# CZECH UNIVERSITY OF LIFE SCIENCES

Faculty of Tropical AgriSciences

Department of Crop Sciences and Agroforesty


## Micropropagation *in vitro* of native potato *Solanum jaenense Ochoa.*

Bachelor Thesis

Prague 2015

**Supervisor:**

doc.Dr. Ing. Eloy Fernández Cusimamani

**Autor:**

Kelly M Delgado Rivera

## Declaration

Here I confirm that this bachelor thesis "Micropropagation *in vitro* of native potato *Solanum jaenense Ochoa*" is original result of my own work and that I have used no other resources than referenced.

Prague 17th April 2015

_____

Kelly M.Delgado Rivera

## Acknowledgement

I would like to thank and express my sincere gratitude to my supervisor of my thesis doc. Dr. Ing. Eloy Fernández Cusimamani for his professional help, patience and his valuable guide during all the process of the thesis and the opportunity to work in the Laboratory of Plant Tissue Cultures (Faculty of Tropical AgriSciences) CULS Prague.

Also I am grateful with, the Ing. Iva Viehmanová, Ph.D (Department of Crop Sciences and Agroforestry in Tropics and Subtropics, Faculty of Tropics and Subtropics, Czech University of Life Sciences Prague) for her extensive knowledge provided during the laboratory work.

## Abstract

The present study describes a successful report on *in vitro* propagation of a native potato species *Solanum jaenense* Ochoa from Peru.

Nodal segments (30) were cultivated in MS medium with growth regulators in different concentrations of cytokinins KIN and BAP (0.5, 1.0 and 1.5 $mg.l^{-1}$) in order to investigate their effects on shoot induction. Whereas the same amount of nodal segments were cultivated in MS medium with auxins IAA and NAA (0.25, 0.5 and 1.0 $mg.l^{-1}$) to evaluate root induction. It was also established an experiment in MS medium supplemented with combinations of auxins and cytokinins: IAA (1.0, 0.5, 0.25 $mg.l^{-1}$) + KIN and BAP (0.5, 1.0, 1.5 $mg.l^{-1}$). The control treatment was MS medium without regulator growth. The explants were kept in permanent conditions of photoperiod 16/8, temperature 25°C, and 3000 lx of luminary. The number of root, number of nods, root length, height stem and height plant were evaluated in each treatment once a week during 4 weeks cultivation.

The data revealed significant effects of each treatment ($P \leq 0.05$) in all evaluated parameters. However MS medium supplemented with IAA 0.5 $mg.l^{-1}$ and BAP 1.0 $mg.l^{-1}$ showed the best effects on shoot induction (8.74 ± 3.05). The higher average of height plant (7.21 ± 3.84) was taken from the combinations of IAA and KIN in concentrations of 0.5 $mg.l^{-1}$ and 1.0 $mg.l^{-1}$ respectively. The optimal treatment for root induction was IAA in concentration of 0.5$mg.l^{-1}$.

The successful shoot and root induction under *in vitro* conditions indicated that *Solanum jaenense* could be successfully multipropagated by tissue culture technology.

**Keywords :** Growth regulators, *in vitro* culture, nodal segments, *Solanum jaenense* Ochoa.

## Abstrakt

Cílem této práce bylo zhodnocení vlivů růstových hormonů na regeneraci a růst původních druhů brambor *Solanum jaenense* Ochoa v *in vitro* podmínkách.

Nodální segmenty rostlin (30) byly kultivovány v MS médiu s regulátory růstu o různých koncentracích růstových hormonů cytokininů KIN a BAP (0,5;1,0 a 1,5 $mg.l^{-1}$), aby se zjistily jejich účinky na růst výhonků. Stejný počet nodálních segmentů byl kultivován v MS médiu s auxiny IAA a NAA o různých koncentracích (0,25; 0,5 a 1,0 $mg.l^{-1}$) pro vyhodnocení kořenového růstu. Další experiment probíhal v MS médiu doplněném o kombinací auxinů a cytokininů: IAA (1,0; 0,5; 0,25 $mg.l^{-1}$) + KIN a BAP (0,5; 1,0; 1,5 $mg.l^{-1}$). Jako kontrolní médium bylo zvoleno MS médium bez růstových regulátorů. Explantáty byly udržovány ve stálých podmínkách: fotoperioda 16/8, teplota 25 °C a při světelné intenzitě 3 000 lux. Množství kořenů, počet výhonků, délka kořenů, výška stonků a výšky rostlin byly hodnoceny jednou týdně během 4 týdnů kultivace pro každý vzorek koncentrace zvlášť.

Získaná data odhalila významný vliv u každé koncentrace růstových hormonů ($P \leq 0.05$) na všechny hodnocené parametry. Nicméně MS médium doplněné s IAA 0,5 $mg.l^{-1}$ a BAP 1,0 $mg.l^{-1}$ ukázalo nejlepší účinek na růst výhonků (8,74 ± 3,05). Nejvyšší průměrná výška rostliny (7,21 ± 3,84) byla získána kombinací IAA a KIN v koncentraci 0,5 $mg.l^{-1}$ a 1,0 $mg.l^{-1}$. Optimální koncentrace pro růst kořenů byla zjištěna v médiu s IAA o koncentraci 0,5$mg.l^{-1}$ .

Úspěšný růst výhonků a kořenů značí, že za *in vitro* podmínek, jsou *Solanum jaenense* Ochoa vhodné pro mikropropagaci.



**Klíčová slova**: Regulátory růstu, kultura *in vitro*, nodální segmenty, *Solanum*

**Table of content** **Page N$^0$**

## List of Abbreviations

ANOVA-Analysis of Variance

BAP- 6-benzylaminopurine

CULS-Czech University of Life Sciences Prague

$GA_3$-gibberellic acid

IAA- Indole-3-acetic acid

KIN-kinetin

MS-Murashige and Skoog (1962) medium

NAA-naphthalene acetic acid

PGRs-Plant growth regulators

BA-6-benzyladenine

MH- Maleic hydrazide

CIP-International Potato Center ( centro Internacional de la Papa)

IBCH- Interdependency of indigenous bio cultural heritage

**List of figures:** **Page N$^0$**

**List of tables:** **Page N$^0$**

# 1. Introduction

The potato is one of the fourth most important foods in the world ranking with the corn, wheat and rice, making it the principal food fundamental for the fight against hunger in the world.

The potato originated in the Andes of South America (southern Peru and north western Bolivia), domesticated more than 7000 years before Christ under the knowledge of the Andean farmer.

In the world are more than 5000 varieties of potatoes of which 3000 are native varieties, mostly found in South America between Peru and Bolivia. These native potatoes have been preserved in altitude over than 4000 meters, through many generations by the inhabitants of the Andean region.

One of the way of propagation of the native potato is asexually (vegetative) which consist in sow by tubers, but with this brings promotion to more disease and weaker seed, because it lost genetically efficiency by the time and therefore there is a low rate of productivity. One way to lead this is use quality seed.

The native potatoes have not yet strongly entered the market and are not well recognised. Means should be sorted to presents this product which high nutrients to the market and thereby improve the living standards of farmers in the poorest regions of Peru producers of native potatoes.

The micropropagation in *vitro* is a safer way to produce and preserve seeds in better condition and free of disease which would help to better harvest.

This thesis consists of micropropagation by nodal segments of native species potato *Solanum jaenense* Ochoa, in MS media with different concentrations of growth regulators auxiny and cytikinins and evaluated once a week for 4 weeks the influence in the regeneration of the explant under *in vitro* conditions.

## 2. Literature review

### 2.1 Botany and morphology of *Solanum jaenense* Ochoa

The potato plant is characterised with erect, small, 25-35 cm tall, subglabrous .Stem slender, 3-4 mm in diameter at base, usually simple, slightly sinuous, very narrowly straight-winged, internodes 1.5-2.0 cm long, glabrous or subglabrous, lightly pigmented on the basal one-third of its length and in the leaf axilis. Stolons with 40cm long by 1.5 mm in diameter, white the tubers round to ovoid 1.5-2.0cm long with semi-deep eyes. Leaves long petioled, light green, subglabrous or glabrous, with a smooth surface, (6-)9-13(-17) cm long by (2.5-).

Are round to ovoid , 1.5-3.0 cm long ,with semi-deep eyes.leaves long petiole , light green , subglabrous or glabrous , with a smooth surface, (6-)9-13(-17) cm long by (2.5-) 4.0-7.0 (-10.0) cm broad including the petiole, imparipinnate, with (2-)3-4(-5) pairs of leaflets and (1-)2-3(-4) pairs of sessile interjected leaflets, ovate tp orbicular, 2-5 mm long. Terminal leaflet slightly larger and broader than the laterals ,(3.0-)3.5-4.5(-5.0) cm long by 1.7-2.2 (-2.8) cm broad , elliptic- lanceolate ,the apex pointed or shortly acuminate and the base slightly attenuate to cuneate , narrowly decurrent about the rachis and with 0-1 pairs of small interjected leaflets on the petiolule, lateral leaflets narrowly lanceolate, subsessile or shorthly acuminate and the base slightly asymmetrical or symmetrical rounded petiolulate 1-2 mm long; the first pair of leaftles larger than leaflets of the second and following pairs , 2.3-4.0 cm long by 1.0-1.4 cm broad. Pseudostipular leaves subfalcate or asymmetrical and narrowly elliptic, 5-10 mm long by 2.5-7.0 mm broad. Infloescence cymose, lateral or terminal 4-6(-10) floweres.Penducle 5-9 mm long by 1.5-2.0 mm in dia . at base, forked, light green, generally glabrous like the pedicels and calyx; pedicels short, 15-20 mm long; inconspicuously articulated near the midpoint or occasionally slightly the below center, usually about 7-9 mm below the base calyx. Calyx is symmetrical or asymmetrical, small, 5-6 mm long, subglabrous; lobes narrowly elliptic-lanceolate, broadly membranous apex very shortly acuminate. Corolla rotate, white, small, 2.0-2.5 cm in dia. The internal star white-hyaline and lobes puberulent externally, margins of the acumens finely pilose. Anthers pale yellow, narrowly ellipticlanceolate or lanceolate, 5.5 mm long; filaments 1,0-1.8 mm long , slender glabrous. Style 8.0-8.5 mm long, exerted 2 mm, glabrous or occasionally very shortly and

sparsely papillose on the basal one- third of its length; stigma capite, small, slightly anlarged at apex. Fruit long-conical 1.8-2.2 cm long, light purple-green. Chromosome number: 2n=72. EBN=4 (Ochoa C, 2004).

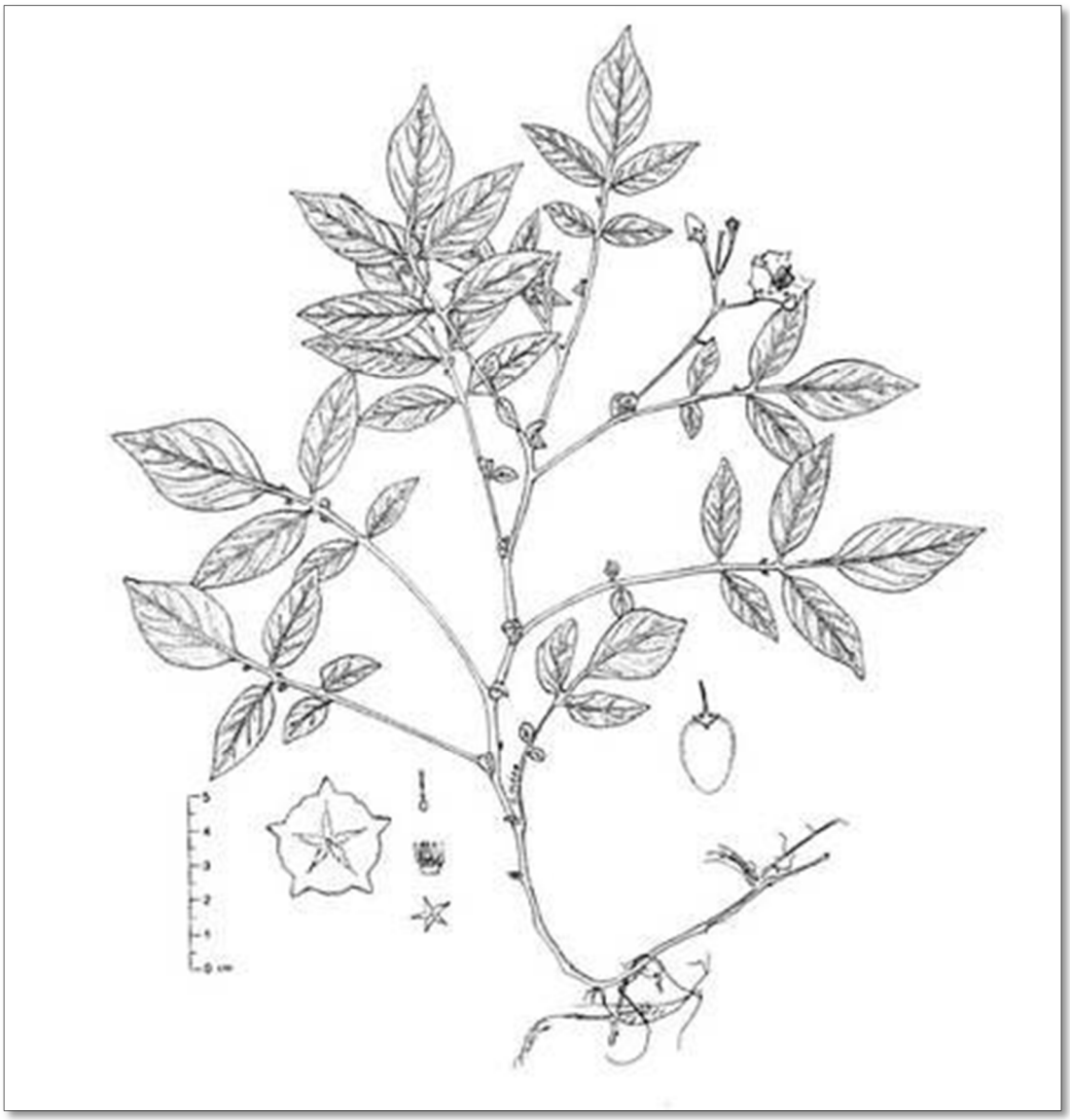


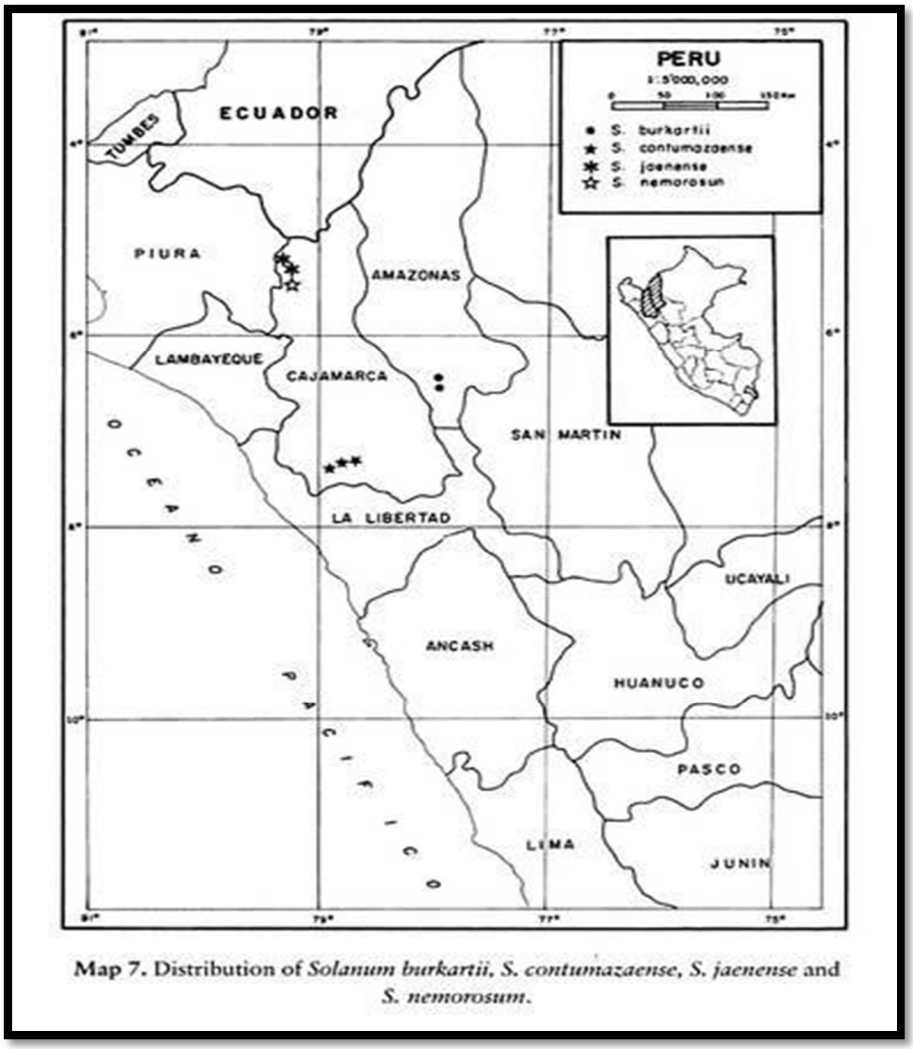
**Figure 1**. *Solanum jaenense* Ochoa 2328, holotype (Ochoa et al., 2004).

## 2.2. Affinities

Contrary to what Correll Stated in his 1962 monograph, *S. Jaense* is more closely related to the Ecuadorian *S.tundalomense* than to S.*chomatophium*. Both species hexaploid (2n=72) and have small white corollas and similarly-appearing fruits and leaves. *S.tundalomense*, however, is a larger and more vigorous plant. More extensive field collections and comparative studies of living plants are needed, however, in order to better clarify the existing relationships between these two (Ochoa , 2004).

## 2.3. Habitat and distribution

Solanum *jaenense* thrives in the cool and rainy climate of the Huascarai Mountains that form borderline between the Departments of Piura and Cajamarca in northern Peru (figure. 2).It grows along the margins of tree forest and thickets formed by such herbs and shrubs as *Hedyosomum huascarai, Miconia caelata Gleichenia affine, Slavia florida* and various calceolarias, composites and grasses, at elevations between 2700 and 2800 m (Ochoa, 2004).




Map 7. Distribution of *Solanum burkartii, S. contumazaense, S. jaenense* and *S. nemorosum*.

**Figure 2**. Distribution of *Solanum burkartii*, *S.contumazaense*, *S.jaenense* and S.*nemorosum* (Ochoa, 2004)

## 2.4 Nutritional importance of the potatoes

The potato (*Solanum tuberosum*) is the fourth most consumed food in the world and its production globally is about 320 million tons per year. This amount tends to increase while the other three most consumed foods like corn, wheat and rice, is decreasing. North America and Europe are the major producers, although in the last decades there has been an extraordinary growth of these plantations in Asia, Africa and Latin America (Borba N, 2008).

The potato has nutritive components (energy, macro- and micronutrients) and non-nutritive components (water, cellulose, hemicellulose, pectin, glycoalkaloids, organic acids, enzymes between other minorities. After harvesting the tubers contain on average 80% water and 20% dry matter (60% of dry matter corresponds to starch) ( Reinoso, 2009).

**Energy**: The tubers have an important role in the alimentation for the energy supply and therefore the dry material corresponds to the starch, but has lower caloric density compared with the banana and cassava (Pertuz, 2003).

Carbohydrates: The potato is a tuber that contains high percentages of carbohydrates which mostly are : Starch, in small percentage, sucrose, fructose and glucose ( Pertuz, 2003).

Poteins The potato protein has a high content of lysine and low in sulfur amino acids. The protein quality is lower by the presence of glycoalkaloids and proteinase inhibitors ( Pertuz, 2003).

**Fat:** The fat content in the potatoes is very low that is an advantage for individuals with restricted calorie.

**Vitamins:** The potatoes contain vitamins, but are not considered source of this nutrient. The vitamins found in potatoes are ascorbic acid niacin, B1, B6, mainly is concentrated in the peel. The vitamin C has a high reactivity and high losses due to oxidation (Jimenez et al., 2014)

**Minerals**: The potato contains potassium, especially in the peel, and moderate amounts of phosphorus 44mg for 100g of potato, chlorine, sulfur, magnesium and iron (Nutrientes de la papa, 2011)

**Fiber:** The fibre is composed by cellulose, pectin substances and hemicelluloses .Actually exist resistance starch which is the starch not digested in the upper tube intestinal in humans

Although fiber is not a strict nutrient, but it is important to complete the cycle of digestion, also it has the advantage to increase the volume of food waste, which favours the elimination of waste and the ability to absorb water, which favours the intestinal flow (Sanchez, 2004).

**Table 1:** Nutritional values for 100g of potato (Borba, 2008).

| **Nutritional values for 100g of potato** (these values are different, according to the type of cooking and the variety of potato) | |
|---|---|
| Water | 77g |
| Fiber | 1.80g |
| Calorie value | 87 kcal |
| Protein | 1.87g |
| Carbohydrates | 20.13 g |
| Lipids | 0.10g |
| Vitamin C | 13 mg |
| Iron | 0.31 mg |
| Calcium | 5 mg |
| Phosphor | 44mg |

## 2.5 Important characteristics of the native potato

Native potatoes have not only shapes and attractive colours; they also provide significant amounts of nutrients and functional components, which are very beneficial, for the body functions more than the adequate nutritional ( López, 2012).

Native potatoes are especially rich in polyphenols an average of four more times than the other varieties of potatoes, for example the native potato Tushpa (646.3 $mg.100^{-1}$) and the Super chola (311. 2 $mg.100^{-1}$) in dry base (Table 2).The polyphenol is a natural antioxidant which is find in some purple and red vegetables, it protects the body, help fight a degenerative diseases and inhibit the formation and growth of tumours (Yany, 2001 and Andre, 2007).

In the same way the native potatoes are rich in carotenoids, the Super chola registered 5.4$ug.g^{-1}$) table 3. The carotenoides are the pigments responsible to give yellow colour and orange to the vegetables. A diet with height value of carotenoid can be good for prevent degenerative diseases that can cause colour blindness (Andre, 2007).

**Table 2:** Content of polyphenol in native potatoes in dry base (Quilca, 2008).

| No | Native potatoes | Dry base $PE^{-1} \pm D.E^2$ mg galic acid/100 | Range[3] |
|---|---|---|---|
| 1 | Tushpa | 646.3 ± 10.6 | a |
| 2 | Dolores | 516.2±4.7 | b |
| 3 | Macholulo | 518.6±9.4 | b |
| 4 | Wagrasinga | 326.0±6.6 | c |
| 5 | Super chola | 71.8±3.1 | l |

**Table 3:** Content of carotene in native potatoes (Qulica, 2008).

| No | Native potatoes | $Ug.g^{-1}$ dry base |
|---|---|---|
| 1 | Chaucha amarilla | 11.4 |
| 2 | Quillu | 10.0 |
| 3 | Ovaleña | 5.8 |
| 4 | Chivolulo | 5.4 |
| 5 | Super chola | 7.4 |

The potato and its different varieties have an important social and cultural role in the daily life of Andean families (Catacora, 2006)

Socially the potato is one of the crop which demand most jobs in rural areas (production and marketing). This crop is also one of the most important in the daily diet in the Andean communities. The importance that the potato has in the food allows the conservation of native varieties as a form of provision in the production ( Catacora, 2006).

In this relation diversity-alimentation, the participation of women is very important. They influence in the native varieties should be planted and search the food security and balance provided in different varieties (Iriarte et al., 1999).

As already mentioned the majority of native potatoes are destined for the auto-consumption, for example a study realized in a small community in Bolivia showed that from 19 native varieties all are destined for their consumption, 5 varieties for the market and 1 for the cultural activities (Iriarte et al., 1999). Another example is the community of Ayllu Majasaya Mujlli also in Bolivia, where 82 varieties were planted bitter, semi-bitter and sweet of which 10% are commercial varieties and 90% for the auto-consumption (Saravia et al., 2002).

Due to the globalization of markets, the native potatoes are presented like an interesting alternative to the potato markets thanks to its unique characteristics which provides a strategy to compete in the market. The Andean community has been preserved this cultural legacy and now they have the opportunity to enter in the modern and urban markets (Monteros et al.,2011).

In these days the producers of this native potatoes are entering to the modern and dynamic market which are recognized with interesting prices directly with the farmers, which contribute a directly revenue with the farmers. (Jimenez, 2014)

### 2.5.1 Tradicionally cultive of native potatoes

The process of potato planting is done in two steps first is the preparation of land between the months of February and March and then end with the plowing of the land which is done 6-7 times and planting is realized between May and June. By the end of May irrigated is often with the purpose of counteracting the adverse climatic factors such as the frosts (Sánchez and Charan, 2006).

The harvest of native potato is usually a family activity, because the farmer recognized the value of these potatoes which requires some care. During the harvest one tool called asho is used with the purpose of not damage the potato. The participation of the whole family in harvesting, selecting and the storage of native potatoes allows the transfer of practical knowledge from farmers to their generations, this is important for the conservation of native potatoes (Malaver, 2002).

The storage of potatoes natives is realized in various districts and periods. Some farmers after their finish the harvest proceed to store the potato is sack bag called collona it is a kind of little straw house located in the land. Some of the farmers prefer to first select the potato and storage it separate according to the final purpose of the potato (Sánchez and Charan, 2006).

## 2.6 Conservation and multiplication of the potato.

### 2.6.1 Conservation of Potato Genetic Resources in the CIP (Center international of the potato)

The potato possesses more related wild species than any other crop plant and 235 native species are recognized as composing a polyploid series from diploid (2n=2x=24) to hexaploid (2n=2x=72) (Hawkes, 1990). These wild potato species are widely distributed from the southwestern United States to most countries in Central and South America. Two clear centers of diversity are recognized: one in central Mexico and the second in the high Andes from Peru through Bolivia to Northwest Argentina. The range of elevation where these potatoes are distributed is from sea level to 4500 m. The largest numbers of wild potato species are in Mexico with 36 species, Bolivia with 39 and Peru with about 100 species (Hawkes et al., 1990).CIP maintains a total of 1,712 wild potato accessions, including 140 different tuber-bearing *Solanum taxa*, including species, sub-species, varieties and forms, collected in 11 countries in the Americas. Biosystematic studies on the wild and cultivated potatoes from this collection have been published in a comprehensive monograph for Bolivia (Ochoa, 1990) and on the wild potatoes of Peru (Ochoa, 1999).

The degree of representativeness of the wild potato species that are maintained worldwide is far from being adequate. Thus, in the major potato genebanks in the world out of the 235 tuber-bearing potato species recognized by Hawkes (1990), 188 taxa are represented. From the 5,547 accessions with known origin maintained in these genebanks, about 45% comprise only 12 *Solanum* species. On the other extreme, 33 species are represented by 5 to 2 accessions each (5%) and 34 species are presented by only one accession (1%) (Huaman et al., 2000).

### 2.6.2 *Conservation* ex situ

The problem of the collection of the field and the necessity to conserve some species of plants that are almost to disappear led to the development of some conservationmethodologies in vitro by cutting nodal it means a part of the plant (organ, tissue, cell or protoplast) is aseptically cultivate in a nutrient medium under Controlled conditions of light and temperature (Benítez, 2001).

One of the advantages of maintaining *in vitro* is the conservation of a large number of plants in reduced spaces there is more control, reducing time multiplication, ease change of genetic material and increased rate of cloned multiplication of valuable germplasm (Lopez, 2012).

The disadvantage of this technique is that requires periodic subcultures (Engelmann M, 1997). But are many alternatives to prolong the *in vitro* preservation period. These are techniques preservation at low temperature (+5 C), the use growth regulators, reduced of oxygen tension, defoliation of shoots, and the manipulation of osmotic pressure of the culture media.

The conservation *in vitro* of the potato has been done for more than two decades, because has allowed a rapid multiplication, strict control of pests and diseases (Paez and Gonzales, 2011), it means help to the plant to be in better conditions. The micropropagation of the potato is mainly used for the production of seed for the collection and distribution of germplasm and for find clones for agriculture interest (Ludergan et al.,1979).

### 2.6.3 Conservation *in situ*

The conservation of plant genetic *in situ* is ‘’the continuing maintenance of a population in the community’’ which belongs into the environment which is adapted. (Frankel, 1970).

In the Andes the conservation *in situ* is practiced since always not like systematic way but like a consequence of the vision of their settlers, for them the diversity is synonym of live.

The conservation of plant genetic has the aim to preserve the genetic variation between and inside of particular species. The strategies of conservation in *situ* depend of the environment and the resource of plant genetic (Revilla C, 2012).

One benefit of *in situ* conservation is that it maintains recovering populations in the surrounding where they have developed their distinctive properties (Tapia, 1998).
Another is that this strategy helps ensure the ongoing processes of evolution and adaptation within their environments.

Institutions in South America as well as new accessions obtained by joint CIP-NARS collecting expeditions in Mexico, Guatemala, Venezuela, Colombia, Ecuador, Peru, Bolivia, Argentina and Chile (Huaman et al., 2000).

### 2.6.3 The potato park

The potato park focuses on protecting and preserving the critical role and interdependency of indigenous bio cultural heritage (IBCH), for local rights, livelihoods and conservation and sustainable use of agro biodiversity.

The park is located in Cuzco which is one of the microcentre of native potatoes, one of the world is major food crops which has been protected for centuries by the deeply rooted local food systems of Quechua peoples. The potato park, celebrates the diversity of native potato varieties and other native Andean crops characteristic of Andean food systems.
The Potato Park is dedicated to safeguarding and enhancing these food systems and native agrobiodiveristy .

In the case of the potato park, the epistemological bridges prescribed by the IBCH approach the traditional and science-based understandings of the multiple functions of agricultural biodiversity – including the close interaction between wild and domestic plant and how they sustain local.

The traditional knowledge, innovations, and practices of Quechua peoples are show cased in the Park for their essentially modern significance and utility including for the

purposes of pharmaceuticals, biotechnologies, agro ecotourism activities, and contributions for their community. The Potato Park is concerned with indigenous people and their traditional knowledge (The potato park, 2009).

**Figure 3.** The Potato Park: where science and traditional knowledge meet. Zoraida P. Cuzco, Peru. Source: Global Landscapes Forum

## 2.7 Propagation and micropropagation of *Solanum tuberosum L.*

Micopropagation is the fast propagation of the plant, under a controlled environment, using an appropriate culture medium. This kind of cultivation is very useful way in the improvement of the plant, because it has a potentially produce a quality plants. This propagation is done by the properties of *totipotency* **,** which is a characteristic of plant cells to generate a new a completed plant under stimuli conditions. Means that the somatic cells of any plant tissue could form stems, roots or somatic embryos according to competition and encouragement they receive (Wilder and Cabrera, 1999).

### 2.7.1 Vegetative propagation

Potatoes are mainly propagated by vegetative methods (cloning). This is the primary commercial propagation method. Vegetative reproduction ensures a uniform crop, contraire to what would happen with sexual propagation. Sexual propagation of potato is accomplished by planting the true seeds, but a high variability exist between this seed and that is why is not commonly used. However, sexual seed is becoming more and more popular; especially in places were disease pressure is very high and maintaining disease free seed is becoming a problem (Chiwon , 2003).

From biologic view, the youngest tisues (meristems) is the best material for vegetative propagation. Tissue and organs of potatoes we can use for propagation can be listed subsequently: meristem, sprouts from tubers, buds from tubers, parts of tubers, tubers, clons from stolons, clons from udergrounde stalk and clons from aerial stalk (Moravec, 2009).

Potato is well adapted to an average temperature of 17 °C. The growth stems increases by increasing temperature up to 20-25 ° C (Ingram and McCloud, 1984). High temperatures can delay the formation of stolon and tuber growth start early, the optimum temperature is 15-19 ° C (Dam et al., 1996). Means that lower temperature helps to the yields of tubers.

### 2.7.2 Generative propagation

Potato seed is an assemblage of a small tuber used for planting the crop and only potato breeders are accustomed to handling the botanical article properly referred to as seed. Hence the practice of talking and writing of true potato seed (TPS) for what should simply be called seed. I therefore define the terminology carefully so that no ambiguity attaches to the world. Here seed means sexual seed and the contrasting system is referred to as vegetative propagation (Simmonds, 1997).

Potatoes grown in the field are propagated only vegetatively via tubers, is the only way to preserve the traits valued by retailers and consumers. New shoots emerge from the 'eyes' of the mother tuber, making the ensuing daughter tuber a clone of the mother, in other words it is genetically identical (Safety, 2012).

Seed dormancy probably served the survival of potato species by increasing the chances of germination under conditions more favourable to plant growth and development in nature (Taylorson W et al., 1982). Nevertheless, seed dormancy is a nuisance for crop production and considerably lowers the possibilities of using the true seed for producing potatoes directly (Pallais, 1987).

The vegetative propagated potato must be domesticated for sexual propagation by sufficiently reducing dormancy in the TPS progeny through breeding and selection. Evidence in some wild species suggests that seed dormancy may be selected out of a population in as few as three generations (Thompson et al., 1981).

### 2.7.3 Micropropagation in vitro of *Solanum tuberosum L*.

Micropropagation is a tissue culture *in vitro* method used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment. The controlled and aseptic environment of the tissue culture laboratory provides optimum conditions for multiplication of plant cultures. Further, the culture medium, the light and the temperature can be adjusted to meet specific requirements for growth and development of specific plants and plant parts. Micropropagation is the most commercially exploited area of plant tissue culture, having been widely used for production of quality planting material in vegetatively propagated species (Naik and Karihaloo ,2007).

*In vitro* propagation of the potato by serial culture of axillary shoots (a leaf and its associated axillary bud) inseparated nodes (Goodwin et al., 1980). Is becoming established as an effective means of rapidly multiplying new or existing cultivars in disease-free conditions. (Selection of an efficient *in vitro* micropropagation, 2012).

The explant culture can be used to for produce plants from normal plants free of viral and bacterial infection. The infections are eliminating in the establishment sterile culture. For eliminate the bacterial infection and viral can be realized eliminated the isolated meristem that lack vascular bundles which represents the main paths of propagation of many plant . Bacterial contamination of explants is also possible eliminated by the addition of antibiotics to the culture medium but this antibiotics produce a toxic effect on the cell of the explants so that is why is use less frequently than the simple techniques of sterilization.

Is also possibly eliminated the virus in the explants raising the temperature (thermotherapy) thermotherapy plant ( Nyland and Goheen, 1969). Anothers autors carried out the temperature check-up directly to the meristem of the culture and with this method could be more optimal elimated the frequency of virus in the explants (Thomson et.,al 1956).

Many potato seed programs use *in vitro* pathogen-tested plantlets as starting material. The initial stages of the potato seed program can make use of varying amounths of *in vitro* micropropagation depending on the size and location of it. The basic methods used, however, are very similar in most instructions and are based on the rapid growth on solid or liquid culture media single node cutting or stems with multiple nodes (Espinoza et al., 1984).

The micropropagation of *solanum* is also propagated with grow regulators .A week of setting up the cultures two of the controls had controls had formed very small tubers .IAA treated cultures formed tubers earlier and produced larger tubers than did the control stem pieces .In contrast to the effects of maleic hydrazide (MH) and gibberellic acid (IAA) inhibeited tuberisation and caused the production of shoots. Thus it appears that IAA promotes tuber initiation and enlargement while maleic hydrazide (MH), mainly affects tuber enlargement. GA inhibits the formation of tubers and counteracts the the tuber promoting activity of both IAA and maleic hydrazide MH (Harmey et al., 1966).

Exogenous cytokinins supplementation, especially BA (6-benzyladenine),to the standard MS medium containing highconcentration of sucrose promote potato tuberization andare considered to be tuber-inducing factors (Gopal et al.,1998). Cytokinin causes stolon formation *in vitro* followed by tuberization (Forsline and Langille, 1976). Starch accumulation required for tuber initiation anddevelopment is strengthened by the finding that cytokinins influence starch formation by their inhibitoryeffect on amylase activity (Sanz et al., 1996). A relatively low concentration of auxin in the medium is required for root formation

on the culture. Indole-3acetic acid (IAA) is markedly superior to any other auxin in root forming activity. 1-Naphthaleneacetic acid (NAA) is less effective than IAA, and 2, 4-dichlorophenoxyaceticacid (2,4-D) shows no stimulation on root formation (Okazawa et al.,1967).

(Webb et al., 1983). Reported that the micropropagation was sustainable on MS media for six cultivars of potato and the shoot formation and how was the dependence of hormonal compositions of the medium employed as well as genotype used.

The percentage of regenerate roots, on the contrary, is quite high (Bancilhon and Nozeran, 1987). It has also been observed that best results concerning rhizogenesis are shown by internodes placed in inverse polarity, and on the medium in which the concentration of BA is the lowest.

Depending upon the genotype, the origin and type of the explant and the culture conditions, it is often necessary to alter the composition and /or concentration PGRs in the culture medium (Kut et al.,1984). Generally, a low ratio of auxin to cytokinin is required for adventitious shoot development (Saker et al., 2012).

From the results it can be seen that growth regulators highly affected the growth of the cells. The best growth was recorded in the medium containing NAA and KIN quite good growth was also exhibited in the medium containing NAA and BAP and in the medium with 2,4-D and KIN, poor growth was measured after cultivation in media without any regulators containing only 2,4-D (Macková et al.,1997). According to (Mejia and González , 2006).The best multiplication scheme for *Oxalis tuberous* Mol. Includes Murashige and Skoog (1962) supplemented with 1.0 $mg.l^{-1}$ BAP and 1.0 $mg.l^{-1}$ of NAA in the multiplication phase.

## 3. Objectives of the study

The aim of this thesis is the micropropagation in vitro by segments of a native species Solanum jaenense Ochoa. Evaluated the influences of two different growth regulators in different concentrations of cytokinins kinetin (KIN) and 6-benzylaminopurine (BAP) and auxins indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) for shoot enlogation and rooting.

# 4. Materials and methods

## 4.1 Plant material

The material selected for this study was a native potato *Solanum jaenense* Ochoa which is a native species of the department of Cajamarca. The main fenotypic characteristics of this species are ; its thick stems purple, green limb and purple underside and reddish-purple tuber.

This plant material as *in vitro* culture was granted from the National University Pedro Ruiz Gallo, Lambayeque, 2012. Peru. They were brought and multiplicated by nodal segments on MS medium without growth regulators in the laboratory of plant tissue cultures at the faculty of tropical Agrisiences, Czech University of live science Prague.

Jaen is a province located in the north part of the department of Cajamarca (figure 4) which take part of the Andes, highlands and jungle. According to the topographical characteristics Jaen presents altitude lower than 1,000 m.a.s.l, which make it a climate medium-wet and macro-thermal Topographical. Juan Luis podesta Losa. 2005). Jaen has diversity microclimates with an average temperature of 8.6 ºC minimu m and 36ºC maximum.

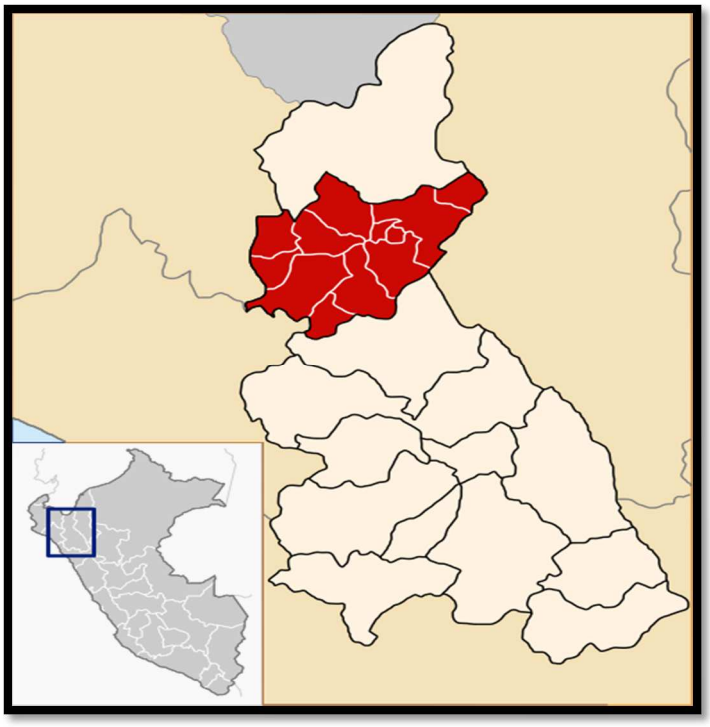


**Figure 4**. Map of Cajamarca, Jaén (Departamentos del Peru, 2015).

## 4.2 Initial experiment conditions

All the materials required for each experiments, such ass; Petri dishes, scalpels and tweezers to tinfoil were previously sterilized, (hot-air sterilizer for 3 hours), in 160°C. All the medium tested had to be autoclaved for 20 minutes, with temperature of 120°C .For the flow box, first it was necessary to cleaned it with 70% ethanol, after turn on UV lamp for at least 2 hours and turn on FATRAN at least 1 hour before use it.

Before working in the flow box the hands and all the instruments like tweezers, wrap Petri dishes, scalpels and the culture media must be disinfected with 70% ethanol.

## 4.3 Multiplication experiment.

The plant material was transferred into the flow box , get out from the culture media and put in the Petri dish already stelized , then processed to cut in nodal segments of about 1cm (Figure 5, after cutting the nodal segments were put individually in the tubs with MS medium ( Murashige and Skoog, 1962) containing 30 g. $l^{-1}$ sucrose, 100 mg.$l^{-1}$ myo-inositol, 200 mg $l^{-1}$ casein, 6 g.$l^{-1}$ agar, 200 mg.$l^{-1}$ glutamine Ph 5.7 (Table 8). All tubs were labelled and organize for future evaluated; all the cultures were maintained in a cultivation room at temperature 25±2º C, under a photoperiod 18/6 hours under light/dark conditions, with a light intensity 2000 lx (fluorescent lamps NARVA LT 36 W/010).

### 4.3.1 Root induction

For multiple root induction, 30 nodal segments were cultivated on MS medium (Musharige and skoog, 1962) supplemented with growth regulators 1naphthaleneacetic (NAA) and Indole-3-acetic acid (IAA) with different concentrations (0.25, 0.5 and 1.0 mg $l^{-1}$) (Table 4). And another 30 nodal segments were cultivated only on MS medium (control) without grow regulators.

### 4.3.2 Shoot induction

Nodal segments were cultivate on MS medium (Musharige and skoog, 1962) containing 0.5, 1.0 and 1.5 mg.$l^{-1}$ of BAP (6- benzylaminpurine) and the same concentrations were use to cultivate in MS medium with KIN cytokinin (Table 4). Thirty nodal segments were cultivated only on MS medium (control) without grow regulators.

Also were cultivate 30 nodal segments on MS medium with auxins and cytokinins together. The auxin IAA in concentrations of 0.25, 0.5 and 1.0mg$l^{-1}$ and the cytokinins KIN and BAP in concentration of 0.5, 1.0, and 1.5 mg.$l^{-1}$ (Table 5). This cultive was performed for evaluated the influence of auxins and cytokinins together, in the explants.

## 4.4 Experiment evaluation

The evaluation of measurement was done in the Laboratory of Plant Tissue Cultures (Facultyof Tropical AgriSciences), CULS Prague.

After the cultivation, once a week during one month were controlled the number of nodes, number of roots, height of the plant, height of the stem and root length.

The statistical evaluation was evaluated by analysis of variance (ANOVA) with least significant ($P \leq 0.05$) were identified by the Tukey's test [StarSoft STATISTICA 9.0].

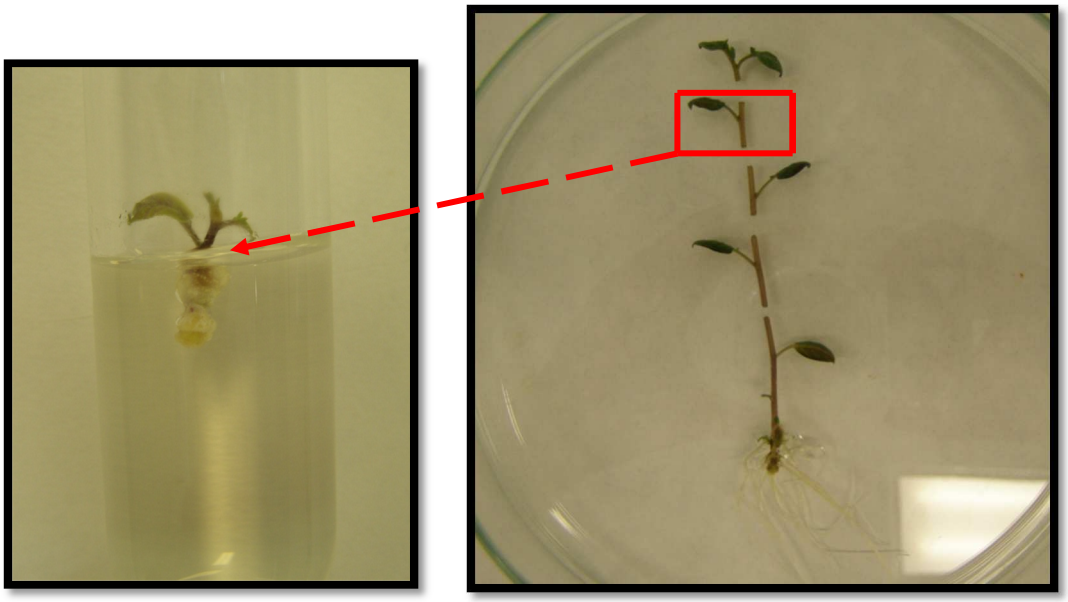


**Figure 5**. Multiplication of *Solanum jaenense* by nodal segments.

**Table 4**: Treatments with and without grow regulators

| Culture media | Growth regulators mg.l$^{-1}$ | | | | Number of explants |
|---|---|---|---|---|---|
| | Cytokinins | | Auxins | | |
| | KIN | BAP | IAA | NAA | |
| MS + | 0.5 | | | | 30 |
| | 1.0 | | | | 30 |
| | 1.5 | | | | 30 |
| | | 0.5 | | | 30 |
| | | 1.0 | | | 30 |
| | | 1.5 | | | 30 |
| | | | 0.5 | | 30 |
| | | | 1.0 | | 30 |
| | | | 1.5 | | 30 |
| | | | | 0.5 | 30 |
| | | | | 1.0 | 30 |
| | | | | 1.5 | 30 |
| | 1.5 | | 0.25 | | 30 |
| | 1.0 | | 0.5 | | 30 |
| | 0.5 | | 1.0 | | 30 |
| | 1.5 | | 0.25 | | 30 |
| | 1.0 | | 0.5 | | 30 |
| | 0.5 | | 1.0 | | 30 |
| Control medium | - | - | - | - | |

## 5. Results and discussion

The result were based in the evaluation of the following parameters: Number of nods, Number of roots, root length , length stem and height of the plant.The height average of number of shoots (7.73±4.62), length stem (3.76±1.74) and height of the plant (2.69 ± 2.44), were; respectly from cytokinins and auxins in concentrations of KIN 1.5 $mg.l^{-1}$, BAP 1.0 $mg.l^{-1}$ and NAA 0.5 $mg.l^{-1}$. Registered the higher average (8.24 ±3.62) in KIN 1.5 $mg.l^{-1}$ according to the number of nods and the lowest was registed in IAA 1.0 $mg.l^{-1}$ (4.86 ±2.51).

In shoot and root induction of auxins and citokynins (combinated) the most optimal average for the number of nods and highest of the plant were registed in concentrations of IAA 0.25 $mg.l^{-1}$ + KIN 1.5 $mg.l^{-1}$ IAA 0.5 $mg.l^{-1}$ + BAP 1.0 $mg.l^{-1}$and IAA 1.0 $mg.l^{-1}$ + NAA 0.5 $mg.l^{-1}$.

The obtain data for each concentration was optimal to conduct static analysis optimal, besides the nodal segments responded positively to the explants into the growth regulators. For the work done in the laboratory on can say that was important to be careful with the contamination like a virus or bacteria that could affect the lost of plant material. As said by Sakongo (1997), the explants materials for micropropagation are liable to be influenced by pathogens *in vitro* culture micropropagation observed in many plants. In this experiment was possibly to see the presences of pathogens in the stem of the plant.

**Shoot induction**

The main explants multiplied in these researches were nodal segments such as in this study. The cytokinins have an important role in developmental processes of shoot (koleva et al., 2012). This was also found in this study in different concentrations of KIN. The higher average of number of shoots (7.73 ±4.62) was registered in concentration of 1.5 $mg.l^{-1}$. Moreover the higher concentrations of this regulator also influence the formation of microtuber (Table 6; Figure 6). MS medium supplement with cytokins especially BA in high concentration of sucrose promote potato tuberization, therefore is consider a tuber induction factor (Gopal et al., 1998).

**Induction of rooting**

According to Okazawa et al. (1967), a relatively low concentration of auxin in the medium is required for root formation on the culture. Indole-3acetic acid (IAA) is markedly superior to any other auxin in root forming activity and 1-Naphthaleneacetic acid (NAA) is less effective than IAA. Similar results were found in this study, since a low concentration of auxins and IAA were markedly superior than the other auxin in root forming with an average of IAA (8.82± 5.62 ) and for NAA (4.75 ±8.99) (table 6) both in the same concentration of 0.5 $mg.l^{-1}$.

Badoni and Chauhan (2009) evaluated root length with $GA_3$-gibberellic acid + 0.01 $mg.l^{-1}$ NAA in MS media, their results showed higher root length in lower concentrations of 0.25 $mg.l^{-1}$ likewise NAA 0.25 $mg.l^{-1}$ showed higth root length in the present study.

**Shoot and root induction**

Another work in with *Solanum nigrum* with growth regulator reported high effect on growth of the cells (Macková et al., 1997). The optimal treatment for the induction of shoots

(8.74±3.05) and roots ( 7.00 + 2.68) in the combination of 0.5 mg.l$^{-1}$ IAA and 1.0 mg.l$^{-1}$ BAP. Compared with separate supplementation, we found better results of root induction in

IAA 0.5 mg.l$^{-1}$(8.82+5.65). However Koleva et al.,(2012) evaluated the effect of cytokinins and combination of citokinins and auxins on *in vitro* microtuber formation and growth of potato (*Solanum tuberosum L*) where combination of citokinins and auxins in higher concentrations of 2.00 mg.l$^{-1}$ BAP and NAA 1.0 mg.l$^{-1}$ showed better rooting and shoots.

The supplement with IAA in concentration of 1.0 mg.l$^{-1}$ enhanced tuber growth while cytokinin at 1.0 mg.l$^{-1}$ increased the number of tubers at 8% sucrose, both phytohormones produced a positive effect (Romanov et al., 2000). The cytokinins had a different result in this study but in both cases it was registered the induction of roots and shoots however it was not possible to see a tuber under this treatment

.

**Table 5**: Characteristics of *in vitro* cultivated plants measured after 4 weeks on MS-derived on

| Media mg.l$^{-1}$ | Days | Number of roots | Number of nods | Root length (cm) | Stem(cm) | Height plant (cm) |
|---|---|---|---|---|---|---|
| MS+KIN 0.5 mg.l$^{-1}$ | 30 | 4.66±3.56$^{de}$ | 5.79 ±2.76$^{abc}$ | 3.19±2.63$^{de}$ | 3.31±2.32$^{c}$ | 5.63 ±3.57$^{ef}$ |
| MS+KIN 1.0 mg.l$^{-1}$ | 30 | 5.0 ±3.43$^{cde}$ | 7.49±3.24$^{defg}$ | 3.34±2.27$^{def}$ | 4.50±2.56$^{def}$ | 5.29 ±2.68$^{ef}$ |
| MS+KIN 1.5 mg.l$^{-1}$ | 30 | 2.20± 1.27$^{bc}$ | 7.73±4.62$^{efg}$ | 2.42±3.27$^{cde}$ | 3.11±2.02$^{bc}$ | 4.59± 2.89$^{de}$ |
| MS | 30 | 6.39 ±2.86$^{def}$ | 6.40±4.19$^{abcd}$ | 3.55±2.79$^{ef}$ | 3.55±2.49$^{cd}$ | 3.63±2.86$^{bcde}$ |
| MS + BAP 0.5 mg.l$^{-1}$ | 30 | 0.84 ±1.09$^{a}$ | 5.99±2.62$^{abcd}$ | 1.94±2.42$^{bc}$ | 2.82±1.20$^{bc}$ | 3.02±1.27$^{bc}$ |
| MS + BAP 1.0 mg.l$^{-1}$ | 30 | 0.74 ± 1.09$^{a}$ | 6.52±2.48$^{bcdef}$ | 0.82±1.10$^{ab}$ | 3.76±1.74$^{cde}$ | 3.77±1.74$^{bcd}$ |
| MS + BAP 1.5 mg.l$^{-1}$ | 30 | 0.74± 1.21$^{a}$ | 6.06±2.96$^{abcd}$ | 0.74±1.13$^{a}$ | 2.93±1.65$^{bc}$ | 3.40± .68$^{bcd}$ |
| MS | 30 | 6.40± 4.18$^{def}$ | 6.39±2.86$^{abcde}$ | 3.55±2.79$^{ef}$ | 3.55±2.49$^{cd}$ | 3.63±2.68$^{bcd}$ |
| MS + IAA 0.25 mg.l$^{-1}$ | 30 | 3.28 ± 2.20$^{bc}$ | 8.24 ±3.62$^{g}$ | 3.24±2.17$^{de}$ | 4.71 ±2.65$^{dfg}$ | 5.26±2.92$^{ef}$ |
| MS + IAA 0.5 mg.l$^{-1}$ | 30 | 8.82± 5.65$^{def}$ | 7.95 ±3.18$^{fg}$ | 4.43 ±2.87$^{f}$ | 5.66 ±3.19$^{g}$ | 5.91 ±3.19$^{f}$ |
| MS + IAA 1.0 mg.l$^{-1}$ | 30 | 7.93 ± 5.43$^{fg}$ | 8.08 ±3.42$^{fg}$ | 2.41±2.20$^{cd}$ | 5.47 ±3.46$^{fg}$ | 6.01 ±3.59$^{f}$ |
| MS | 30 | 6.53 ± 4.58$^{efg}$ | 6.86 ±3.45$^{cdef}$ | 4.43 ±2.26$^{f}$ | 3.47 ±1.94$^{cd}$ | 3.88±2.10$^{cd}$ |
| MS + NAA 0.25 mg.l$^{-1}$ | 30 | 4.11 ±7.05$^{bcd}$ | 4.95 ±2.58$^{a}$ | 0.53 ±0.44$^{a}$ | 1.34±1.31$^{a}$ | 1.71 ± 1.54$^{a}$ |
| MS + NAA 0.5 mg.l$^{-1}$ | 30 | 4.75±8.99$^{cde}$ | 5.14 ±2.41$^{ab}$ | 0.23 ±6.44$^{a}$ | 1.59±1.18$^{a}$ | 2.69 ± 2.44$^{abc}$ |
| MS + NAA 1.0 mg.l$^{-1}$ | 30 | 2.81 ±4.99$^{abc}$ | 4.86 ±2.51$^{a}$ | 0.44 ±0.70$^{a}$ | 2.02±1.49$^{ab}$ | 2.53 ± 1.82$^{ab}$ |
| MS | 30 | 4.11 ±4.72$^{bcd}$ | 5.12 ±2.27$^{ab}$ | 2.26±3.24$^{cd}$ | 3.35±2.23$^{c}$ | 3.67 ±2.31$^{bcd}$ |

media of auxins and cytokinins.

Each value represents the mean ± SD. Values within the same column followed by different lower-case letters are significantly different at $P \leq 0.05$ according the Tukey's test.

**Table 6**: Characteristics of *in vitro* cultivated plants measured after 4 weeks on MS-derived on media combinated with cyotkinins and auxins together.

| Media | Days | Number of roots | Number of nods | Root length (cm) | Stem (cm) | Higth plant (cm) |
|---|---|---|---|---|---|---|
| MS + 0.5 mg.l$^{-1}$ IAA + 1.0 mg.l$^{-1}$ KIN | 30 | 4.04± 2.72$^{a}$ | 7.43±3.44$^{ab}$ | 3.84±2.38$^{a}$ | 6.79±3.81$^{a}$ | 7.21± 3.84$^{d}$ |
| MS +1.0 mg.l$^{-1}$ IAA + 0.5 mg.l$^{-1}$ KIN | 30 | 4.37± 3.28$^{a}$ | 7.45±4.51$^{ab}$ | 4.18±2.41$^{ab}$ | 5.41±3.58$^{b}$ | 5.85± 3.66$^{c}$ |
| MS + 1.5 mg.l$^{-1}$ IAA+ 0.25 mg.l$^{-1}$ KIN | 30 | 3.26 ±2.58$^{a}$ | 7.92± 3.79$^{b}$ | 3.22±2.12$^{c}$ | 6.07±3.62$^{a}$ | 6.46 ±3.69$^{b}$ |
| MS | 30 | 5.35± 2.19$^{a}$ | 6.37 ±2.50$^{a}$ | 4.29±1.68$^{b}$ | 3.56±1.99$^{c}$ | 3.96 ±1.81$^{a}$ |
| MS + 1.0 mg.l$^{-1}$ IAA + 0.5 mg.l$^{-1}$ BAP | 30 | 6.93± 3.88$^{b}$ | 7.25±4.07$^{c}$ | 3.72±1.73$^{a}$ | 6.34±2.88$^{a}$ | 7.08 ±2.97$^{d}$ |
| MS +0.25 mg.l-1 IAA + 1.5 mg.l-1 BAP | 30 | 5.78±5.32$^{ab}$ | 5.63±3.16$^{ab}$ | 2.04±2.16$^{c}$ | 3.61±2.42$^{b}$ | 4.00 ±2.56$^{b}$ |
| MS + 0.5 mg.l$^{-1}$ IAA+ 1.0 mg.l$^{-1}$ BAP | 30 | 7.00 ±2.68$^{b}$ | 8.74±3.05$^{d}$ | 5.01±1.58$^{b}$ | 5.86±2.51$^{a}$ | 6.32± 2.51$^{c}$ |
| MS | 30 | 5.35±2.19$^{ab}$ | 6.39±2.49$^{b}$ | 4.29±1.68$^{b}$ | 3.56±1.99$^{c}$ | 3.99 ±1.81$^{a}$ |

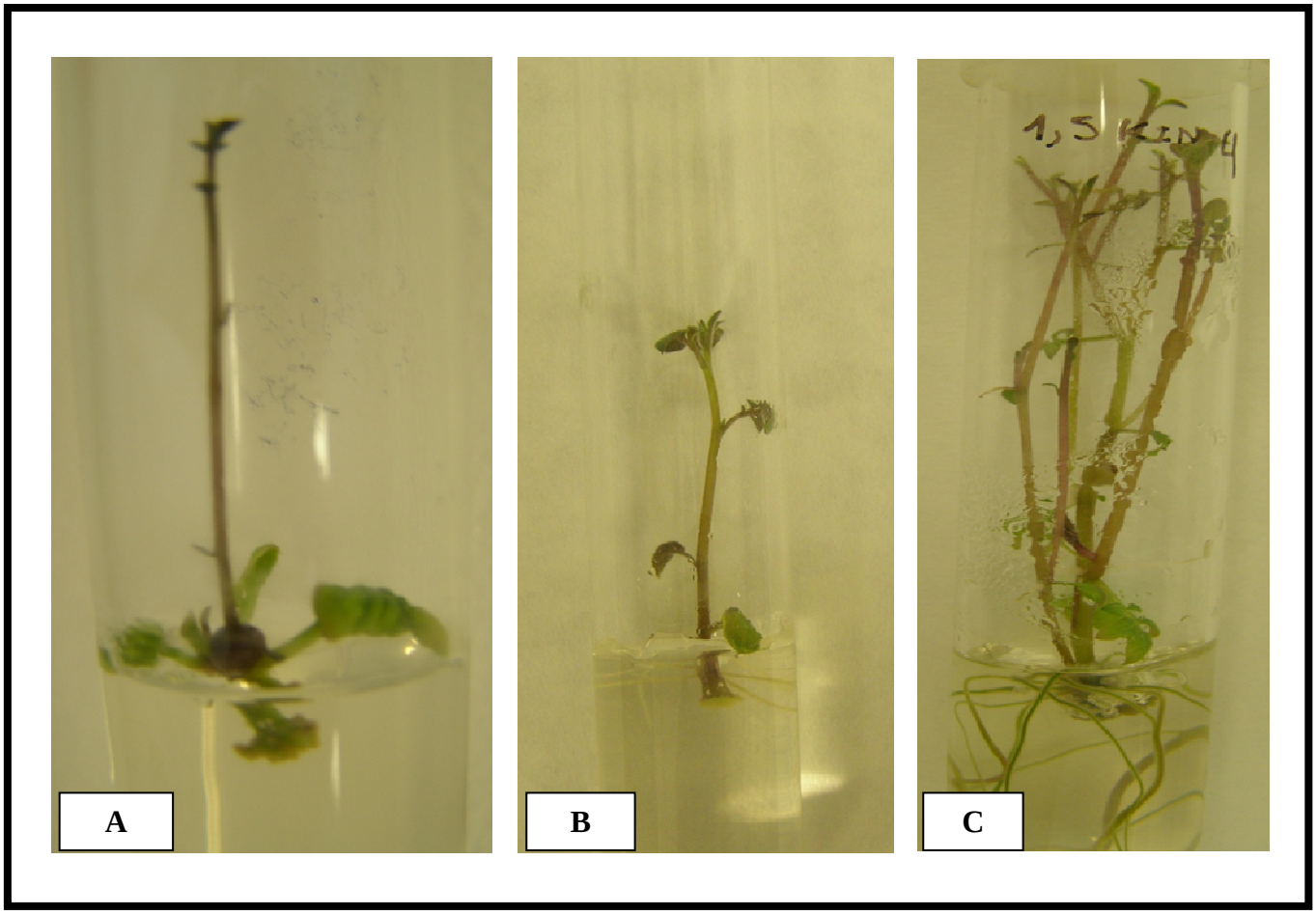



**Figure 6.** Micropropagation of *Solanum jaenense* Ochoa in MS with content of kinetin A) 1.0 mg.l$^{-1}$ presence of tuber , B) Concentration of 0.5 mg.l$^{-1}$ C) Concentration of 1.5 mg.l$^{-1}$.

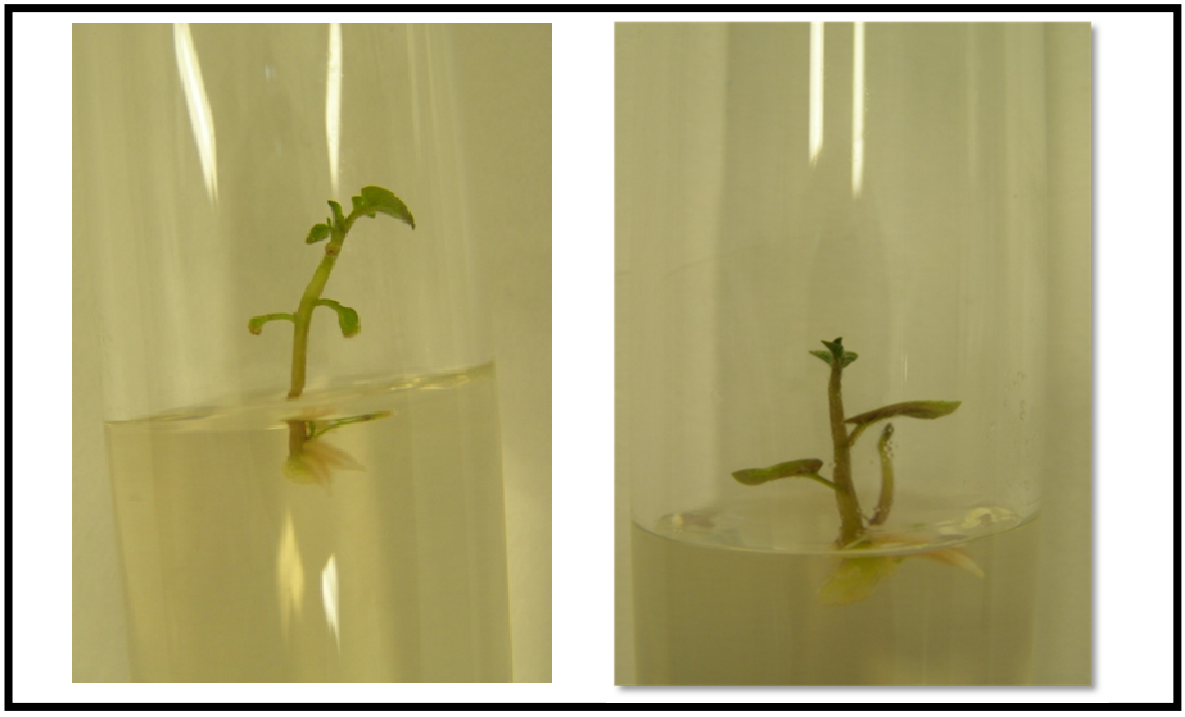


**Figure 7.** The beginning of rooting before 1 week in MS media with auxin IAA on the right with 1.0 mg.l$^{-1}$ and on the left 1.5 mg.l$^{-1}$ .

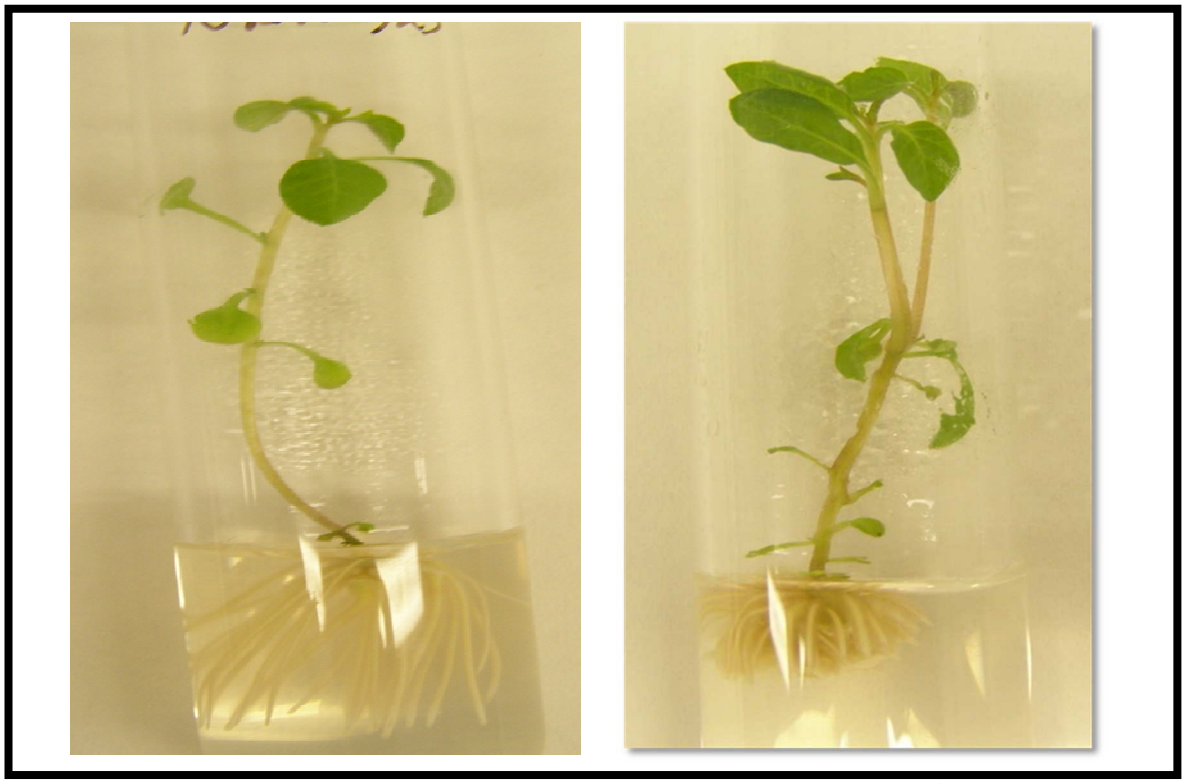


**Figure 8**. Presence of rooting in MS media and the auxin NAA 0.5 mg.l$^{-1}$ the first figure for 1.0 mg.l$^{-1}$ and the second for 0.5 mg.l$^{-1}$. After 3 weeks.

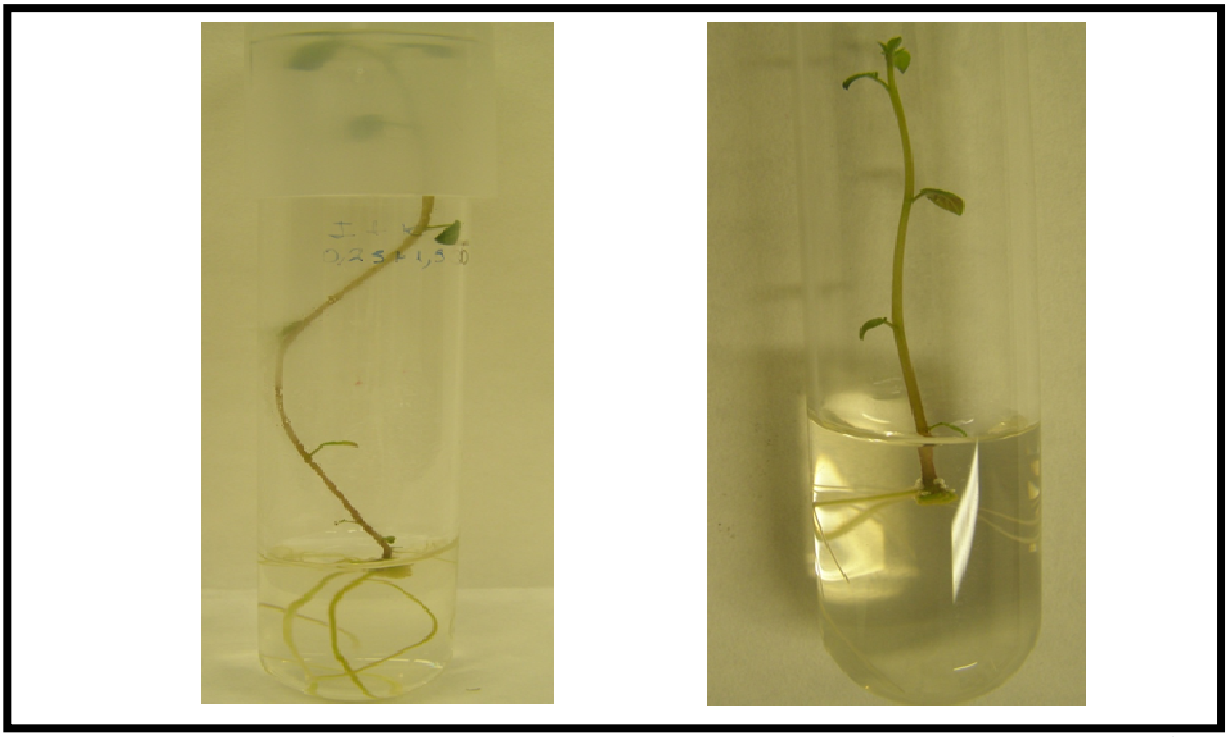


**Figure 9.** The first figure shows the growth of the plant after 4 weeks with auxin IAA 0.5 mg.$l^{-1}$ and cytokinin KIN 1.5 mg.$l^{-1}$ the second figure shows the plant in concentrations of IAA 1.0 mg.$l^{-1}$ and KIN 0.5 mg.$l^{-1}$ .

**Figure 10**. *S.jaenense* Ochoa, after four week on BAP 1.0 mg.$l^{-1}$ and IAA 0.5 mg.$l^{-1}$

# 5. Conclusion

The results obtained in this study showed that for the micropropagation of native potato species *Solanum jaenense* Ochoa by nodal segments, it is necessary to be supplemented with growth regulators.

Kinetin resulted to be a better cytokin than BAP for the regeneration of shoots and for the develop of a new shoot. For the auxins, IAA registered a better effect than NAA for root induction.

And in the combinations of auxins and cytokinins resulted with a positive effect for the regeneration of the plant propagated by nodal segments.

The optimal development of the shoots was taken in the treatment IAA 0.5 $mg.l^{-1}$ + BAP 1.0 $mg.l^{-1}$. While for root induction (8.82 ± 5.65), IAA in concentration of 0.5 $mg.l^{-1}$ was found to be adequate.

# 6. References


Badoni and J.-S. Chauhan (2009) Effect of growth regulators on meristem-tip development and in vitro multiplication of potato cultivar Kufri Himalini. Nature and Science 7: pp. 31–34.

Bancilhon L ,Nozeran R (1987) Effect of the origen of explants on callus initiation and differentiation in potato: Biotechnology in Agriculture and Foresty 3: 244-255.

Deposito de documentos de la FAO (2013) Estrategias para conservación. Available at http://www.fao.org/docrep/006/ad111s/AD111S02.htm: Accessed 2015-02-02.

Estrategia en recursos fitogenéticos para los países del Cono Sur. PROCISUR. 2001 Conservacion de recursos fitogenicos *EX SITU*.Chile. 12p.

Forsline D, Langille A, (1976) An assessment of the modifying effect of kinetin on an *in vitro* tuberization of induced and non-inducedtissue *of Solanum tuberosum* . Can. J. Bot 54: 2513-2516.

Gopal J, Minocha L, Dhaliwal HS, (1998) Microtuberization in potato( *Solanum tuberosum L*.). Plant Cell Rep 17: 794-798.

Hossain M and Sultana N (1998) Effect of Benzyl Amino Purine (BAP) and Chloro Choline Chloride (CCC) on *in vitro* tuberization of potato. Bangladesh J Ag Res. 23(4): 685-690.

Herrera C, Humberto L, Dilner J, Manejo Integrado Del Cultivo de la Papa, Colombia: Corpica. 48-49p.

Huaman Z. 2011.Semilleros comunales de papas nativas del Peru : Repatriación de semilla saneada de variedades nativas a los agricultores andinos . Mexico. Biodiversidad 30. 23p.

Herrera C, Humberto L, Dilner J, (2011) Leopoldo H. Longitude del dia Manejo integrado Del Cultivo de la papa: Corpoica. Pp 47-48.

Harmey A, Crowley M and Clinich P, ( 1966) The effects of growth regulators on tuberisation of cultured stem pieces os *solanum tuberosum* .Botany department, university college, Dublin 150: 146-147.

Hawkes, J.G (1970). Potatoes. In Genetic resources in plants – their exploration and conservation. International Biological Programme Handbook 11: 311-319.

Hawkes, J.G. and J.P. Hjerting (1969). The potatoes of Argentina, Brazil, Paraguay, and Uruguay. A biosystematic study. Oxford University Press, Oxford. pp. 525.

Huaman Z, Salas A, Gomez R, Panta A and Toledo J( 2000) Conservation of Potato. Genetic Resources at CIP International Potato Center (CIP), 1558, PP 103-111.

International potato center (2012) Comer papas nativas es nutrición. Available at http://cipotato.org/es/press-room/press-releases/comer-papas-nativas-es-comer-nutricion/: Accessed 2014-12-11.

Jaramillo A, Santos M, González H, Rodríguez M and García J (2007) El cultivo de la papa, taxonomía y morfología. Avaible at http://fflugsa.tripod.com/papa.htm. Accessed 2015-03-10.

Jimenez P, Chaparro A, Blanco J (2009) Regeneracion de plantas de papa (*Solanum tuberosum L.*) a partir de tejido foliar en las variedades diacol capiro y parda pastura. Colomia. Revista Papa. Colombia.17 p.

Jimenez A. 2014. Red electrónica de la papa, Redepapa. Available at http://redepapa.org/2014/12/12/tres-componentes-de-las-papas-nativas-que-nos-ayudan-a-estar-sanos/: Accessed 2015-01-02.

La papa y la biodiversidad (2008) Avaible at http://www.fao.org/potato 2008/es/lapapa/IYP-1es.pdf . Accessed at 2015-13-02.

Lopez E, (2012) Conservación *in vitro* de germoplasma de papas nativas de pulpa de color de la Región La Libertad, Perú. Revista Científica de la Facultad de Ciencias Biológicas Universidad Nacional de Trujillo. Trujillo. Pp. 11-13.

Malaver M. 2002 Conservacion de semilla de papa utilizando productos naturales (Chanta alta, Cajamarca), Peru: Ali arte grafico S.R.L. 2002-3836.

Moravec. I .2009. Micropropagation of wild species of potatoes, Czech Republic. Bachelor thesis. Czech University of Life Sciences, 38p.

Macková M, Macek T, kučerova T, Burkhard J , Pazlarova J and Demnerova K (1997). Degradation of polychlorinated biphenyls by hairy root culture of *Solanum nigrum*. Biotechnology letters vol 19: 787-790.

Okazawa Y, Katsura N, Tagawa T, (1967) Effects of auxin and kinetinon the development and differentiation of potato tissue cultured *in vitro.* Physiol Plant 20: 862-869.

Ocha C, 2004. The potatoes of south America: Peru. Part 1: The wild species. International Potato Center. 1036p.

Páez J, González R (2001) Conservación *In Vitro* de Dos Variedades de Papa (*Solanum tuberosum* L) bajo Condiciones de Crecimiento Mínimo. Revista Latinoamericana de la Papa 12: 121-129

Peru ecológico (2012) La papa y la alimentación mundial. Available at http://www.peruecologico.com.pe/tub_papa.htm: Accessed 2015-02-01.

Perez N, Castro D, Montoya C, Diaz C, Rios G (2008) Tuberización *in vitro* de papa (*Solanum tuberosum* L), variedad Diacol Capiro, en biorreactores de inmersión temporal y evaluación de su comportamiento en campo. Ciencia 16: 75-79.

Paksenova N, konstatanntinova T, Golznovskaya, Kossmann J (2000) Effect of indole-3acetic acid and kinetin on tuberasation parameters of different cultivars and transgenic lines of potato *in vitro*. Institut of plant physology Russian academy 32: 245-251

Paksenova N, konstatanntinova T, Golznovskaya, Kossmann J (2000) Effect of indole-3acetic acid and kinetin on tuberasation parameters of different cultivars and transgenic lines of potato *in vitro*. Institut of plant physology Russian academy 32: 245-251

Quilca N, Muñoz R, Villacres E, Monteros C, Reinoso I, (2009) Caracterizacion química y funcional de papas nativas para orientar sus usos futuros. Revista Latinoamericana de la Papa 15: 52-54.

Revilla C (2012) Centros de servicios Agropecuarios. Peru. Biodiversity. PER/OP4/RAF/08/02.

Saker M, Moussa T, Heikal N, Ellil R, Rahman A. 2012. **S**election of an efficient *in vitro* micropropagation and regeneration system for potato ( *Solanum tuberosum* L.) African Journal of Biotechnology Vol 11: 16388–16404.

Sanz J, Mingo-Castel M, van Lammeren A, Vreugdenhil D, (1996) Changes in the microtubular cytoskeleton precede *in vitro* tuber formation in potato. Protoplasma Vol.191: 46-54

Salisbury F. B. and C. W. Ross. 1992. Plant Physiology. 4th Edition. Wadsworth Publishing Co., Belmont, CA. 682 pp.

Seabrook J, .Percy L, Douglas K, and George C. Tai (1995) Photoperiod *in vitro* affect subsequent yield of greenhouse-grown potato tubers. Agriculturel and agri-food Canada 72: 365-367.

Simmonds N (2005) Patentes y biodiversidad: el control de la ingeniería genética. Available at http://www.ecologistasenaccion.org/article3174.html: Accessed 2015-02-06

Teisson C, Alvard D ,(1999) *In vitro* production of potato microtubers inliquid medium using temporary immersion. Potato Research 42: 499-504

Tapia M, Rosas A, (1998) Sistematizacion de la Experiencias en Conservacion *In Situ* de los recursos fitogenicos, Cajamrca. Peru. Condesan-Aspaderuc-CIP-GTZ. 28p

Webb K, Osifo E, Henshaw GG (1983) Shoot regeneration fromleaflet discs of six cultivars of potato (*Solanum tuberosum* subsp. *Tuberosum*). Plant Science letters. Lett 30:18

**Table 7**: Components of MS medium (Murashige and Skoog) 1962.

| Medium Murashige – Skoog | | | |
|---|---|---|---|
| supply solution to 1L destille water | | Weight to 1L supply solution | For 1L is need (pH 5,7) |
| A | NH4NO3 | 16,5 g | 100 ml |
| | KNO3 | 19 g | |
| | CaCl2 | 3,3 g | |
| | MgSO4 x 7H2O | 3,7 g | |
| | KH2PO4 | 1,7 g | |
| B | H3BO3 | 0,62 g | 10 ml |
| | MnSO4 x 4 H2O | 2,23 g | |
| | ZnSO4 x 4 H2O | 0,86 g | |
| C | KI | 0,083 g | 10 ml |
| | Na2MoO4 x 4H2O | 0,025 g | |
| D | CuSO4 x 5 H2O | 0,0025 g | 10 ml |
| | CoCl x 6 2O | 0,0025 g | |
| E | Na2EDTA | 3,72 g | 10 ml |
| | FeSO4 x 7 H2O | 2,78 g | |
| V | nicotin acid | 0,05 g | 10 ml |
| | pyridoxin | 0,05 g | |
| | thiamin | 0,01 g | |
| | glycin | 0,2 g | |

**Direct weight to media:**

Myoinositol 0,1 g

Sacharosa 30 g

Agar 8 g