomy, evolutionary biology and population genetics. Hasan *et al.* (2010) developed 8 cpSSR primers for further utilization in characterization of genetic variation among cultivated and wild *Lepidium meyenii* plants and for study of related taxa. Emshwiller *et al.* (2009) used AFLP markers with the aim to find origins of polyploidy of octoploid oca. They compared AFLP data of *Oxalis tuberosa* with populations of wild tuber-bearing *Oxalis* taxa from different Andean regions. Results suggested one unnamed *Oxalis* species found in Bolivia and *O. chicligastensis* as probable genome donors for polyploid *O. tuberosa*.

Genetic relationships among five different yacon landraces using RAPD and AFLP markers were evaluated by Millela *et al.* (2005, 2011). In their first study (Millela *et al.*, 2005)

using 61 decamer primers a total of 282 RAPD bands were scored with 28.7% of polymorphism observed within landraces. One marker (OBP14) showed various pattern of bands between all landraces. Millela *et al.* (2011) compared RAPDs and AFLPs application for the analysis of yacon diversity. Using 61 RAPD primers 85 informative markers were distinguished, corresponding to 28.7% of polymorphism. A similar percentage of polymorphism (23.4%) was revealed using six selected AFLP primer pairs generating 84 informative markers. Both markers proved to be reliable tools to investigate genetic variability among yacon landraces.

Sharma *et al.* (2007) evaluated the genetic and phenotypic stability of potato plants derived from various methodologies of propagation - axillary-bud proliferation, microtuberisation, through true potato seeds (TPS) and somatic embryogenesis. They found out a very low level of variation in AFLP marker in plants propagated through somatic embryogenesis (3 out of 451 bands) and microtuber derived plants (2 out of 451 bands). No polymorphism was found in axillary-bud-proliferation derived plants.

2.4.2. *Arracacia xanthorrhiza*

Diversity of arracacha gene bank collections in Peru with help of molecular markers were evaluated by Biondi *et al.* (2009a). Three hundred and thirty four accessions from 5 genebanks were assessed using 143 polymorphic AFLP markers (from total of 206 bands= polymorphism from 71%) obtained from five primer combinations. By analysis it was found out that arracacha germplasm in Peru is diverse and no duplicated accessions were identified. The UPGMA dendogram showed according to arracacha geographical distribution patterns 3 clusters (northern, central and southern Peru) from which the first one (northern Peru) showed significantly higher diversity than others.

Blas *et al.* (2008) studied genetic diversity among 4 wild *Arracacia* species with help of morphological descriptors and AFLP markers. Five primer combinations generating 202 AFLP reliable and reproducible markers were studied. No unique fragments were found in *Arracacia xanthorrhiza*. Polymorphism presented among arracacha populations varied from 23.2 to 26.7%. The relatively high degree of variation within the populations could be a result of widespread occurence of wind pollination and systems of breeding promoting outcrossing. On this basis arracacha seems to be facultative outbreeder which gives progenies with genotypes of new genetic combinations. Nevertheless, such pronounced variation could also result from hybridizations between cultivated and wild arracacha forms or between *A. xanthorrhiza* and its relative species.

Morillo *et al.* (2004) developed microsatelite marker for arracacha crop. In the *A. xanthorrhiza* complex, from 18 successfully amplified primers 14 primers showed polymorphic pattern. Diversity of alleles was low, nevertheless ability to transfer the primers to closely related wild forms showed to be good.

2.5. Evaluation of genetic stability in *in vitro* regenerants using molecular markers

The term "somaclonal variation" is now widely adopted for the variation caused by culturing of plants in *in vitro* conditions. Alternatives like "protoclonal " or "gametoclonal", which describe variation originating from protoplast and anther culture, respectively, can be also used. It is possible to regenerate plants from somatic cells due to their totipotent character (Karp, 1994).

According to Karp (1994) three classes of variation can be recognized: heritable stable variation, heritable unstable variation and non-heritable (epigenetic) vatiation. Somaclonal variation can have basis on change in karyotype, chromosome structure, on single-gene mutations, cytoplasmic genetic changes, mitotic crossing over, gene amplification and nuclear changes and on transposable elements (Chawla, 2002).

The genetic variability is restricted mainly on some types of cultures. The only culture system, in which somaclonal variation is not common and which can be considered free from this problem is in-meristem tip culture, where meristems remains undisturbed during the *in vitro* cultivation (Chawla, 2002; Karp, 1994; Reed *et al.*, 2004). Somaclonal variation is related to disorganized growth and is common in plants regenerated from single cells, callus or adventitious buds (Reed *et al.,* 2004). Chawla (2002) mentioned 4 main factors influencing somaclonal variation - plant genotype, source of explant, duration of cell culture and conditions of culture cultivation. Genotype of plant can influence the frequency of regeneration as well as the frequency of somaclones. Source of explant and duration of culture are also important variables influencing somaclonal variation. Karp (1994) mentioned the fact that the longer the duration of the disorganized phase and the greater the departure from organized structures the greater the chances of somaclonal variations. This is true when plants are regenerated through organogenic or embryogenic cultures (with intermediate callus phase). Nevertheless, direct formation of such structures from cultivated plant tissues without callus formation minimizes the occurence of genetic instability. Although majority of variations originates from the culturing phase, somaclonal variability may arise from mutations already present in the mother plant

(Karp, 1994). Medium supplemented with different hormone concentrations affects development of shoot and growth rates, and high concentrations, particularly of cytokinins, can affect genetic stability (Reed *et al.*, 2004). It has been known that frequency of karyotypic alterations in cultured cells is greatly influenced by growth regulators forming composition of the culture medium (Chawla, 2002).

It is of fundamental importance that genome of plants multiplicated through *in vitro* cultures remain stable and no variation occurs (Karp, 1994; Sharma *et al.*, 2007). Somaclonal variability is highly undesirable in cultures cultivated with the aim of clonal propagation of plants (Sharma *et al.*, 2007). Maintanance of genetic stability is important particularly in preserving of plant germplasm in genebanks (Reed *et al.,* 2004).

On the contrary, somaclonal variation is a desirable novel source of variation utilizable in plant breeding. It retain promise especially in species with limited breeding approaches (assexually propagated species) and in crops cultivated in developing countries with restricted breeding efforts due to the paucity of funds (Karp, 1994). They can be applied in several breeding programmes. Agronomically used novel variants can arise from somaclonal variation. As a greatest contribution of somaclonal variation towards plant improvement development of disease resistant crops is considered. Somaclonal variants can also serve as a source of insect, herbicid and abitotic stress resistant plants. By this way varieties with improved seed quality can arise (i.e.lowering of neurotoxin content in lathyrus seeds). And finally the increase in genome rearrangement during cultivation provides a new opportunity for alien gene introgression widening the crop germplasm base (Chawla, 2002).

Somaclonal variation does not provide easy alternative to conventional breeding. Although there are many attractions of this source of variability, there are also many issues associated with its application. Nature of variations is unpredictable, uncontrollable and variations are mostly without any apparent use. The variation is cultivar-dependent and changes obtained are not always stable, heritable and not all changes are a novel. Most of variants improved through somaclonal variation have not been selected for breeding purposes (Chawla, 2002).

There is lack of the universal system for detecting somaclonal variation. Nowadays changes of genome are evaluated by morphological, cytogenetical, biochemical and molecular descriptors (Karp, 1994). Quite recently, molecular genetic techniques have been developed to obtain informations about genome looking and to reveal variations between original plant and plant after culturing. Molecular marker methods are highly multiplex tools for evaluation of uniformity regardless of limitations of any profiling methodology (Sharma *et al.*, 2007).

Studies on application of molecular markes for detection of genome stability in plants regenerated via different methods of micropropagation, are listed in Table 3.

Table 3: Utilization of various molecular markers for assessment of genome stability in examples of plants regenerated through different methods of micropropagation

species	type of culture	explant source	molecular technique detecting variability	variable /stable genome	Reference
Aloe vera	embryogenesis	inflorescence axis	RAPD, ISSR	variable	Rathore *et al.* (2011)
	axillary bud regeneration	axillary shoot bud		stable	
Arabidopsis thaliana	organogenesis	roots	AFLP	variable	Polanco and Ruiz (2002)
Asparagus officinalis	organogenesis	spear sections devoid of lateral buds	AFLP	variable	Pontaroli and Camadro (2005)
Cannabis sativa	shoot multiplication	nodal segments	ISSR	stable	Lata *et al.* (2010)
Curcuma longa	shoot multiplication	axillary buds from rhizome	RAPD, SDS-PAGE	stable	Das *et al.* (2010)
Cymbidium giganteum	induction of protocorm-like bodies	pseudostem segments	RAPD	variable	Roy *et al.* (2012)
Dioscorea prazeri	shoot multiplication	axillary buds	RAPD	stable	Thankappan and Morrawala-Patell (2011)
Freesia hybrida	embryogenesis	inflorescences with rachillae	AFLP, MSAP	variable	Gao *et al.* (2010)
Gentiana straminea	embryogenesis	leaf explant	ISSR	stable	He *et al.* (2011)
Gerbera jamesonii	shoot multiplication	capitulum explants	RAPD, ISSR	stable	Bhatia *et al.* (2011)
Glycine max	embryogenesis	immature zygotic cotyledons	RAPD	variable	Gesteira *et al.* (2002)
Guadua angustifolia	shoot multiplication	axillary buds	RAPD, ISSR	stable	Nadha *et al.* (2011)
Jatropha curcas	axillary bud proliferation	shoot buds	RAPD, AFLP	stable	Sharma *et al.* (2011)
Sapindus trifoliatus	shoot multiplication	nodal explants	RAPD	stable	Asthana *et al.* (2011)
Simmondsia chinensis	shoot multiplication	nodal segments	RAPD, ISSR	stable	Kumar *et al.* (2011)

3. Aims of the thesis

The main objective of this work is development of an appropriate protocol for micropropagation through indirect morphogenesis of *Arracacia xanthorrhiza* and detection of genetic stability/variability of *in vitro* regenerants by means of molecular markers.

The main objective can be divided into the following partial goals:

1. Finding optimal concentrations and combinations of plant growth regulators in cultivation medium for induction of morphogenic callus in *Arracacia xanthorrhiza.*
2. To prove an ability of arracacha callus to recover plants via indirect morphogenesis (i.e. organogenesis or embryogenesis).
3. Evaluation of genetic stability or variability of regenerants using simple sequence repeat markers (ISSR).

Indirect morphogenesis offer an effective tool in propagation of plant material, however involved intermediate callus phase in this type of propagation and use of media supplemented with higher concentrations of plant growth regulators leads to higher probability of somaclonal variation obtained. Nevertheless, potential somaclonal variability could serve as a source of valuable traits exploitable in further breeding approaches.

4. Materials and methods

4.1. Plant material

Plant material aquired from Bolivia from Locotal region in 2007 was introduced into *in vitro* conditions by Slíva (2009). Thus, already established *in vitro* culture of multiplied plants was used in this study. Segments of petioles (cca 8-10 mm) of *in vitro* cultivated plants were used for induction of callogenesis.

4.2. Methodology

Schematic diagram showing a complete design of the experiment have been proposed for better orientation (Figure 6). The individual stages are decribed in detail in a single subsections of methodology.

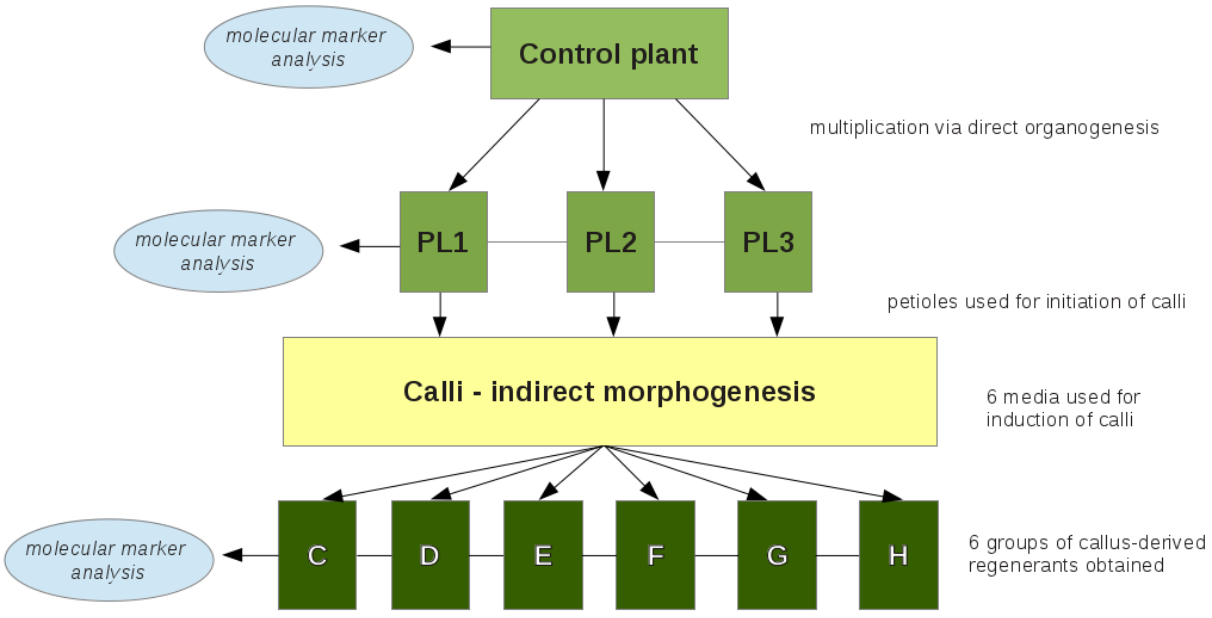

Figure 6: Schematic diagram representing stages of whole methodology. PL1-PL3: plants obtained via direct organogenesis; C-H: 6 groups of plants obtained via indirect morphogenesis according to media used (source: author)

4.2.1. *In vitro* propagation of plant material

For plant multiplication MS (Murashige and Skoog, 1962) medium supplemented with sucrose (30 $g.l^{-1}$), *myo*-inositol (100 $mg.l^{-1}$), plant growth regulators NAA and BAP at concentrations 0.1 and 1 $mg.l^{-1}$, respectively, and agar (8 $g.l^{-1}$) as a solidifying agent were used. The pH was regulated by KOH and ascorbic acid at 5.7. This protocol for plant multiplication via direct shoot production was adopted from Slíva (2009). Using this medium, sufficent plant material for further experiments was obtained. Plants grown on MS media without plant growth regulators were maintained as controls for genetic analysis. Genetic stability among initial plant material and multiplied plants was assessed using ISSR analysis (described in chapter 5.1.)

4.2.2. Callogenesis and plants regeneration

Segments of petioles of 8-10 mm in length, obtained from *in vitro* plants of arracacha, were used as explants for callusing (Figure 7). Explants (3 segments to each Erlenmayer flask with 50 ml of volume) were placed on the surface of basal medium with macro- and micro-elements of MS medium supplemented with *myo*-inositol (100 $mg.l^{-1}$), sucrose (30 $g.l^{-1}$) and agar (8 $g.l^{-1}$). Plant growth regulators from groups of auxins (2,4-D) and cytokinines (BAP) were added prior to autoclaving in various concentrations (Table 4). MS medium without plant growth regulators was used as a control. After the calli formation, they were transferred either onto regeneration medium without PGRs or on fresh maintenance media (medium of the same chemical composition as the induction medium). Cultures were maintaned in incubator with 18 hours of light and 6 hours of dark per day, at temperature 25/23 ^{o}C (light/dark period) and with light intensity of 2000 lx.

The percentage of explants producing callus and the proliferation responses of calli to the various PGRs were recorded 4 weeks after establishment of experiment. Calli characteristics such as color, friability and occurence of meristematic centres were observed.

Regeneration of plants from calli was recorded at 8 weeks of culture. Every stadium of arracacha culture was photographed using the camera Canon PowerShot A570 IS.

Statistical evaluation was carried out using analysis of variance (ANOVA) and the significantly different means were identified by using the Tukey´s HSD test ($p = 0.05$) [StatSoft STATISTICA 9.0].

Table 4: Various treatments of callus inducing media

Treatment	2,4-D (mg.l^{-1})	BAP (mg.l^{-1})
A		1
B		2
C	0.1	
D	1	
E	1	0.5
F	1	1
G	1	3
H	1	5
Control	0	0

These experiments took place in the Laboratory of plant tissue cultures of the Department of Crop Sciences and Agroforestry in Tropics and Subtropics of Faculty of AgriSciences of Czech University of Life Sciences Prague in 2012-2013.

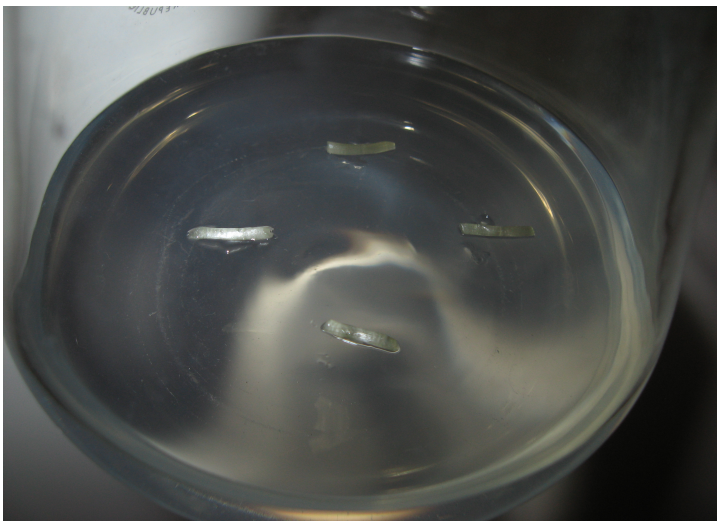

Figure 7: Segments of petioles used for induction of callus (photo: author)

4.2.3. DNA extraction

At first, molecular analysis using ISSR markers were applied in initial plant material and plants multiplied via direct shoot formation, in order to exclude occurence of somaclonal variation before beginning of the experiment.

Secondly, ISSR markers were applied in regenerants obtained via indirect morphogenesis on different media, where as a control plant initial material was used. All analyse samples are listed in Table 5.

Table 5: *In vitro* regenerants from which DNA was extracted

Regenerants	type of media (mg.l^{-1})	number of accessions
C	0.1 2,4-D	2
G	3 BAP + 1 2,4-D	1
H	5 BAP + 1 2,4-D	4
Multiplication medium	1 BAP + 0.1 NAA	3
Control	without PGRs	1

Invisorb® Spin Plant Mini Kit (Invitek Company, Germany) was used for extraction of genomic DNA from fresh leaves of *in vitro* cultivated plants.

The common practice is extraction of DNA from the fresh plant material about 100 mg of weight. In the case of arracacha weight of samples used for extraction was mostly under this limit. Due to small amount of fresh leaves in obtained regenerants was available.

The samples were homogenized under liquid nitrogen with help of pestle and mortar and the DNA was from them extracted following instructions in manual of the commercial kit. No modification of extraction procedure was done.

The DNA [solution containing 5.5 µl of DNA and 2 µl of loading dye (Fermentas, Germany)] was tested on a gel composed from 1.5 g of agarose dissolved in 100 ml of 1% TBE buffer. For staining 1 µl of SYBR® Safe DNA Gel Stain (Invitrogen, USA) was used. Gels were run at 55V for about 1 hour. It was visualized on a gel with a UV transilluminator and gel pictures were displayed using CSL-MICRODOC System (CLEAVER, Great Britain). The gel was during electrophoresis immersed in 500 ml of 1% TBE solution.

The experiment took place in the Laboratory of molecular biology of the Department of Crop Sciences and Agroforestry in Tropics and Subtropics of the Faculty of Tropical AgriSciences of CULS in 2012.

4.2.4. ISSR analysis

DNA amplifications using Polymerase Chain Reaction (PCR) were carried out in a reaction volume of 20 µl with following composition: 10 µl of PPP Master Mix (Top-Bio, Czech Republic), 8.3 µl of PCR H2O (Top-Bio, Czech Republic), 1 µl of template DNA, 0.5 µl of primer and 0.2 µl of BSA (Fermentas, Germany).

A set of 11 ISSR primers obtained from Biotechnology Laboratory of UBC (University of British Columbia, Canada) was used for screening (Table 6).

Table 6: Sequences of primers used

primer name	primer sequence
'UBC 809'	5'-$(AG)_8$G-3'
'UBC 810'	5'-$(GA)_8$T-3'
'UBC 812'	5'-$(GA)_8$A-3'
'UBC 823'	5'-$(TC)_8$C-3'
'UBC 834'	5'-$(AG)_8$YT-3'
'UBC 836'	5'-$(AG)_8$YA-3'
'UBC 841'	5'-$(GA)_8$YC-3'
'UBC 843'	5'-$(CT)_8$RA-3'
'UBC 844'	5'-$(CT)_8$RC-3'
'UBC 845'	5'-$(CT)_8$RG-3'

Annealing temperature in PCR was optimized in each primer (Table 7).

After DNA amplification, amplified products were electrophoretically separated on gel composed from 1.5 g of agarose dissolved in 100 ml of 1% TBE buffer. Staining capability of EtBr and SYBR® Safe DNA Gel Stain (Invitrogen, USA) was verified. To dye agarose gel 1 µl of each stain was used. Gels were run at 55V for about 2.5 hours. The amplified stained products were visualized on a gel with a UV transilluminator and gel pictures were displayed using CSL-MICRODOC System (CLEAVER, Great Britain). The gel was during electrophoresis immersed in 500 ml of 1% TBE solution. ISSR fragments were scored as a presence (1) or absence (0) of bands in the gel profile. The size of fragments was compared with the DNA standard 100 bp ladder (Fermentas, Germany).

Table 7: Conditions of PCR cycles for selected primers

number of cycles	step		temperature	duration of cycle
1	Initial denaturation		94°C	5 min
40	Denaturation		94°C	1 min
	Annealing		specific	1 min
	Primer:	'UBC 809'	50°C	
		'UBC 810'	50°C	
		'UBC 812'	50°C	
		'UBC 823'	49°C	
		'UBC 834'	53°C	
		'UBC 836'	50°C	
		'UBC 841'	53°C	
		'UBC 843'	50°C	
		'UBC 844'	49°C	
		'UBC 845'	49°C	
	Elongation		72°C	2 min
1	Extension		72°C	2 min
1	Final extension		72°C	10 min

5. Results and discussion

5.1. Multiplication of plant material

Protocol for multiplication of plant material was adopted from the study of Slíva *et al.* (2010). Plantlets were multiplied to obtain enough material (petioles) for the experiment (induction of callogenesis and indirect morphogenesis). Through this type of propagation - direct organogenesis - satisfactory number of plants for further research was obtained. This method proved to be suitable for multiplication of plant material. Genetic stability was assessed in these plants using ISSR markers, as it was essential to exclude somaclonal variation before experiment initiation. Results are described in chapter 5.4.1.

Propagation via direct organogenesis has been previously reported in apioid species such as *Crithmum maritimum* L. (Grigoriadou and Maloupa, 2008) and *Thapsia garganica* L. (Makunga *et al*., 2005). Nevertheless, most species of this botanical family are being propagated via callus intermediate phase (Anzidei *et al.,* 2000; Tawfik and Noga, 2002; Dave and Batra, 1995; Fiore *et al.,* 2012; Hedrawati *et al.,* 2012; Irvani *et al.,* 2010; Maatar and Hunault, 1997; Makunga *et al.,* 2003; Osuga *et al.,* 1997; Paramageetham *et al.,* 2004).

5.2. Indirect morphogenesis

5.2.1. Induction of callogenesis

Formation of calli started in petiole explants within 2 weeks after inoculation in all treatments. This fact corresponds with paper of Slíva *et al.* (2010), who observed initiation of callogenesis in arracacha two weeks after placing of petioles on media supplemented with PGRs. Makunga *et al.* (2003) observed the initiation of callogenesis also after 2 weeks of culture in leaflet explants of *Thapsia garganica* L., a medicinal plant from family *Apiaceae* using media containing NAA and BAP at concentrations of 1 and 3 $mg.l^{-1}$, respectively.

In present study, calli started to spread from the cuts of petioles. The type and texture of the callus depended on the type of PGRs used. Textures of calli were soft and grainy, from light yellow-green to light-brown colored and meristematic centres in the form of light spherical formations on the surface of calli sometimes appeared (Figure 8). Calli on media supplemented with 2,4-D at concentration of 1 $mg.l^{-1}$ had a grainy surface and were light-brown when up to 30 days old and then they tend to change the color into light-green or yellow. Calli were usually light green-brown on the media supplemented with lower concentration of 2,4-D (0.1 $mg.l^{-1}$) and

meristematic centres appeared at higher frequency in comparison with previously mentioned treatment. Calli on media combining BAP and 2,4-D were soft, compact and from light-brown to green-brown colored. The occurence of spherical structures on the surface of calli on these media increased with the age of cultures and the higher frequency of these structures was observed on media containing lower concentrations of BAP (0.5 and 1 $mg.l^{-1}$).

Figure 8: Occurence of spherical meristematic centres on the arracacha callus surface (photo: author)

The data on callus initiation is given in Table 8. Process of dedifferentiation failed to occur on the control medium devoid of PGRs and petiole explants on this medium became necrotic within 4 weeks of culture. Calli on media supplemented with higher concentration of 2,4-D (1 $mg.l^{-1}$) alone grew faster, when compared with calli grown on media with lower concentration of 2,4-D (0,1 $mg.l^{-1}$). At media combining BAP and 2,4-D, production of callus increased with higher concentration of BAP in the initiation medium. Callus was not initiated from petioles placed on two types of media (treatments A, B) supplemented with BAP in concentrations of 1 and 2 $mg.l^{-1}$, respectively. Likewise, Makunga *et al.* (2003) used various concentrations of BAP to initiate callus in petiole explants of *Thapsia garganica* L.*,* an apioid medicinal plant. Nevertheless, they observed the elongation of explants only. Based on these results, can be suggested that BAP may induce callogenesis only in combination with auxin, in our case 2,4-D. However, 2,4-D when used individually in induction medium, succesfully

produced callus on explants.

The frequency of callus induction in other variants (C-H) varied from 64-100% in dependence on BAP and 2,4-D concentrations (Table 8). The highest percentage (100%) of callus was observed in variant of medium F containing BAP and 2,4-D at concentrations 1 and 1 $mg.l^{-1}$, respectively. Combination of these PGRs was found out highly effective for callus formation also in *Juncus effusus* L. (Xu *et al.,* 2009), *Hypericum perforatum* L.(Pretto and Santarém, 2000), *Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson (Corrêa *et al.,* 2009), *Curcuma amada* Roxb. (Raju *et al.,* 2013), *Clivia miniata* Regel (Wang *et al.,* 2012a, 2012b), *Dorem ammoniacum* D. Don. (Irvani *et al.,* 2010), *Centella asiatica* L. (Martin, 2004b; Paramageetham *et al.,* 2004), *Foeniculum vulgare* Miller (Maatar and Hunault, 1997), *Eriophorum vaginatum* L. (Rewers *et al.,* 2012), and *Aloe vera* L. (Rathore *et al.,* 2011).

Table 8: Effect of various concentrations of BAP and 2,4-D on callus induction from petiole explants of arracacha

Treatment	**2,4-D ($mg.l^{-1}$)**	**BAP ($mg.l^{-1}$)**	**callus induction frequency (%)***
A		1	0
B		2	0
C	0.1		72.7
D	1		63.6
E	1	0.5	81.8
F	1	1	100
G	1	3	81.8
H	1	5	81.8
* frequency of callus induction was recorded after 4 weeks of culture			

In this thesis, calli on petioles on the induction medium supplemented with 1 $mg.l^{-1}$ of 2,4-D were initiatied from 63.6%. These results correspond with study of Slíva *et al.* (2010), who reported as the best variant for induction of callogenesis from petiole explants in arracacha, medium supplemented with 1 $mg.l^{-1}$ 2,4-D, in which 70% of explants produced callus. Das *et al.* (2013) studied the effect of various concentrations of auxins 2,4-D, IBA and NAA on induction of callus in Indian valerian (*Valeriana jatamansi* Jones.). They found out the best callus induction frequency of petiole explants in medium with addition of 2,4-D (0.5 $mg.l^{-1}$). At such a concentration 64.1% of explants involved in their experiments proliferated callus. As the second best result of callus induction frequency from petiole explants (62.7%), the variant of medium

supplemented with 1 mg.l^{-1} 2,4-D was recorded. Auxin 2,4-D was considered to be highly effective also in *Pennisetum glaucum* (L.) R. Br. (Jha *et al.,* 2009).

Percentage of callus formation depends on the following factors: explant source (Tawfik and Noga, 2002; Dave and Batra, 1995; Irvani *et al.,* 2010; Jha *et al.,* 2009; Kallak *et al.,* 1997), types and concentrations of cytokinins and auxins used (Anzidei *et al.,* 2000; Tawfik and Noga, 2002; Irvani *et al.,* 2010; Kallak *et al.,* 1997; Martin, 2004b), and plant genotype (Anzidei *et al.,* 2000; Bairu *et al.,* 2008; Jha *et al.,* 2009; Kallak *et al.,* 1997; Pourhosseini *et al.,* 2013).

All calli on every initiation medium had a tendency to brown after 3 weeks of culture. These calli were not removed. Despite browning they showed to be viable and capable of growth and shoot initiation. The browning of the culture that was also observed in other species from family *Apiaceae* (Irvani *et al.,* 2010; Makunga *et al.,* 2003; Martin, 2004a) may occur due to the activation of secondary metabolite synthesis (Irvani *et al.,* 2010; Martin, 2004a). Chand *et al.* (1997) prevented the browning of the culture by the shortening of the interval between subcultures into 15-17 days. In this study callus seemed to be active and proliferating even when left without subculturing for more than 70 days. This corresponds with study of Martin (2004a) who observed the growth of callus of medicinal plant *Eryngium foetidum* L. (*Apiaceae*) even when it was maintained 80 days without subculturing.

5.2.2. Regeneration of *in vitro* plantlets

Plantlets started to regenerate from callus when replaced on media without PGRs. If the callus was subcultured on the medium with the same plant growth regulators, organized tissue also appeared, nevertheless in lower frequency. Medium supplemented with the lowest concentration of 2,4-D (0.1 mg.l^{-1}) in comparison with other induction media showed the earliest regeneration of plantlets. Callus replaced from this induction medium into medium without PGRs started to regenerate shoots after 60 days of age, which was the first regeneration response in cultures. Moreover, it provided the greatest quantity of shoots and thus it proved to be the most effective. The difference compared to other treatments was statistically significant (Table 9). Satisfactory response was observed also on medium supplemented with 2,4-D at concentration 1 mg.l^{-1}. Callus on this medium started to differentiate onto organized tissue at 80 days of culture, but regeneration of plantlets was in comparison with the treatment C (0.1 mg.l^{-1} 2,4-D) lower. The treatments of media combining concentrations of BAP and 2,4-D proved to be also capable of plantlets regeneration from callus, nevertheless organogenesis occured later and in lower frequency than in variants with 2,4-D alone. The fact that the regeneration of plants was

obtained with inclusion of a single plant growth regulator does not correspod with study of Makunga *et al.* (2003) regarding micropropagation of *Thapsia garganica* L., who mentioned that the influence of one PGR could not support further development of plantlets. This hypothesis is also confirmed by study of Pretto and Santarém (2000), in which cultivation of explants in the presence of 2,4-D did not result in callogenesis of *Hypericum perforatum* L. leaf explants. Nevertheless, preliminary paper of Slíva *et al.* (2010) and present study confirm the fact, that indirect organogenesis in presence of 2,4-D alone is possible. For the count of regenerated plantlets from calli of various induction media see Table 9.

Table 9: Effect of various concentrations of 2,4-D and BAP on shoot regeneration from petiole-derived calli of arracacha

Treatment	PGRs used in callus initiation phase		Average number of plants per callus (mean ± S.E.)*
	2,4-D (mg.l^{-1})	BAP (mg.l^{-1})	
C	0.1		14.13 ± 1.90d
D	1		11.22 ± 1.47c
E	1	0.5	7.11 ± 1.52ab
F	1	1	6.64 ± 1.23a
G	1	3	9.11 ± 1.2bc
H	1	5	7.43 ± 1.59ab
* Number of plants regenerated from the callus was recorded after 90 days of culture ** In the same column, numbers followed by the same letter are not significantly different (Tukey test, $p < 0.05$)			

To date, many studies regarding micropropagation of valuable taxa from family *Apiaceae* are available (i.e. Anzidei *et al.,* 2000; Tawfik and Noga, 2002; Fiore *et al.,* 2012; Grigoriadou and Maloupa, 2008; Hedrawati *et al.,* 2012; Irvani *et al.,* 2010; Makunga *et al.,* 2003; Paramageetham *et al.,* 2004). Nevertheless, systems of tissue-culture regeneration optimized for various species and genera from the *Apiaceae* family may not be applicable for all members of this group. This study regarding assessment of genetic stability of *in vitro* propagated arracacha follows the study of Slíva *et al.* (2010).

It is of essential importance that genotypes of plants propagated with help of *in vitro* culture remain stable and the most crucial requirement in such cultures is to retain genetic uniformity with respect to original plant genotype (Fiore *et al.,* 2012; Makunga *et al.,* 2003). Numerous reports describing the lack of genotypic and phenotypic uniformity among callus-

derived plants exist (Aversano *et al.,* 2011; Gao *et al.,* 2010; Gesteira *et al.,* 2002; Linacero *et al.,* 2011; Pontaroli and Camadro, 2005; Rathore *et al.,* 2011; Sharma *et al.,* 2007).

The process of *in vitro* gereneration of arracacha plants via indirect morphogenesis is provided on Figure 9.

The occurence of somaclonal variability was verified in following arracacha regenerants: 2 accessions regenerated from callus induction medium supplemented with 0.1 $mg.l^{-1}$ 2,4-D, 1 plant regenerated from callus cultivated on induction medium containing 3 $mg.l^{-1}$ and 1 $mg.l^{-1}$ of BAP and 2,4-D respectively and 4 regenerants obtained from induction medium supplemented with 5 $mg.l^{-1}$ and 1 $mg.l^{-1}$ of BAP and 2,4-D, respectively. Such a small number of regenerants was verified due to limitation of time available for the experiment. Results of genetic analysis of regenerants using molecular markers are provided in chapter 5.4.2..

Figure 9: Regeneration of arracacha plantlets through intermediate callus phase. A- petiole-derived callus (60 days of culture), B - proliferating callus on medium lacking PGRs subcultured from induction medium supplemented with 0.1 mg.l-1 of 2,4-D, C - proliferating callus divided into smaller parts, D - mature arracacha plantlets (photo: author)

5.3. DNA extraction

There were no problems with DNA extraction from plant material using the Invisorb® Spin Plant Mini Kit. Despite the small amount of plant material, DNA of satisfactory concentration was extracted. The DNA was electrophoretically separated on an agarose gel to test its capability for using in further molecular analysis. All DNA samples showed to be suitable for further analysis (Figure 10)

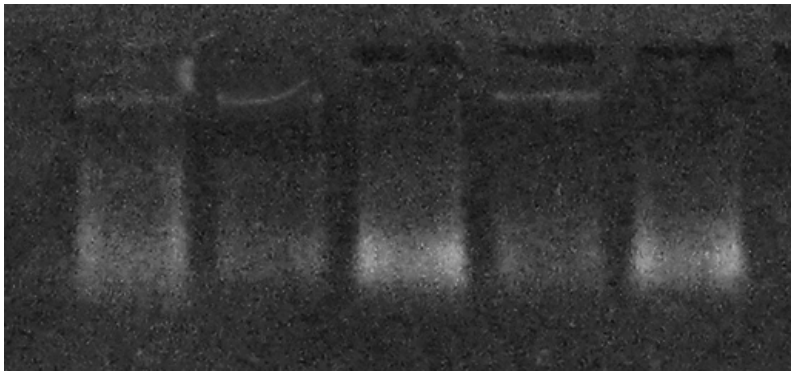

Figure 10: DNA samples screened on agarose gel to verify suitability of DNA for further molecular analysis (photo: author)

In various apioid species DNA extraction was mostly done with help of Cetyl trimethylammonium bromide - CTAB (Bahmani *et al.,* 2012; Dogan *et al.,* 2010, Fiore *et al.,* 2012). This method of DNA extraction is popular also in other species out of *Apiaceae* family (Bhatia *et al.,* 2011; Cuesta *et al.,* 2010; Gao *et al.,* 2010; Gesteira *et al.,* 2002; He *et al.,* 2011; Khoddamzadeh *et al.,* 2010; Kumar *et al.,* 2011; Mansilla *et al.,* 2006; Milella *et al.,* 2005; Milella *et al.,* 2011; Ortega *et al.,* 2007; Pissard *et al.,* 2006, 2008a, 2008b). The popularity of this method is probably due to the cost, which is in comparison with commercial kits relatively low. Moreover this method can be modified considering the purpose for which DNA is extracted. On the contrary, this method is more time consuming than commercial kits (Čurn *et al.,* 2008). The same method of DNA extraction was used in study of Blas *et al.* 2008 regarding diversity of wild arracacha species using AFLP markers.

Despite the relatively high cost per sample in extraction methods using commercial kits, the DNA samples obtained from these methods are standard, reproducible, repeatable and easy to make (Čurn *et al.,* 2008). These methods were used for DNA extraction in many species throughout the plant kingdom (Aversano *et al.,* 2011; Bennici *et al.,* 2004; Emshwiller *et al.,* 2009; Fernández *et al.,* 2002; Khatri *et al.,* 2011; Lata *et al.,* 2010; Linacero *et al.,* 2011).

5.4. ISSR analysis

5.4.1. Verification of genetic stability in plants propagated via direct organogenesis

First step of ISSR analysis involved the verification of genetic stability in plants propagated via direct organogenesis in order to exclude occurence of somaclonal variation before beginning of the indirect morphogenesis experiment.

The amplified DNA products were screened in three plants obtained via direct organogenesis and compared with DNA template of control plant. A total of 10 ISSR primers were screened, out of which 9 ISSR primers produced a total of 216 clear distinct and reproducible bands (Table 10). The amplified products were monomorphic throughout all the selected multiplicated plants and were similar to the control plant (Figure 11). The multiplication protocol adopted from Slíva *et al.* (2010) proved to be suitable for plants micropropagation and thus enough of genetically stable plant material was obtained for further experiments.

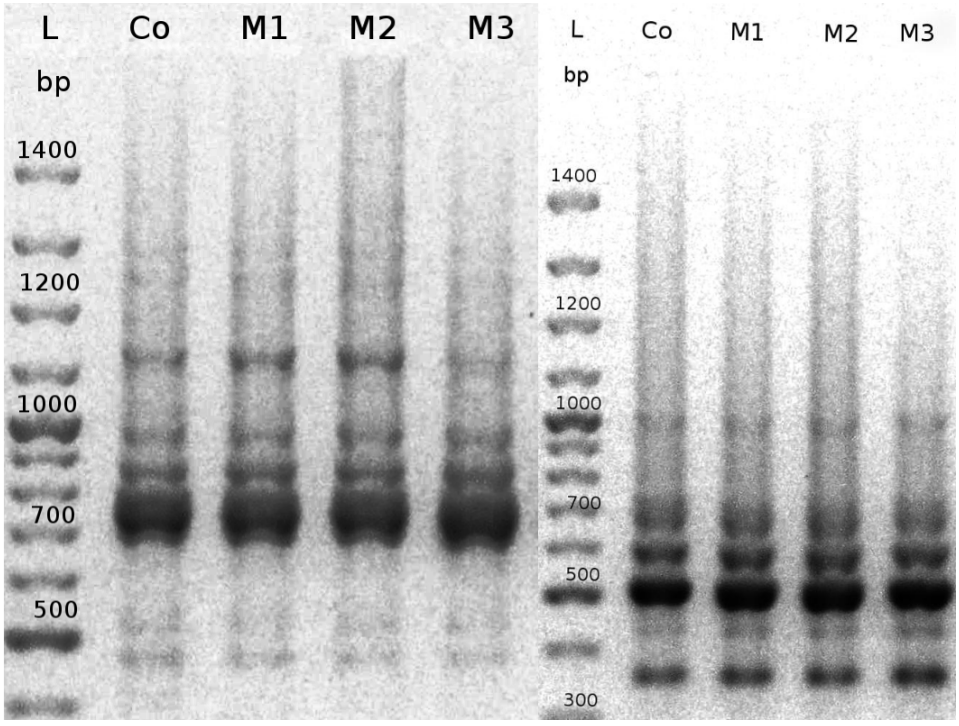

Figure 11: Examples of monomorphic banding patterns of primers 'UBC 836' (on the left) and 'UBC 812' (on the right) screened to verify stability of plantlets multiplicated through direct organogenesis. bp: base pairs; L: 100bp DNA ladder; Co: control; M1-M3: plants multiplicated through direct organogenesis (photo: author)

Table 10: Scorable ISSR bands amplified by each of 9 selected primers screened to verify stability of plants multiplicated via direct organogenesis

Primers code	Primer sequence (5'-3')	Total number of bands amplified	Number of scorable bands per primer (total/ polymorphic)	Range of amplification (pb)	Approximate size of each band (bp)
'UBC810'	$(GA)_8T$	32	8/0	350-1200	350, 580, 780, 900,950, 1000, 1160, 1210
'UBC812'	$(GA)_8A$	24	6/0	360-1000	360, 440, 500, 600, 700, 1000
'UBC823'	$(TC)_8C$	16	4/0	290-580	290, 450, 500, 550
'UBC834'	$(AG)_8YT$	48	12/0	200-1280	200, 280, 420, 460, 480, 540, 700, 750, 820, 1000, 1100, 1280
'UBC836'	$(AG)_8YA$	24	6/0	480-1120	480, 520, 700, 850, 950, 1140
'UBC841'	$(GA)_8YC$	28	7/0	200-1270	200, 280, 500, 620, 720, 1000, 1270
'UBC843'	$(CT)_8RA$	8	2/0	1020-1200	1020, 1120
'UBC844'	$(CT)_8RC$	24	6/0	250-600	250, 300, 420, 500, 600, 800
'UBC845'	$(CT)_8RG$	12	3/0	320-580	320, 500, 580
Total		216	-		-

In literature, genetic stability/instability of plants propagated via direct organogenesis is assessed mainly by combination of two molecular marker techniques - ISSR and RAPD. Genetic fidelity of plants propagated via direct organogenesis have been proved using these two markers in following plant species: *Gerbera jamesonii* Bolus (Bhatia *et al.,* 2011), *Simmondsia chinensis* (Link) Schneider (Kumar *et al.,* 2011), *Olea europaea* L. (Leva and Petruccelli, 2012), *Prunus dulcis* (Mill.) D. A. Webb. (Martins *et al.,* 2004), *Guadua angustifolia* Kunth (Nadha *et al.,* 2011), *Withania somnifera* (L.) Dunal (Nayak *et al.,* 2013), *Aloe vera* L. (Rathore *et al.,* 2011), *Cordyline terminalis* (L) Kunth. (Ray *et al.,* 2006), *Pongamia pinnata* (L.) Pierre (Kesari *et al.,* 2012), *Zingiber rubens* Roxb (Mohanty *et al.,* 2011a), *Aerides vandarum* Reichb.f x *Vanda stangeana* Reichb.f (Kishor and Devi, 2009), *Eucalyptus tereticornis* Sm. (Aggarwal *et al.,* 2010), *Kaempferia galanga* L. (Mohanty *et al.,* 2011b), *Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne (Singh *et al.,* 2013). Nevertheless, some studies proving the genetic fidelity of plants propagated directly without intermediate callus phase using ISSR markers alone are also available (Lata *et al.,* 2010; Negi and Saxena, 2011; Piña-Escutia *et al.,* 2010; Gangopadhyay *et al.,* 2010; Bopana and Saxena, 2009).

Although the genetic fidelity has been proved in most of species propagated by this way it is not a rule and some studies revealing somaclonal variability in plants propagated through direct organogenesis are also available (Bairu *et al.*, 2006; Chandrika *et al.,* 2008; Cuesta *et al.,* 2010; Guo *et al.,* 2006a; He *et al.,* 2011; Sharma *et al.,* 2011). Somaclonal variability obtained with multiplication of plants through direct organogenesis can be influenced by rate of multiplication (the higher the rate of multiplication the higher the somaclonal variation), number of subcultures (age of culture) (Bairu *et al.,* 2006; Skirvin *et al.,* 1994), plant genotype (Sharma *et al.,* 2011; Skirvin *et al.,* 1994), concentrations of PGRs (Bairu *et al.,* 2006; Sharma *et al.,* 2011; Skirvin *et al.,* 1994) and the number of initial explants, which means that with a large number of multiplication cycles, if the number of initial explants is small, the final rate of variants in the population will be very high (Bairu *et al.,* 2006; Skirvin *et al.,* 1994).

5.4.2. Assessment of genetic variability in regenerants obtained via indirect morphogenesis

In this thesis amplified products obtained by amplification of DNA extracted from 7 plants regenerated from calli cultivated on 3 different induction media (C - MS medium supplemented with 0.1 $mg.l^{-1}$ 2,4-D; G - MS medium containing 3 $mg.l^{-1}$ BAP, 1 $mg.l^{-1}$ 2,4-D; H - MS medium supplemented with 5 $mg.l^{-1}$ BAP and 1 $mg.l^{-1}$ 2,4-D) and the donor DNA template were screened with aim to detect the genetic variability/stability in plants propagated via intermediate callus phase. Out of 10 ISSR primers screened, 9 generated 418 amplified fragments of which 6 (1.4%) were polymorphic (Table 11). The average number of bands generated by each primer was 6 (range from 2 to 13) and the size of fragments ranged from 200 to 1,280 bp. The highest number of bands (13) was generated by primer 'UBC834', the lowest (2) by primer 'UBC843'. In four of 9 ISSR primers ('UBC 812', 'UBC 823', 'UBC 844', 'UBC 845') monomorphic profiles were detected (Figure 12).

Table 11: Total number of amplified bands and number of polymorphic fragments generated by PCR using 9 selected ISSR primers in 7 plants regenerated from 3 types of callus-induction media and 1 control plant.

Primer code	Total number of bands amplified	Number of scorable bands per primer	No. and frequency of polymorphic bands per primer	Polymorphic regenerants	Loss of original band	Novel band	No. singleton	Range of amplification (pb)
'UBC810'	61	8	2 (25%)	C1, G1, H4	2		1	350-1,200
'UBC812'	48	6	0					360-1,000
'UBC823'	32	4	0					290-580
'UBC834'	96	13	2 (15.4%)	C2, G1	1	1	2	200-1,280
'UBC836'	48	6	0					480-1,120
'UBC841'	48	6	2 (33.3%)	G1	1	1	2	200-1,270
'UBC843'	16	2	0					1,020-1,120
'UBC844'	48	6	0					250-800
'UBC845'	21	3	0					320-580
Total	418	54	6 (11.1%)	-	4	2	5	

Three out of 9 ISSR primers (33.3%) revealed some polymorphism among regenerated plants and control plant. The highest frequency of polymorphism (33.3%) was detected by primer 'UBC841', however this variation occured in one regenerant only. Primer 'UBC810' showed to be the most efficient in revealing of polymorphism among plants. Using this primer polymorphism was revealed in all types of regenerants (Figure 13). Also in other studies regarding assessment of somaclonal variation obtained via *in vitro* culture this primer proved to be effective in polymorphism revelation (Bahmani *et al.,* 2012;Guo *et al.,* 2006b; Khatri *et al.,* 2011; Yuan *et al.,* 2009). On the contrary Bortlová (2012) screened the set of 4 ISSR primers in order to detect genetic stability/instability of *Smallanthus sonchifolius* plantlets obtained via indirect embryogenesis. She detected no polymorphic bands with primer 'UBC810'. Nevertheless the two other primers ('UBC834' and 'UBC841') generated some polymorphic bands as in the present study.

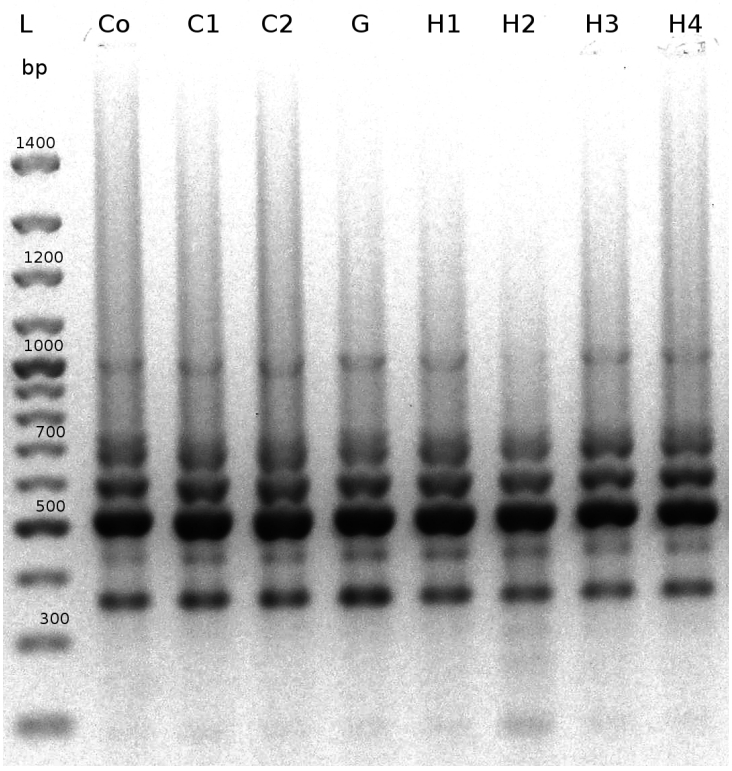

Figure 12: Example of monomorphic pattern screened in primer 'UBC812'. bp: base pairs; L: 100bp DNA ladder; Co: control; C1-H4 regenerants (photo: author)

Polymorphism was detected in regenerants from all types of media used for induction of callus. The distribution of variation among the regenerated plants was uneven and only 1 regenerated plant (from callus cultivated on the induction medium supplemented with 3 $mg.l^{-1}$ BAP and 1 $mg.l^{-1}$ 2,4-D) accumulated 57.1% of the variation observed. The rest of plants (2 regenerated from the callus cultivated on media supplemented with 0.1 $mg.l^{-1}$ 2,4-D and 1 plant regenerated from the callus induced on media supplemented with 5 $mg.l^{-1}$ BAP and 1 $mg.l^{-1}$ 2,4-D) revealing some somaclonal variability were always variable in one single band, which represents 14.3% of the all variations detected. From the studied plants 57.1% revealed at least one variation when compared with control plant. The polymorphism included as the gain of a novel bands as the absence of some fragments and some of them were even singletons that is variant band present or absent in a single regenerant for a given primer (Gao *et al.,* 2010) (Table 12). The average proportion of shared ISSR fragments was higher than 97.7% among the regenerated plantlets and the control plant.

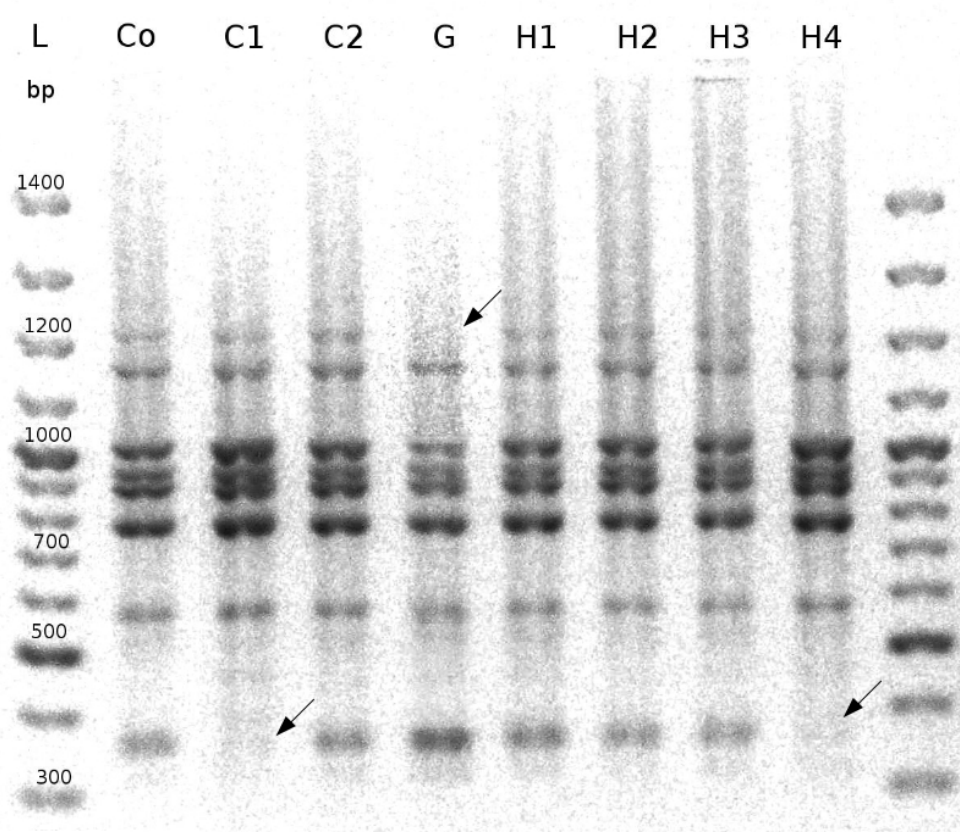

Figure 13: Polymorphic profile using primer 'UBC810', detecting polymorphism in all types of regenerants. Black arrows show loss of original bands; bp: base pairs; L: 100 bp ladder; Co: control plant; C1-H4: regenerants (photo: author)

Many studies regarding genetic stability of plants regenerated via callus confirming the actuality that plants originating from undifferentiated tissue (callus) are more prone to genomic alterations than plants regenerated directly without intermediate callus phase, are available (Bublyk *et al.,* 2012; Gao *et al.,* 2010; Guo *et al.,* 2006b; Hu *et al.,* 2011; Kuznetsova *et al.,* 2005, 2006; Linacero *et al.,* 2011; Peredo *et al.,* 2006; Polanco and Ruiz, 2002; Pontaroli and Camadro, 2005; Sharma *et al.,* 2007; Siragusa *et al.,* 2007; Wang *et al.,* 2012a; Yuan *et al.,* 2009). The occurrence and frequency of somaclonal variation in undifferentiated plant tissue cultures depends on following factors: plant genotype (Chawla, 2002; Kuznetsova *et al.,* 2006; Sharma *et al.,* 2011; Skirvin *et al.,* 2004), source of explant (Chawla, 2002; Cuesta *et al.,* 2010; Khatri *et al.,* 2011; Sharma *et al.,* 2007; Skirvin et al., 2004; Wang *et al.,* 2012b), duration of cell culture (Chawla, 2002; Kuznetsova *et al.,* 2005, 2006; Skirvin *et al.,* 2004; Wang *et al.,* 2012b) and conditions of culture cultivation (Chawla, 2002; Skirvin *et al.,* 2004; Siragusa *et al.,* 2007).

As the somaclonal variation of any kind is undesirable in a micropropagation programs (Bairu *et al.,* 2011; Karp, 1994; Sahijram *et al.,* 2003; Sharma *et al.,* 2007) it can on the other

hand serve as a valuable source of new desirable traits (Bairu *et al.,* 2011; Karp, 1994; Sahijram *et al.,* 2003). Somaclonal variation is promissing source of desirable traits mainly in plant species with limited breeding approaches, such as assexually propagated species have (Karp, 1994). As a greatest contribution of somaclonal variation towards plant improvement development of disease resistant crops is considered (Chawla, 2002; Sahijram *et al.,* 2003). Moreover it can be utilized in breeding programes involving selection of insect, herbicid and abiotic stress resistant plants and improvement of quality or quantity of yield. Nevertheless there are many issues associated with application of somaclonal variation, such as uncontrollable and unpredictable nature of variations, which can be moreover without apparent use (Chawla, 2002).

ISSR proved to be an efficient method for identification of somaclonal variation in *Arracacia xanthorrhiza* plants regenerated from calli cultivated on various induction media.

The ISSR marker has been previously successfully employed to measure genetic stability/instability in many species accross the plant kingdom. At least small variability of plants propagated via intermediate callus phase have been proved using this marker in following plant species: *Codonopsis lanceolata* Benth. et Hook. f. (Guo *et al.,* 2006b), *Amorphophallus rivieri* Durieu (Hu *et al.,* 2011), *Secale cereale* L. (Linacero *et al.,* 2011), *Aloe vera* L. (Rathore *et al.,* 2011), *Lycopersicon hirsutum* f. *typicum* and *Lycopersicon chilense* Dunal. (Rzepka-Plevneš *et al.,* 2010), *Citrus madurensis* Lour. (Siragusa *et al.,* 2007), *Camellia sinensis* (L.) O Kuntze (Thomas *et al.,* 2006), *Cymbopogon pendulus* (Nees ex Steud.) Will. Watson (Bhattacharya *et al.,* 2010), *Gentiana straminea* Maxim (He *et al.,* 2011), *Hydrangea macrophylla* (Thunb.) Ser. (Liu *et al.,* 2011), *Clivia miniata* Regel (Wang *et al.,* 2012a). Genetic stability in such propagated plants using ISSR technique was revealed in *Habenaria edgeworthii* Hook. f. ex. Collett (Giri *et al.,* 2012), *Rhinacanthus nasutus* (L.) Kurz. (Cheruvathur *et al.,* 2012) and *Psidium guajava* L. (Rai *et al.,* 2012).

Leroy *et al.* (2012) assessed the suitability of ISSR markers to detect genetic variations induced by tissue culture. Their results proved that ISSR marker is a fast and efficient technique in the evaluation of somaclonal variations. ISSRs require small amount of DNA, generate a high number of markers and are highly reproducible, which make them a candidate of choice in the study of plant genome stability/variability (Leroy *et al.,* 2012). Moreover, ISSR markers offer other advantages in the detection of the somaclonal variation notably, a high degree of sensitivity, reproducibility, and the dominant representation of polymorphic genetic alleles (Huang *et al.,* 2009). McGregor *et al.* (2000) tested the value of the RAPD, SSR, AFLP and ISSR in ability to distinguish potato cultivars. Their results confirmed that the problems experienced with reproducibility plus the lower informativeness compared to the multi-locus

SSR and AFLP systems limits the use of RAPD and ISSR markers in DNA fingerprinting. Nevertheless they will remain useful where costs exclude the use of AFLPs and SSRs (McGregor *et al.,* 2000).

6. Conclusion

The plants of *Arracacia xanthorrhiza* were successfully multiplicated and callus was initiated from the petiole segments on the MS media containing various concentrations of BAP and 2,4-D. On media supplemented with BAP alone, no callogenic responses were observed. Nevertheless, on other media supplemented with combinations of BAP and 2,4-D or 2,4-D alone, callus occured.

From the calli cultivated on various induction media plants were regenerated. MS medium supplemented with 0,1 $mg.l^{-1}$ 2,4-D proved to be the best for plantlets regeneration from the callus. Plantlets started to proliferate when replaced on medium free of PGRs. Regenerants were obtained from calli cultivated on all 6 types of callus-forming induction media (C-H).

Genetic stability was verified in 3 plants multiplicated via direct organogenesis in order to exclude occurrence of somaclonal variation before beginning of the experiment. The amplified products from 9 ISSR primers were monomorphic accross the multiplicated plants and were identical to the control plant.

Somaclonal variation was assessed in plants obtained via indirect morphogenesis. Amplified products obtained by amplification of DNA extracted from 7 plants regenerated from calli cultivated on 3 different induction media (C, G, H) and the donor DNA template were screened. Out of 10 ISSR primers screened, 9 generated 418 amplified fragments of which 6 (1.4%) were polymorphic. Polymorphism was detected in all regenerants regardless the type of media used for induction of callus. Four from 7 analyzed regenerants were variable at least in one band.

Propagation via intermediate callus phase induces in arracacha somaclonal variation, thus the propagation directly without intermediate callus phase is recommended when is necessary to obtain true-to-type plants. Nevertheless, the somaclonal variability in plants regenerated through indirect morphogenesis can be used in plant breeding to obtain new genotypes with valuable qualitative or quantitative traits. Especially in arracacha, where reproductive capacity is reduced due to rare flowering and low production of seeds, classical breeding methods may be replaced by biotechnological techniques, such as induction of somaclonal variation.

7. References

Aggarwal D, Kumar A, Reddy MS. 2010. *Agrobacterium tumefaciens* mediated genetic transformation of selected elite clone(s) of *Eucalyptus tereticornis.* Acta Physiologiae Plantarum, 33: 1603-1611.

Alves JA, Vilas Boas EVB, Vilas Boas BM, Souza, EC. 2010. Qualidade de produto minimamente processado à base de abóbora, cenoura, chuchu e mandioquinha-salsa. Ciência e Tecnologia de Alimentos, 30(3): 625-634.

Anzidei M, Bennici A, Schiff S, Tani C, Mori B. 2000. Organogenesis and somatic embryogenesis in *Foeniculum vulgare*: histological observations of developing embryogenic callus. Plant Cell, Tissue and Organ Culture, 61: 69-79.

Arbizu C. 2009. *Ex situ* conservation of underutilized Andean roots and tubers. Oral presentation. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources, Conservation and Utilization, Lima, pp. 31-37.

Arbizu C, Huamán Z, Golmirzaie A. 1997. Other Andean Roots and Tubers. In: Fucillo D, Sears L, Stapleton P (eds.). Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centres. New York. Cambridge University Press, pp. 39-56.

Ashmore SE. 1998. The Status of *in vitro* conservation of tropical and subtropical species. Acta Horticulturae, 461: 477–483.

Asthana P, Jaiswal VS, Jaiswal U. 2011. Micropropagation of *Sapindus trifoliatus* L. and assessment of genetic fidelity of micropropagated plants using RAPD analysis. Acta Physiologiae Plantarum, 33: 1821-1829.

Aversano R, Dato FD, Matteo AD, Frusciante L, Carputo D. 2011. AFLP analysis to assess genomic stability in *Solanum* regenerants derived from wild and cultivated species. Plant Biotechnology Reports, 5: 265-271.

Bahmani K, Azadi-Barbandi A, Jafari AA, Noori SAS, Farajpour M. 2012. Assessment of Genetic Diversity in Iranian Fennels Using ISSR Markers. Journal of Agricultural Science, 4(9): 79-84.

Bairu MW, Fennell FW, van Staden J. 2006. The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. '*Zelig*'). Scientia Horticulturae, 108: 347-351.

Bairu MW, Stirk WA, Doležal K, van Staden J. 2008. The role of topolins in micropropagation and somaclonal variation of banana cultivars '*Williams*' and '*Grand Naine*' (*Musa* spp. AAA). Plant Cell, Tissue and Organ Culture, 95: 373-379.

Bairu MW, Aremu AO, van Staden J. 2011. Somaclonal variation in plants: causes and detection methods. Plant Growth Regulation, 63: 147-173.

Bais HP, Vepachedu R, Vivanco JM. 2003. Root specific elicitation and exudation of fluorescent β-carbolines in transformed root cultures of *Oxalis tuberosa*. Plant Physiology and Biochemistry, 41: 345-353.

Barrera V, Espinosa P, Tapia C, Monteros A, Valverde F. 2004. Caracterización de las raíces y los tubérculos andinos en la ecoregión andina del Ecuador. In: Barrera V, Tapia C, Monteros A (eds.). Raíces y Tubérculos Andinos: Alternativas para la conservación y uso sostenible en el Ecuador. Quito. Lima. Agencia Suiza para el Desarrollo y la Cooperación, p. 3 - 30.

Barrella TP, Puiatti M, Santos RHS, Cecon PR. 2011. Production and mass partitioning in Peruvian carrot plants grown under artificial shading period and intensity, Acta Scientiarum. Agronomy, 33(2): 321-326.

Bennici A, Anzidei M, Vendramin GG. 2004. Genetic stability and uniformity of *Foeniculum vulgare* Mill. regenerated plants through organogenesis and somatic embryogenesis. Plant Science 166: 221-227.

Bhattacharya S, Bandopadhyay TK, Ghosh PD. 2010. Somatic embryogenesis in *Cymbopogon pendulus* and evaluation of clonal fidelity of regenerants using ISSR marker. Scientia Horticulturae, 123: 505-513.

Bhatia R, Singh KP, Sharma TR, Jhang T. 2011. Evaluation of the genetic fidelity of *in vitro*-propagated gerbera (*Gerbera jamesonii* Bolus) using DNA-based markers. Plant Cell, Tissue and Organ Culture, 104: 131-135.

Biondi J, Zorilla C, Manrique I, Arbizu C, Roca W, Medina T, Seminario J, Quispe J, Tay D, Blas R. 2009a. Genetic diversity of arracacha (*Arracacia xanthorrhiza*) in Peru. Poster. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources Conservation and Utilization. Lima, pp. 60-66.

Biondi J, Rossel G, Zorilla C, Arbizu C, Roca W. 2009b. Molecular characterization of the *Oxalis tuberosa* Mol. collection maintained in the CIP's genebank. Poster. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources, Conservation and Utilization. Lima, pp. 50-54.

Blas R, Arbizu C. 1995. In: Hermann M. 1997. Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Hermann M, Heller J (eds.). Andean roots and tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. Rome. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, pp. 75-172.

Blas R, Arbizu C, Ghislain M, Herrera R. 1997. In: Hermann M. 1997. Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Hermann M, Heller J (eds.). Andean roots and tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. Rome. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, pp. 75-172.

Blas R, Arbizu C, Ghislain M, Herrera R, Rodrigues GS. 2006. Identificación de los morfotipos de arracacha (*Arracacia xanthorrhiza* Bancroft) Peruana. Anales científicos de la Universidad Nacional Agraria La Molina, 66: 126-139.

Blas R, Ghislain M, Herrera MR, Baudoin J-P. 2008. Genetic diversity analysis of wild *Arracacia* species according to morphological and molecular markers. Genetic Resources and Crop Evolution, 55: 625-642.

Bopana N, Saxena S. 2009. *In vitro* regeneration of clonally uniform plants of *Crataeva magna*: a high value medicinal tree by axillary branching method. New Forests, 38: 53-65.

Bortlová Z. 2012. Induction of somaclones in yacon *Smallanthus sonchifolius* and detection of their variability using molecular markers. Diploma thesis, Czech University of Life Sciences Prague. Prague.

Bublyk OM, Andreev IO, Spiridonova KV, Kunakh VA. 2012. Genetic variability in regenerated plants of *Ungernia victoris*. Biologia Plantarum, 56(2): 395-400.

CGIAR. 1996. Report on the Inter-Centre Review of Root and Tuber Crops Research in the CGIAR. Consultative Group on International Agricultural Research, Food and Agriculture Organization of the United Nations, p. 56 plus appendices.

Chand S, Sahrawat AK, Prakash DVSSR. 1997. *In Vitro* Culture of *Pimpinella anisum* L. (anise). Journal of Plant Biochemistry and Biotechnology. 6(2): 91-95.

Chandrika M, Thoyajaksha, Ravishankar RV, Ramachandra KK. 2008. Assessment of genetic stability of *in vitro* grown *Dictyospermum ovalifolium*. Biologia Plantarum, 52(4): 735-739.

Chawla HS. 2002. Introduction to Plant Biotechnology. Enfield. Science Publishers, p. 538.

Cheruvathur MK, Abraham J, Thomas TD. 2012. Plant Regeneration Through Callus Organogenesis and True-to-Type Conformity of Plants by RAPD Analysis in *Desmodium gangeticum* (Linn.) DC.

Chia JAW, López CFB, Blas RS, Seminario J, Mansilla R, Baudoin JP. 2006. Diversidad genética molecular de *Mirabilis expansa* mediante RAPD. Ecología Aplicada, 5(1,2): 81-86.

Constance L, Affolter J, 1995. *Arracacia ravenii* (Apiaceae), a New Species from Southern Mexico. Novon, 5(1): 22-23.

Corrêa CM, Oliveira GC, Astarita LV, Santarém ER. 2009. Plant Regeneration through Somatic Embryogenesis of Yacón [*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson]. Brazilian Archives of Biology and Technology, 52(3): 549-554.

Coulter JM, Rose JN. 1900. A Synopsis of Mexican and Central American *Umbelliferae* [online]. Proceedings of the Washington Academy of Science, 1: 111-159. Available at http://www.biodiversitylibrary.org/item/35744#page/135/mode/1up

Cuesta C, Ordás RJ, Rodrígues A, Fernández B. 2010. PCR-based molecular markers for assessment of somaclonal variation in *Pinus pinea* clones micropropagated *in vitro*. Biologia Plantarum, 54(3): 435-442.

Čurn V, Šimáčková K, Kubátová B, Nováková A. 2008. Metodika izolace DNA a analýzy molekulárních markerů pro účely popisu genových zdrojů a identifikace odrůd brambor (*Solanum tuberosum* L.). Jihočeská univerzita v Českých Budějovicích. Zemědělská fakulta. České Budějovice, pp 56.

Darlington CD, Wilie AP. 1955. In: Hermann M. 1997. Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Hermann M, Heller J (eds.). Andean roots and tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. Rome. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, pp. 75-172.

Das A, Kesari V, Rangan L. 2010. Plant regeneration in *Curcuma* species and assessment of genetic stability of regenerated plants. Biologia Plantarum, 54(3): 423-429.

Das, J, Mao AA, Handique PJ. 2013. Callus-mediated organogenesis and effect of growth regulators on production of different valepotriates in Indian valerian (*Valeriana jatamansi* Jones.). Acta Physiologiae Plantarum, 35: 55-63.

Dave A, Batra A. 1995. Somatic tissues leading to embryogenesis in cumin. Current Science, 68(7): 754-755.

Degtjareva GV, Logacheva MD, Samigullin TH, Terentieva EI, Valiejo-Roman CM. 2012. Organization of Chloroplast *psb*A *trn*H Intergenic Spacer in Dicotyledonous Angiosperms of the Family *Umbelliferae*. Biochemistry, 77(9): 1056-1064.

Dogan B, Duran A, Bagci Y, Dinc M, Martin E, Cetin O, Ozturk M. 2010. Phylogenetic relationships among the taxa of the genus *Johrenia* DC. (*Apiaceae*) from Turkey based on molecular method. Bangladesh Journal of Plant Taxonomy, 17(2): 113-120.

Emshwiller E. 2002. Ploidy Levels among Species in the '*Oxalis tuberosa* Aliance' as Inferred by Flow Cytometry. Annals of Botany, 89: 741-753.

Emshwiller E, Theim T, Grau A, Nina V, Terrazas V. 2009. Origins of domestication and polyploidy in oca (*Oxalis tuberosa*; Oxalidaceae): 3. AFLP data of oca and four wild, tuber-bearing taxa. American Journal of Botany, 96(10): 1839–1848.

Evergetis E, Michaelakis A, Haroutounian SA. 2013. Exploitation of *Apiaceae* family essential oils as potent biopesticides and rich source of phellandrenes. Industrial Crops and Products, 41: 365–370.

FAO. 2005. Status of research and application of crop biotechnologies in developing countries. Rome. Food and Agriculture Organization of the United Nations, p. 60.

Favoretto P, Veasey EA, Tavares de Melo PC. 2009. Molecular characterization of potato cultivars using SSR markers. Poster. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources, Conservation and Utilization, Lima, pp. 85-91.

Fernández ME, Figueiras AM, Benito C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theoretical and Applied Genetics, 104: 845-851.

Fernández CE, Viehmannová I, Milella L, Robles CD. 2007. Micropropagación del yacón [*Smallanthus sonchifolius* (Poepp. et Endl.) H.Robinson] a partir de segmentos nodales. Congreso Internacional de Biotecnología. Quevedo-Los Ríos. Universidad Técnica Estatal de Quevedo, Unidad de Investigación Científica y Tecnología, pp. 93-99.

Fiore MC, Carimi F, Carra A, Sunseri F. 2012. Efficient plant regeneration via somatic embryogenesis in bulbing fennel using immature flower explants. *In Vitro* Cellular & Developmental Biology - Plant, 48: 440-445.

Fletcher PJ, Fletcher JD. 2001. *In vitro* virus elimination in three Andean root crops: Oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*), and arracacha (*Arracacia xanthorrhiza*). New Zealand Journal of Crop and Horticultural Science, 29(1): 23-27.

Flores HE, Walker TS, Guimarães RJ, Bais HP, Vivanco JM. 2003. Andean Root and Tuber Crops: Underground Rainbows. HortScience, 38(2): 161-167.

Gamborg OL, Miller RA, Ojima O. 1968. Nutrient requirements of suspension cultures of soybean root cell. Experimental Cell Research, 50: 151-158.

Gangopadhyay M, Chakraborty D, Dewanjee E, Bhattacharya S. 2010. Clonal propagation of *Zephyranthes grandiflora* using bulbs as explants. Biologia Plantarum, 54(4): 793-797.

Gao X, Yang D, Cao D, Ao M, Sui X, Wang Q, Kimatu JN, Wang L. 2010. *In Vitro* Micropropagation of *Freesia hybrida* and the Assessment of Genetic and Epigenetic Stability in Regenerated Plantlets. Journal of Plant Growth Regulation, 29: 257-267.

García A, Pacheco-Delahaye E, Tovar J, Pérez E. 2007. Caracterización fisicoquímica y funcional de las harrinas de arracacha (*Arracacia xanthorrhiza*) para sopas instáneas. Ciencia Y Tecnologia Alimentaria., 5(5): 384-393.

Gesteira AS, Otoni WC, Barros EG, Moreira MA. 2002. RAPD-based detection of genomic instability in soybean plants derived from somatic embryogenesis. Plant Breeding, 121: 269-271.

Giri L, Jugran A, Rawat S, Dhyani P, Andola H, Bhatt ID, Rawal RS, Dhar U. 2012. *In vitro* propagation, genetic and phytochemical assessment of *Habenaria edgeworthii*: an important Astavarga plant. Acta Physiologiae Plantarum, 34: 869-875.

Golmirzaie AM, Salazar LF. 1995. Application of Biotechnology for Conservation and Utilisation of Andean Root and Tuber Crops and Biosafety Quidelines. African Crop Science Journal, 3(3): 341-344.

González-Benito ME, Mendoza-Condori VH, Molina-García AD. 2007. Cryopreservation of *in vitro* shoot apices of *Oxalis tuberosa* Mol.. CryoLetters, 28(1): 23-32.

Górecka K, Krzyżanowska D, Kowalska U, Kiszczak W, Górecki R. 2009. Yacon - *in vitro* propagation trials. Acta Biologica Cracoviensia Series Botanica, 51(1): 40-40.

Granados PC, Escalante ZB. 1997. Limpieza, manejo y conservación *in vitro* de germoplasma de olluco (*Ullucus tuberosus* Caldas), oca (*Oxalis tuberosa* Mol.), mashua (*Tropaeolum tuberosum* R. et P.) y papas nativas (*Solanum tuberosum* subsp. *andigena*). Cusco. IX. Congreso Internacional de Cultivos Andinos, p. 16.

Granate MJ, Silva DJH, Sinval WN, Pinto FSA, Sediyama MAN, Puiatti M. 2009. Competição de clones de mandioquinha-salsa em quatro épocas de colheita. Horticultura Brasileira, 27: 414-419.

Grigoriadou K, Maloupa E. 2008. Micropropagation and salt tolerance of *in vitro* grown *Crithmum maritimum* L.. Plant Cell, Tissue and Organ Culture, 94: 209-217.

Guo W, Li Y, Gong L, Li F, Dong Y, Liu B. 2006a. Efficient micropropagation of *Robinia ambigua* var. *idahoensis* (Idaho Locust) and detection of genomic variation by ISSR markers. Plant Cell, Tissue and Organ Culture, 84: 343-351.

Guo WL, Gong L, Ding ZF, Li YD, Li FX, Zhao SP, Liu B. 2006b. Genomic instability in phenotypically normal regenerants of medicinal plant *Codonopsis lanceolata* Benth. et Hook. f., as revealed by ISSR and RAPD markers. Plant Cell Reports, 25: 896-906.

Handro V, Mello CM, Manzano MA, Floh EIS. 1997. Enhancement of stem elongation and flower bud regeneration by salicylic acid. Revista Brasileira de Fisiologia Vegetal, 9(2): 139-142.

Hasan NA, Mummenhoff K, Quiros CF, Tay CD, Bailey CD. 2010. Polymorphic chloroplast microsatellite markers in the octoploid *Lepidium meyenii* (Brassicaceae) and cross-species amplification in *Lepidium*. American Journal of Botany, 97: e85-e88.

He T, Yang L, Zhao Z. 2011. Embryogenesis of *Gentiana straminea* and assessment of genetic stability of regenerated plants using inter simple sequence repeat (ISSR) marker. African Journal of Biotechnology, 10(39): 7604-7610.

Hedrawati O, Hille J, Woerdenbag HJ, Quax WJ, Kayser O. 2012. *In vitro* regeneration of wild chervil (*Anthriscus sylvestris* L.). *In Vitro* Cellular & Developmental Biology - Plant, 48: 355-361.

Hedrick UP. 1919. Sturtevant's notes on edible plants [online]. Albany. J.B. Lyon Company, State Printers. Available at: http://www.biodiversitylibrary.org/item/65930#page/7/mode/1up

Henry RJ. 1998. Practical applications of molecular markers to tropical and subtropical species. Acta Horticulturae, 461: 107-111.

Henz GP. 2002. Doenças da mandioquinha-salsa e sua situação atual no Brasil. Horticultura Brasileira, 20(2): 135-144.

Henz GP, Reifschneider FJB, Duarte V. 2006. *Erwinia chrysanthemi*: bactéria pectolítica envolvida na "mela" da mandioquinha-salsa no Brasil. Pesquisa Agropecuária Brasileira, 41(10): 1567-1571

Hermann M. 1997. Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Hermann M, Heller J (eds.). Andean roots and tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. Rome. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, pp. 75-172.

Hodge WH. 1954. The Edible Arracacha - A Little-Known Root Crop of the Andes. Economic Botany, 8(3): 195-221.

Hu J-B, Li Q, Li J. 2011. ISSR Analysis of Somaclonal Variation in Callus-Derived Plants of *Amorphophallus rivieri* Durieu. Acta Biologica Cracoviensia Series Botanica, 53(1): 120-124.

Huang WJ, Ning GG, Liu GF, Bao MZ. 2009. Determination of genetic stability of long-term micropropagated plantlets of Platanus acerifolia using ISSR markers. Biologia Plantarum, 53(1): 159-163.

Ide S, Yuki VA, Takada HM, Delfino MA, Hojo H, Peronti ALBG, Souza-Silva CR, Kuniyuki H, Bueno SCS, Yamakawa W. 2011. *Semiaphis dauci* (Fabricius) (Insecta, Hemiptera, Aphididae) - Formal Record of Occurrence on Arracacha (*Arracacia xanthorrhiza* Bancr.) (*Apiaceae*) in Brazil, Morphological Characterization, Description of Damages and Arthropods Associated to the Culture. Arquivos do Instituto Biológico, 78(1): 53-61.

Irvani N, Solouki M, Omidi M, Zare AR, Shahnazi S. 2010. Callus induction and plant regeneration in *Dorem ammoniacum* D., an endangered medicinal plant. Plant Cell, Tissue and Organ Culture, 100: 293-299.

Jha P, Yadav CB, Anjaiah V, Bhat V. 2009. *In vitro* plant regeneration through somatic embryogenesis and direct shoot organogenesis in *Pennisetum glaucum* (L.) R. Br.. *In Vitro* Cellular & Developmental Biology - Plant, 45: 145-154.

Jimenez R, Faviola S. 2005. Características nutricionales de la arracacha (*Arracacia xanthorrhiza*) y sus perspectivas en la alimentación. Lima. Publicación virtual red peruana de alimentación y nutrición, p. 22.

Jordan M, Amenábar A, Roveraro C. 2002. Rapid *in vitro* Propagation and Microtuber Production in *Ullucus tuberosus* (Basellaceae). Gartenbauwissenschaft, 67(2): 50-54.

Kallak H, Reidla M, Hilpus I, Virumäe K. 1997. Effects of genotype, explant source and growth regulators on organogenesis in carnation callus. Plant Cell, Tissue and Organ Culture, 51: 127-135.

Karp A. 1994. Origins, causes and uses of variation in plant tissue cultures. In: Vasil IK (ed.). Plant Cell and Tissue Culture. Dordrecht. Kluwer Academic Publishers, p. 593.

Kesari V, Ramesh AM, Rangan L, 2012. High frequency direct organogenesis and evaluation of genetic stability for *in vitro* regenerated *Pongamia pinnata*, a valuable biodiesel plant. Biomass and Bioenergy, 44: 23-32.

Khatri A, Bibi S, Dahot MU, Khan IA, Nizamani GZ. 2011. *In vitro* mutagenesis in banana and variant screening through ISSR. Pakistan Journal of Botany, 43(5): 2427-2431.

Khoddamzadeh AA, Sinniah UR, Kadir MA, Kadzimin SB, Mahmood M, Sreeramanan S. 2010. Detection of somaclonal variation by random amplified polymorphic DNA analysis during micropropagation of *Phalaenopsis bellina* (Rchb.f.) Christenson. African Journal of Biotechnology, 9(40): 6632-6639.

Kishor R, Devi HS. 2009. Induction of multiple shoots in a monopodial orchid hybrid (*Aerides vandarum* Reichb.f x *Vanda stangeana* Reichb.f) using thidiazuron and analysis of their genetic stability. Plant Cell, Tissue and Organ Culture, 97: 121-129.

Kumar S, Mangal M, Dhawan AK, Singh N. 2011. Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensi*s (Link) Schneider using RAPD and ISSR markers. Acta Physiologiae Plantarum, 33: 2541-2545.

Kuznetsova OI, Ash OA, Hartina GA, Gostimskij SA. 2005. RAPD and ISSR Analyses of Regenerated Pea *Pisum sativum* L. Plants. Russian Journal of Genetics, 41(1): 60-65.

Kuznetsova OI, Ash OA, Gostimsky SA. 2006. The Effect of the Duration of Callus Culture on the Accumulation of Genetic Alterations in Pea *Pisum sativum* L.. Russian Journal of Genetics, 42(5): 555-562.

L'Heureux L, Bastin R. 1936. In: Hermann M. 1997. Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Hermann M, Heller J (eds.). Andean roots and tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. Rome. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, pp. 75-172.

Lata H, Chandra S, Techen N, Khan IA, ElSohly MA. 2010. Assessment of the Genetic Stability of Micropropagated Plants of *Cannabis sativa* by ISSR Markers. Planta Medica, 76: 97-100.

Leroy XJ, Leon K, Hily JM, Chaumeil P, Branchard M. 2012. Detection of *in vitro* culture-induced instability through inter-simple sequence repeat analysis. Theoretical and Applied Genetics, 102: 885-891.

Leterme P, Buldgen A, Estrada F, Londoño AM. 2006. Mineral content of tropical fruits and unconventional foods of the Andes and the rain forest of Colombia. Food Chemistry, 95: 644-652.

Leva AR, Petruccelli R. 2012. Monitoring of cultivar identity in micropropagated olive plants using RAPD and ISSR markers. Biologia Plantarum, 56(2): 373-376.

Libreros D. 2001. Bibliography on underutilized roots and tubers crops. Cali. International Plant Genetic Resources Institute, p. 27.

Linacero R, Rueda J, Esquivel E, Bellido A, Domingo A, Vázquez AM. 2011. Genetic and epigenetic relationship in rye, *Secale cereale* L., somaclonal variation within somatic embryo-derived plants. *In Vitro* Cellular & Developmental Biology - Plant, 47: 618-628.

Liu F, Huang L-L, Li Y-L, Reinhoud P, Jongsma MA, Wang C-Y. 2011. Shoot organogenesis in leaf explants of *Hydrangea macrophylla* 'Hyd1' and assessing genetic stability of regenerants using ISSR markers. Plant Cell, Tissue and Organ Culture, 104: 111-117.

Maatar A, Hunault G. 1997. Influence des régulateurs de croissance sur la teneur des tissus en polyamines libres lors de l' induction de l' embryogenese somatique chez le fenouil (*Foeniculum vulgare* Miller). Biologie et pathologie végétales, 320: 245-251.

Madeira NR, Teixeira JB, Arimura CT, Junqueira CS. 2005. Influência da concentração de BAP e AG no desenvolvimento *in vitro* de mandioquinha-salsa. Horticultura Brasileira, 23(4): 982-985.

Makunga NP, Jäger AK, van Staden J. 2003. Micropropagation of *Thapsia garganica*—a medicinal plant. Plant Cell Reports, 21: 967-973.

Makunga NP, Jäger AK, van Staden J. 2005. An improved system for the *in vitro* regeneration of *Thapsia garganica* via direct organogenesis – influence of auxins and cytokinins. Plant Cell, Tissue and Organ Culture, 82: 271-280.

Malice M, Vogt V, Licht C, Pissard A, Arbizu C, Baudoin J-P. 2009. Genetic diversity of the andean tuber crop species *Ullucus tuberosus* as revealed by molecular (ISSR) and morphological markers. Belgian Journal of Botany, 142(1): 68-82.

Mansilla RCS, López CB, Blas RS, Chia JW, Baudoin J. 2006. Análisis de la Variabilidad Molecular de una Colección Peruana de S*mallanthus sonchifolius* (Poepp & Endl) H. Robinson "Yacón". Ecología Aplicada, 5(1,2): 75-80.

Martin KP. 2004a. Efficacy of different growth regulators at different stages of somatic embryogenesis in *Eryngium foetidum* L. - a rare medicinal plant. *In Vitro* Cellular & Developmental Biology - Plant, 40: 459-463.

Martin KP. 2004b. Plant regeneration through somatic embryogenesis in medicinally important *Centella asiatica* L.. *In Vitro* Cellular & Developmental Biology - Plant, 40: 586-591.

Martins M, Sarmento D, Oliveira MM. 2004. Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. Plant Cell Reports, 23: 492-496.

Mathias ME, Constance L. 1962. Flora of Peru [online]. Chicago. Field Museum of Natural History. Available at: http://www.biodiversitylibrary.org/item/19731

Matsubara S, Ohmori Y, Takada Y, Komasadomi T, Fukazawa H. 1990. Vegetative propagation of yakon [*Polymnia sonchifolia*] by shoot apex, node and callus cultures. Scientific Reports of the Faculty of Agriculture, 76: 1-6.

McGregor CE, Lamber CA, Greyling MM, Louw JH, Warnich L. 2000. A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica, 113: 135-144.

Menolli LN, Finger FL, Barbosa JM, Correia TD, Vieira LM. 2011. Peroxidase activity in roots of arracacha affected by pH and temperature. Acta Scientiarum. Agronomy, 33(3): 513-518.

Mesquini RM, Schwan-Estrada KRF, Godoy CV, Vieira RA, Zarate NAH, Vieira MC. 2009. Escala diagramática para a quantificação de Septoria apiicola e Cercospora arracacina em mandioquinha-salsa. Tropical Plant Pathology, 34(4): 250-255.

Millela L, Salava J, Martelli G, Greco I, Cusimamani EF, Viehmannová I. 2005. Genetic Diversity between Yacon Landraces from Different Countries Based on Random Amplified Polymorphic DNAs. Czech Journal of Genetics and Plant Breeding, 41(2): 73-78.

Millela L, Martelli G, Salava J, Fernandez CE, Ovesná J, Greco I. 2011. Total phenolic content, RAPDs, AFLPs and morphological traits for the analysis of variability in *Smallanthus sonchifolius*. Genetic Resources and Crop Evolution, 58: 545-551.

Mohanty S, Panda MK, Sahoo S, Nayak S. 2011a. Micropropagation of *Zingiber rubens* and assessment of genetic stability through RAPD and ISSR markers. Biologia Plantarum, 55(1): 16-20.

Mohanty S, Parida R, Singh S, Joshi RK, Subudhi E, Nayak S. 2011b. Biochemical and molecular profiling of micropropagated and conventionally grown *Kaempferia galanga*. Plant Cell, Tissue and Organ Culture, 106: 39-46.

Morillo E, Second G, Pham JL, Risterucci AM. 2004. Development of DNA microsatellite markers in the Andean root crop arracacha: *Arracacia xanthorrhiza* Banc. (Apiaceae). Molecular Ecology Notes, 4: 680-682.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Nadha HK, Kumar R, Sharma RK, Anand M, Sood A. 2011. Evaluation of clonal fidelity of *in vitro* raised plants of *Guadua angustifolia* Kunth using DNA-based markers. Journal of Medicinal Plants Research, 5(23): 5636-5641.

National Research Council, 1989. Lost Crops of the Incas: Little - known plants of the Andes with promise for worldwide cultivation. Washington, DC.. National Academy Press, p. 428.

Nayak SA, Kumar S, Satapathy K, Moharana A, Behera B, Barik DP, Acharya L, Mohapatra PK, Jena PK, Naik SK. 2013. *In vitro* plant regeneration from cotyledonary nodes of *Withania somnifera* (L.) Dunal and assessment of clonal fidelity using RAPD and ISSR markers. Acta Physiologiae Plantarum, 35: 195-203.

Negi D, Saxena S. 2011. *In vitro* propagation of *Bambusa nutans* Wall. ex Munro through axillary shoot proliferation. Plant Biotechnology Reports, 5: 35-43.

Niwa M, Arai T, Fujita K, Maruhashi W, Inoue E, Tsukihashi T. 2002. Plant Regeneration through Leaf Culture of Yacon. Journal of the Japanese Society for Horticultural Science, 71(4): 561-567.

Noguera Y, Pacheco-Delahaye E. 2000. Caracterización Física, Química y Sensorial de Hojuelas Fritas de Arracacha. Agronomía Tropical, 50(2): 241-252.

Nunes EE, Vilas Boas EVB, Piccoli RH, Xisto ALRP, Vilas Boas BM. 2010. Efeito de diferentes temperaturas na qualidade de mandioquinha-salsa minimamente processada. Horticultura Brasileira, 28: 311-315.

Orílio AF, Dusi AN, Madeira NR, Inoue-Nagata AK. 2009. Characterization of a member of a new Potyvirus species infecting arracacha in Brazil. Archives of Virology, 154: 181-185.

Ortega OR, Duran E, Arbizu C, Ortega R, Roca W, Potter D, Quiros CF. 2007. Pattern of genetic diversity of cultivated and non-cultivated mashua, *Tropaeolum tuberosum*, in the Cusco region of Perú. Genetic Resources and Crop Evolution, 54(4): 807-821.

Osuga K, Kamada H, Komamine A. 1997. Frequency Improvement of Somatic Embryogenesis at High Embryo Density by Partial Replacement of Medium in Carrot Suspension Cultures. Journal of Fermentation and Bioengineering, 84(3): 275-278.

Panta A, Tay D, Gomez R, Zea B, Rojas E, Simon R, Roca W. 2009. Status and impact of *in vitro* conservation of root and tubers at the International Potato Center (CIP). Oral presentation. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources, Conservation and Utilization, Lima, pp. 15-24.

Paramageetham C, Babu GP, Rao JVS. 2004. Somatic embryogenesis in *Centella asiatica* L. an important medicinal and neutraceutical plant of India. Plant Cell, Tissue and Organ Culture, 79: 19-24.

Parra-Quijano M, Panda S, Rodríguez N, Torres E. 2012. Diversity of *Ullucus tuberosus* (*Basellaceae*) in the Colombian Andes and notes on ulluco domestication based on morphological and molecular data. Genetic Resources and Crop Evolution, 59(1): 49-66.

Patiño EJL, Szeszko DR, Pérez JR, Retis ASB. 2012. The Flora of the Tenancingo-Malinalco-Zumpahuacán Protected Natural Area, State of Mexico, Mexico. Harvard Papers in Botany, 17(1): 65-167.

Pence VC, Sandoval JA, Villalobos VMA, Engelmann F. 2002. *In vitro* collecting techniques for germplasm conservation. Rome. International Plant Genetic Resources Institute, p. 100.

Peredo EL, Revilla MA, Arroyo-García R. 2006. Assessment of genetic and epigenetic variation in hop plants regenerated from sequential subcultures of organogenic calli. Journal of Plant Physiology, 163: 1071-1079.

Pessoa ACS, Vieira RC, Esquibel MA. 1994. Induction and Histogenesis of Calli from Petiole Explants of Peruvian Carrot (*Arracacia xanthorrhiza* Banc.). Arquivos de Biologia e Tecnologia, 37(2): 231-246.

Piña-Escutia JL, Vázquez-García LM, Arzate-Fernández AM. 2010. *In vitro* regeneration and genetic fidelity of *Tigridia pavonia* (L.f.) DC.. Electronic Journal of Biotechnology, 13(1): 1-7.

Pissard A, Ghislain M, Bertin P. 2006. Genetic diversity of the Andean tuber-bearing species, oca (*Oxalis tuberosa* Mol.), investigated by inter-simple sequence repeats. Genome, 49: 8-16.

Pissard A, Arbizu C, Ghislain M, Faux A-M, Paulet S, Bertin P. 2008a. Congruence between morphological and molecular markers inferred from the analysis of the intra-morphotype genetic diversity and the spatial structure of *Oxalis tuberosa* Mol.. Genetica, 132: 71-85.

Pissard A, Rojas-Beltran JA, Faux A-M, Paulet S, Bertin P. 2008b. Evidence of intra-varietal genetic variability in the vegetatively propagated crop oca (*Oxalis tuberosa* Mol.) in the Andean traditional farming system. Plant Systematics and Evolution, 270: 59-74.

Polanco C, Ruiz ML. 2002. AFLP analysis of somaclonal variation in *Arabidopsis thaliana* regenerated plants. Plant Science, 162: 817-824.

Pontaroli AC, Camadro EL. 2005. Somaclonal variation in *Asparagus officinalis* plants regenerated by organogenesis from long-term callus cultures. Genetics and Molecular Biology, 28(3): 423-430.

Pourhosseini L, Kermani MJ, Habashi AA, Khalighi A. 2013. Efficiency of direct and indirect shoot organogenesis in different genotypes of *Rosa hybrida*. Plant Cell, Tissue and Organ Culture, 112: 101-108.

Pretto FR, Santarém ER. 2000. Callus formation and plant regeneration from *Hypericum perforatum* leaves. Plant Cell, Tissue and Organ Culture, 62: 107-113.

Rai MK, Phulwaria M, Harish, Gupta AK, Shekhawat NS, Jaiswal U. 2012. Genetic homogeneity of guava plants derived from somatic embryogenesis using SSR and ISSR markers. Plant Cell, Tissue and Organ Culture, 111: 259-264.

Raju CS, Kathiravan K, Aslam A, Shajahan A. 2013. An efficient regeneration system via somatic embryogenesis in mango ginger (*Curcuma amada* Roxb.). Plant Cell, Tissue and Organ Culture, 112: 387-393.

Rathore MS, Chikara J, Mastan SG, Rahman H, Anand KGV, Shekhawat NS. 2011. Assessment of Genetic Stability and Instability of Tissue Culture-Propagated Plantlets of *Aloe vera* L. by RAPD and ISSR Markers. Applied Biochemistry and Biotechnology, 165: 1356-1365.

Ray T, Saha P, Roy SC. 2006. Commercial production of *Cordyline terminalis* (L) Kunth. from shoot apex meristem and assessment for genetic stability of somaclones by isozyme markers. Scientia Horticulturae, 108: 289-294.

Reddy MP, Sarla N, Siddiq EA. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

Reed BM, Engelmann F, Dulloo ME, Engels JMM. 2004. Technical guidelines for the management of field and *in vitro* germplasm collections. IPGRI Handbooks for Genebanks No. 7. Rome. International Plant Genetic Resources Institute, p. 107.

Reis A, Nascimento WR. 2011. New apiaceous hosts of *Sclerotinia sclerotiorum* in the Cerrado region of Brazil. Horticultura Brasileira 29: 122-124.

Rewers M, Kisiala A, Drouin J, Sliwinska E, Cholewa E. 2012. *In vitro*-regenerated wetland sedge *Eriophorum vaginatum* L. is genetically stable. Acta Physiologiae Plantarum, 34(6): 2197-2206.

Riberio RA, Finger FL, Puiatti M, Casali VWD. 2007. Vida útil e metabolismo de carboidratos em raízes de mandioquinha-salsa sob refrigeração e filme de PVC. Pesquisa Agropecuária Brasileira, 42(4): 453-458.

Rolfs PH. 1916. Subtropical Vegetable-Gardening [online]. New York. The Macmillan Company, p. 276. Available at: http://www.biodiversitylibrary.org/item/92376

Roy AR, Sajeev S, Pattanayak A, Deka BC. 2012. TDZ induced micropropagation in *Cymbidium giganteum* Wall. Ex Lindl. and assessment of genetic variation in the regenerated plants. Plant Growth Regulation, 68: 435-445.

Rzepka-Plevneš D, Kulpa D, Palka E, Wisniewska M. 2010. Somaclonal variability in callus culture of *Lycopersicon hirsutum* f. *typicum* and *Lycopersicon chilense.*Hortorum Cultus - Acta Scientiarum Polonorum, 9(4): 63-73.

Sahijram L, Soneji JR, Bollamma KT. 2003. Invited Review: Analyzing Somaclonal Variation in Micropropagated Bananas (*Musa* spp.). *In Vitro* Cellular & Developmental Biology - Plant, 39: 551-556.

Sánchez DF, Panta A, Tay D, Roca W. 2011. Cryopreservation of Ulluco (*Ullucus tuberosus* Cal.) and Oca (*Oxalis tuberosa* Mol.) Shoot Tips Using the PVS2 Droplet-vitrification Method. Acta Horticulturae, 908: 339-346.

Santacruz S, Koch K, Svensson E, Ruales J, Eliasson A-C. 2002. Three underutilised sources of starch from the Andean region in Ecuador Part I. Physico-chemical characterisation. Carbohydrate Polymers, 49: 63-70.

Santacruz S, Ruales J, Eliasson A-C. 2003. Three under-utilised sources of starch from the Andean region in Ecuador. Part II. Rheological characterisation. Carbohydrate Polymers, 51: 85-92.

Santos FF, Hermann M. 1994. Processing Arracacha (*Arracacia xanthorrhiza* Bancroft) in Brazil. Bahia. Proceedings of the Tenth Symposium of the International Society for Tropical Root Crops, pp. 23-29.

Seminario J, Valderrama M. 2004a. Conservación de germoplasma de arracacha con campesinos. In: Seminario J (ed.). Raíces andinas: contribuciones al conocimiento y a la capacitación. Lima. Centro Internacional de la Papa (CIP), p. 376.

Seminario J, Valderrama M. 2004b. Variabilidad morfológica y distribución geográphica de la colección de arracacha (*Arracacia xanthorrhiza* Bancroft.) de la Universidad Nacional de Cajamarca, Perú. Arnaldoa, 11(2): 79-104.

Sharma SK, Bryan GJ, Winfield MO, Millam S. 2007. Stability of potato (*Solanum tuberosum* L.) plants regenerated via somatic embryos, axillary bud proliferated shoots, microtubers and true potato seeds: a comparative phenotypic, cytogenetic and molecular assessment. Planta, 226: 1449-1458.

Sharma S, Pamidimarri DVNS, Anand KGV, Reddy MP. 2011. Assessment of genetic stability in micropropagules of *Jatropha curcas* genotypes by RAPD and AFLP analysis. Industrial Crops and Products, 34: 1003-1009.

Sirarusa M, Carra A, Salvia L, Puglia AM, Pasquale FD, Carimi F. 2007. Genetic instability in calamondin (*Citrus madurensis* Lour.) plants derived from somatic embryogenesis induced by diphenylurea derivatives. Plant Cell Reports, 26: 1289-1296.

Singh SR, Dalal S, Singh R, Dhawan AK, Kalia RK. 2013. Evaluation of genetic fidelity of *in vitro* raised plants of *Dendrocalamus asper* (Schult. & Schult. F.) Backer ex K. Heyne using DNA-based markers. Acta Physiologiae Plantarum, 35: 419-430.

Skálová I, Viehmannová I, Vítámvás J. 2012. *In vitr*o Conservation of *Smallanthus sonchifolius* under Slow-growth Conditions. Agricultura tropica et subtropica, 45(3): 147-150.

Skirvin RM, McPheeters KD, Norton M. 1994. Sources and Frequency of Somaclonal Variation. HortScience, 29(11): 1232-1237.

Slíva Š. 2009. *In vitro* cultures of *Arracacia xanhorrhiza*. Diploma Thesis. Czech University of Life Sciences Prague. Prague.

Slíva S, Viehmannová I, Vítámvás J. 2010. Micropropagation and morphogenesis of arracacha (*Arracacia xanthorrhiza* Bancroft). Agricultura Tropica et Subtropica, 43(3): 206-211.

Soto J, Zorilla C, Manrique I, Roca W, Medina T, Blas R, Seminario J, Lizarraga L, Quispe J, Tay D, Arbizu C. 2009. Genetic diversity of yacon (*Smallanthus sonchifolius*) in Peru. Oral presentation. In: Programme of the 15th Triennial Symposium of the International Society for Tropical Root Crops, Session II: Genetic Resources, Conservation and Utilization. Lima, pp. 38-43.

Standley PC, Williams LO. 1966. Flora of Guatemala. Chicago. Chicago Natural History Museum Press, p. 271.

Tapia C, Estrella J, Monteros A, Valverde F, Nieto M, Cordóva J. 2004. Manejo y Conservación de RTAs *in situ* en fincas de agricultores y *ex situ* en el Banco de Germoplasma de INIAP (Capítulo II). In: Barrera V, Tapia C, Monteros A (eds.). Raíces y Tubérculos Andinos: Alternativas para la conservación y uso sostenible en el Ecuador. Quito. Lima. Agencia Suiza para el Desarrollo y la Cooperación, pp. 3-30.

Takeiti C, Fakhouri F, Ormenese R, Steel C, Collares F. 2007. Freeze-thaw Stability of Gels Prepared from Starches of Non-conventional Sources. Starch/Stärke, 59: 156-160.

Taylor M, Hunter D, Ramanatha Rao V, Jackson JVH, Sivan P, Guarino L. 2010. Taro collecting and conservation in the Pacific Region. In: Ramanatha Rao V, Matthews PJ, Eyzaquirre PB, Hunter D (eds.). The Global Diversity of Taro: Ethnobotany and Conservation. Rome. Bioversity International, pp. 157-167.

Tawfik AA, Noga G. 2002. Cumin regeneration from seedling derived embryogenic callus in response to amended kinetin. Plant Cell, Tissue and Organ Culture, 69: 35-40.

Tene V, Malagón O, Finzi PV, Vidari G, Armijos C, Zaragoza T. 2007. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. Journal of Ethnopharmacology, 111: 63-81.

Thomas J, Vijayan D, Joshi SD, Lopez SJ, Kumar RR. 2006. Genetic integrity of somaclonal variants in tea (*Camellia sinensis* (L.) O Kuntze) as revealed by inter simple sequence repeats. Journal of Biotechnology, 123: 149-154.

Toledo J, Dehal P, Jarrin F, Hu J, Hermann M, Al-Shehbaz I, Quiros CF. 1998. Genetic Variability of *Lepidium meyenii* and other Andean *Lepidium* Species (Brassicaceae) Assessed by Molecular Markers. Annals of Botany, 82 : 523-530.

Thankappan SS, Morrawala-Patell V. 2011. *In vitro* propagation studies and genetic fidelity assessment of endangered medicinal wild Yam- *Dioscorea prazeri*. Plant Omics Journal, 4(4): 177-189.

Vásquez N, Medina CI, Lobo M. 2004. Caracterización morfológica de la colección colombiana (Tolima, Huila, Boyacá y Cauca) de arracacha (*Arracacia xanthorrhiza*). In: Seminario J (ed.). Raíces andinas: Contribuciones al conocimiento y a la capacitación. Lima. Agencia Suiza para el Desarrollo y la Cooperación, pp. 165-178.

Viehmannová I, Fernández EC, Bechyně M, Vyvadilová M, Greplová M. 2009. *In vitro* induction of polyploidy in yacon (*Smallanthus sonchifolius*). Plant Cell, Tissue and Organ Culture, 97: 21-25.

Viehmannová I, Trávníčková M, Špatenková E, Černá M, Trávníček P. 2012. Induced polyploidization and its influence on yield, morphological, and qualitative characteristics of microtubers in *Ullucus tuberosus*. Plant Cell, Tissue and Organ Culture, 109: 83-90.

Wang Q-M, Gao F-Z, Gao X, Zou F-Y, Sui X, Wang M, Hui Y-J, Wang L. 2012a. Regeneration of *Clivia miniata* and assessment of clonal fidelity of plantlets. Plant Cell, Tissue and Organ Culture, 109: 191-200.

Wang Q-M, Wang Y-Z, Sun L-L, Gao F-Z, Sun W, He J, Gao X, Wang L. 2012b. Direct and indirect organogenesis of *Clivia miniata* and assessment of DNA methylation changes in various regenerated plantlets. Plant Cell Reports, 31: 1283-1296.

Xu L, Najeeb U, Raziuddin R, Shen WG, Shou JY, Tang GX, Zhou WJ. 2009. Development of an efficient tissue culture protocol for callus formation and plant regeneration of wetland species *Juncus effusus* L. *In Vitro* Cellular & Developmental Biology, 45: 610-618.

Yuan XF, Dai ZH, Wang XD, Zhao B. 2009. Assessment of genetic stability in tissue-cultured products and seedlings of Saussurea involucrata by RAPD and ISSR markers. Biotechnology Letters, 31: 1279-1287.

Zámečníková J, Fernández CE, Viehmannová I, Zámečník J, Faltus M. 2011. Preparation of Shoot Tips by Sucrose and Dehydration Pre-Treatment of *Ullucus tuberosus* Cal. for Cryopreservation. Acta Horticulturae, 908(1): 331-338.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. Genomics, 20: 176-183.