

**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

Faculty of Tropical AgriSciences



Czech University of Life Sciences Prague  
**Faculty of Tropical  
AgriSciences**

**Chlorophyll content in Yacon (*Smallanthus sonchifolius*)  
landraces and its relationships  
with plant characteristics**

**MCs. Thesis**

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## **Declaration**

I hereby declare that this thesis entitled “Chlorophyll content in yacon (*Smallanthus sonchifolius*) landraces and its relationship with plant characteristics” is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague, April 2017

Bc. Alejandro Samper Salvador

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## Abstract

Yacon (*Smallanthus sonchifolius*) is a perennial plant that inhabits the Andean mountains and belongs to the Asteraceae family. The yacon crop has become very popular within some countries for its edible roots as well as for its medicinal and antioxidant properties. Several studies deal with the optimization of the crop for a better yield. To date, there are not studies about the relationships between photosynthetic pigments content and plant characteristics. The main goal of this study was to determine the chlorophyll (a, b) and carotene contents as well as several morphometric parameters in 24 yacon landraces from three different countries (Bolivia, Ecuador, and Peru) and with different ploidy level (octoploids:  $2n=58$  and dodecaploids:  $2n=87$ ). The photosynthetic pigments were firstly assessed with a chlorophyll meter (SPAD) during three months (July, August, and September). For this purpose, three clones of each plant landraces were used and ten SPAD measurements per clone were taken. Furthermore, in September photosynthetic pigments were extracted from fully expanded leaves using 200 mg of fresh material (acetone extraction). Chlorophylls (a, b) and carotene contents were determined with a spectrophotometer using three wavelengths (663, 645, and 470 nm, respectively). At the end of the vegetation period, the morphology of different plant structures and tuberous roots mass were determined. According to the results obtained, the chlorophylls and carotene content were significant lower in September in comparison with July and August ( $p < 0.05$ ). The results also shown that dodecaploids plants tend to have a higher photosynthetic pigments content and leaf area than the octoploids plants. Related with plant morphology, the leaf area and the width of the stems showed a negative relationship with chlorophyll and carotene contents ( $p < 0.05$ ). On the other hand, the mass of the tuberous roots was positively related with the plant height ( $p < 0.05$ ). The plants with a lower leaf size could be more appropriate for its medicinal use due to the higher chlorophyll and carotene contents. It is advisable to harvest the leaves till the end of August. The photosynthetic pigments assessment could be considered as a tool for a better exploitation of the yacon crop.

**Keywords:** crop production, photosynthetic pigments, plant morphology, ploidy level, *Smallanthus sonchifolius*.

## Resumen

Yacón (*Smallanthus sonchifolius*) es un cultivo perenne perteneciente a la familia Asteraceae. Este cultivo tiene su origen en la región Andina. El cultivo del yacón ha llegado a ser muy popular dentro de algunos países por sus raíces comestibles, así como por sus propiedades medicinales y antioxidantes. Varios estudios tratan sobre la optimización del rendimiento del cultivo. No existen estudios sobre la relación entre el contenido de pigmentos fotosintéticos y las características de la planta. El principal logro de este estudio fue determinar el contenido de clorofila (a, b) y caroteno, así como varios parámetros morfológicos en 24 genotipos de yacón de tres países diferentes (Bolivia, Ecuador y Perú) y con diferente nivel de ploidía (octoploides:  $2n=58$  y dodecaploides:  $2n=87$ ). Los pigmentos fotosintéticos fueron primeramente evaluados con un medidor de clorofila (SPAD) durante tres meses (julio, agosto y septiembre). Para este propósito, tres clones de cada genotipo de planta fue usado y diez medidas por clon con el SPAD fueron realizadas. Además, en septiembre los pigmentos fotosintéticos fueron extraídos de las hojas usando 200 mg de materia fresca (extracción con acetona). El contenido de clorofila (a, b) y caroteno fue determinado con un espectrofotómetro usando tres longitudes de onda (663, 645 y 470 nm, respectivamente). Al final del periodo vegetativo, la morfología de las diferentes partes de la planta y la masa de los tubérculos fueron determinadas. El contenido de clorofilas y caroteno fue significativamente más bajo en septiembre, en comparación con julio y agosto ( $p < 0,05$ ). Los resultados también mostraron que las plantas dodecaploides tienden a tener un alto contenido de pigmentos fotosintéticos y área foliar más que las plantas octoploides. En relación con la morfología de la planta, el área foliar y el diámetro del tallo mostraron una negativa relación con los contenidos de clorofila y caroteno ( $p < 0,05$ ). Por otra parte, la masa de los tubérculos tenía una relación positiva con la altura de la planta ( $p < 0,05$ ). Las plantas con hojas pequeñas pueden ser más apropiadas para su uso medicinal debido a sus altos contenidos de clorofila y caroteno. Se aconseja cosechar las hojas hasta finales de agosto. Los pigmentos fotosintéticos pueden ser considerados como una herramienta para una mejor explotación del cultivo del yacón.

**Palabras clave:** producción del cultivo, pigmentos fotosintéticos, morfología de la planta, nivel de ploidía, *Smallanthus sonchifolius*.

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## **List of abbreviations**

FOS = Fructooligosaccharide

dm = dry matter

masl = meter above the sea level

SD = Standard deviation

Gl = Degrees of freedom

FAO = Food and Agriculture Organization

ICFR = International Council for Fitogenetic Resources

LAI = Leaf area index

COB = Canopy cover

## 1 INTRODUCTION

Yacon [(*Smallanthus sonchifolius*) (Poepp. & Endl.) H. Rob.], from Asteraceae family, is a vigorous, herbaceous perennial plant. Firstly was from the family Compositae (Wells, 1967) which was re-established in the genus *Smallanthus* (Robinson, 1978). This tuber crop is closely related to the sunflower family (Asteraceae). The shape of the tuberous roots is similar to sweet potatoes, but they have a much sweeter taste and crunchy flesh (Lachman et al., 2003). Yacon is a domesticated plant which comes from Incas population. It is cultivated from southern Colombia to northern Argentina, between 1800 and 2800 m above the sea level (Svobodová et al., 2013).

It is a tuber crop originated from the Andes, is cultivated for its tuberous roots, which have a high content of fructooligosaccharides (FOS), an special kind of sugar with many benefits to the human health (Seminario et al., 2003). It is also cultivated for medicinal infusions from its leaves, which are rich in phenolic content with strong antioxidant activities. This special root can be eaten in fresh; it has a sweet taste so it is considered as a fruit (Viehmánová et al., 2009).

The vegetative propagation helps in maintaining the genetic stability of the clones, but together with high ploidy level – most yacon clones are octoploids or dodecaploids (Grau and Rea, 1997) – it is a limiting factor for a breeding of yacon. Newly formed polyploids differ in their response to the environment compared with their progenitors and provide understanding of the ecological advantages of polyploidy (Leitch et al., 2008).

The energy that the plants use to grow and support themselves comes from the luminous energy transformed in the process of photosynthesis. This explains the lineal relation existing between the amounts of luminous radiation absorbed by the plant and its growth in dry weight.

The photosynthesis depends equally on the surface area of the crop able to absorb the energy (leaves), and on the activity of the photosynthetic apparatus for the unity of the green area of the crop (converting the radiation into a chemical energy) (Aguirrezábal et al., 2001).

Chlorophyll is an extremely important biomolecule in photosynthesis, a substance that allows plants to absorb energy from the sunlight. It was shown that the use of aqueous extracts of the leaves, besides its antioxidant and antimicrobial properties, can also affect hypoglycemia (Arnao et al., 2011). This is the reason behind the use of leaves as an infusion.

The leaf chlorophyll content is a useful parameter to evaluate the physiological process of the plants. In the 400-700 nm range, all green leaves have major absorption features caused by electron transitions in chlorophyll and carotenoid pigments (Zhang et al., 2007). The content of photosynthetic pigments can change due to factors of stress, photosynthetic capacity or the situation of development of the plant (Ustin et al 1998).

Nowadays the majority of studies about yacon are related with their tuberous roots. However, there are not studies about the content of photosynthetic pigments in yacon.

## **2 LITERATURE REVIEW**

### **2.1 Taxonomy**

#### **2.1.1 The genus**

Yacon and its relatives were originally placed in *Polymnia* (Compositae, Heliantheae, subtribe Melampodinae), a genus founded by Linnaeus in 1751.

In the first modern revision of the genus, Wells (1967) maintained yacon and its relatives within *Polymnia*.

Robinson adopted a different perspective in a study (1978). He also re-established the genus *Smallanthus*, proposed by Mackenzie in 1933.

Robinson separated the species previously considered within *Polymnia* by Wells into two different genera – *Smallanthus* and *Polymnia*- keeping both within the subtribe Melampodinae:

One North American species, most of them, Central American species and all South American species were placed in *Smallanthus*, while a few North American species remained in *Polymnia*.

Some of these differences include striation on the cypsela surface, presence of a whorl of outer involucre bracts, absence of glands on the anther appendages, lack of a particular feature in the lobes of the disk flower corollas (Robinson, 1978).

*Smallanthus* sensu Robinson includes at least 21 species, all from America, ranging mostly through southern Mexico and Central America and the Andes (Grau and Rea, 1997).

#### **2.1.2 The species**

*Smallanthus sonchifolius* [(Poeppig and Endlicher) H. Robinson, Phytologia 39 (1): 47-53, 1978], species was previously known as *Polymnia sonchifolia* (Poeppig and Endlicher, 1845) and *Polymnia edulis* (Weddell, 1857), which were synonyms.

### 2.1.3 The other *Smallanthus* species

According to Robinson, 1978, those species are:

*Smallanthus apus* (Blake) H. Robinson  
*Smallanthus connatus* (Spreng.) H. Robinson  
*Smallanthus fruticosus* (Benth.) H. Robinson  
*Smallanthus glabratus* (DC.) H. Robinson  
*Smallanthus jelskii* (Hieron.) H. Robinson  
*Smallanthus latisquamus* (Blake) H. Robinson  
*Smallanthus lundellii* H. Robinson  
*Smallanthus macroscyphus* (Baker ex. Martius) A. Grau  
*Smallanthus maculatus* (Cav.) H. Robinson  
*Smallanthus macvaughii* (Wells) H. Robinson  
*Smallanthus meridensis* (Steyerm.) H. Robinson  
*Smallanthus microcephalus* (Hieron) H. Robinson  
*Smallanthus oxacanus* (Sch. Bip. ex Klatt) H. Robinson  
*Smallanthus parviceps* (Blake) H. Robinson  
*Smallanthus pyramidalis* (Triana) H. Robinson  
*Smallanthus quichensis* (Coul.) H. Robinson  
*Smallanthus riparius* (H.B.K.) H. Robinson  
*Smallanthus siegesbeckius* (DC.) H. Robinson  
*Smallanthus suffruticosus* (Baker) H. Robinson  
*Smallanthus uvedalius* (L.) Mackenzie

### 2.2 Origin of yacon

Yacon is considered to be originated in the northern and central Andean regions, as several of its wild relatives show preference to disturbed habitats characterized by vegetative gaps such as landslides, river banks and roadsides.

This particular strategy of yacon to colonize areas free of vegetation could have been the way it got discovered by early Andean inhabitants, especially as they were employing slash and burn agricultural practices since the prehistoric times.

This allowed them to discover yacon's nutritional uses and properties, and convert it from its weed state to a managed plant, and later on to a cultivated agricultural plant, this is the reason why it is considered that yacon originated in the Andean regions and has been cultivated since the pre-Inca times (Grau and Rea, 1997).

The Eastern humid slopes of the Andes, extending from northern Bolivia to central Peru regions, are widely considered to be the main areas where these early occurrences took place.

Although the mountain forest of these regions are evergreen and supplied with a lot of rainfall and mist during most of the year, they often suffer a relatively dry and slightly cooler winter which last two to four months.

This particular characteristic could have played a critical role in the evolution and generative conditions under which large tuberous roots could have developed an advantage.

Therefore, it seems likely that a hybrid of two or more *Smallanthus* species had colonized disturbed areas and gave rise to a species ancestral to yacon (Grau and Rea, 1997).

It is consider that the larges germplasm diversity of yacon occurs in Ecuador, Colombia and southern Peru (Seminario el al., 2003).

### **2.3 Geographic distribution**

Yacon grows in the Peruvian valleys around Cusco and Puno with maximal concentration in the northern and southern mountains of Cusco, between altitudes of 2 000 and 3 000 meters above the sea level (Singh, 2011).

It is said that yacon cultivation is centralizes in 18 of the 24 regions of Peru and the total area of cultivation is around 600 ha (Seminario et al., 2003).

Yacon is being grown in many localities scattered throughout the Andes, from northwestern Argentina to Ecuador. It is normal to cultivate yacon for family consumption. Less frecuently yacon is grown as a cash crop to be marketed at the local level. Is not common to find yacon in the north of Argentina (Grau and Rea, 1997).

Thus, this area seems to be the most likely 'origin centre' of the species (Figure 1).





**Figure 1.** Yacon distribution in the Andean region

**Source.** Grau and Rea 1997

The diversity of clones is more reduced in Ecuador, which indicates that the species was introduced to this region with the Inca conquest of Ecuador, decades before the Spanish invasion (Grau and Rea, 1997).

From the humid mountain forest of Peru and Bolivia, yacon might have expanded to the north and south along the humid slopes of the Andes, to the Peruvian coast and to the dry inter-Andean valleys. It is in the coastal archaeological sites of Nazca (500 aC-700 dC), Peru that the oldest phytomorphic representations of yacon have been identified, depicted on textiles and ceramic material (Safford, 1917; Yacovleff, 1933; O’Neal and Whitaker, 1947).

According to Pérez Arbeláez (1956), yacon was exhibited for the first time in Europe at the Paris exhibition at the beginning of the century. European interest was not enough. In Italy there was a serious cultivation attempt in the late 1930s, which faded during World War II (Calvino 1940).

Affected by deep cultural changes, yacon cultivation has declined slowly and steadily throughout the Andes during most of the present century (Grau and Rea, 1997).

According to Fernández (2005), in 1981 yacon was declared an endangered species by FAO (Food and Agriculture Organization), because until the year it was permanently close to disappear likely to other crops. From this point FAO decided to

support research and development of yacon through ICFR (International Council for Fitogenetic Resources).

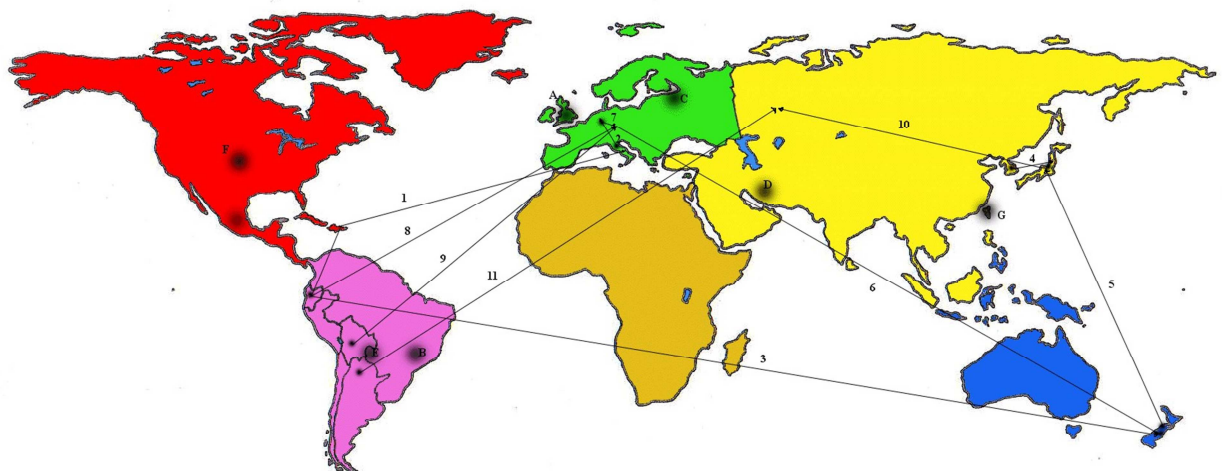
The growing interest in the crop outside the Andes has stimulated a new wave of attention and research on yacon in the Andean countries. In the last three decades yacon cultivation has extended to other continents (Grau and Rea, 1997).

Even so, it still remained a little known crop until it was introduced again in the 1990s to the European continent as a plant with many nutritive values as well as containing fertility enhancing properties (Valentova et al., 2001).

In present time, yacon is distributed in New Zealand, Japan, Korea, Brazil, Czech Republic, China, United States, Paraguay, and Taiwan (Seminario et al., 2003).

Yacon was introduced to the Czech Republic in 1993 (Valentová et al., 2003) to study its medicinal properties, with preliminary results of this study showing that yacon can be grown successfully outside its natural habitat (Lebeda et al., 2003).

The geographic distribution (and year of introduction) of yacon outside the Andes according to Fernández (2005):



**Figure 2.** Geographic distribution (and year of dissemination) of yacon outside the Andes

**Source.** Fernández, C. E. 2005. Habilitation thesis, 154 pp. Prague

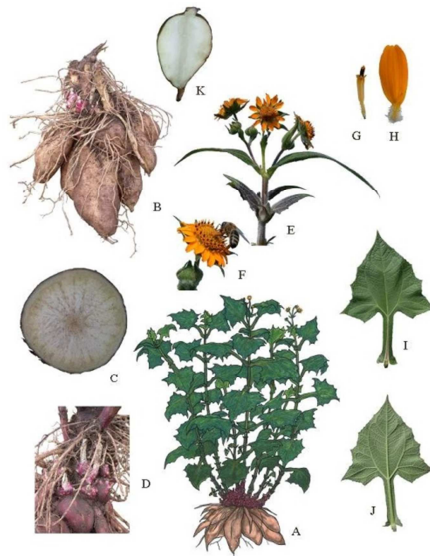
1. From Dominican Republic to Italy, in 1927
2. From Italy to Germany, in 1941
3. From Ecuador to New Zealand, in 1960s
4. From Korea to Japan, in 1970s

5. From New Zealand to Japan, in 1985
6. From New Zealand to Czech Republic, in 1993
7. From Germany to Czech Republic, in 1994
8. From Ecuador to Czech Republic, in 1994
9. From Bolivia to Czech Republic, in 1995
10. From Japan to Russia, in 1994
11. From Argentina to Russia, in 1994-1995

A-G: Other countries where the yacon was disseminated (the UK, Brazil, Estonia, Iran, Paraguay, the United States, and Taiwan).

## 2.4 Morphology

The different parts of the plant are: stem, leaves, root, flowers and fruit.



**Figure 3.** Morphology and anatomy of yacon: (A) yacon plant; (B) tuberous roots; (C) transverse section of tuberous root; (D) rhizomes; (E) flowering branches; (F) flower head; (G) staminate disc flower; (H) pistillate ray flower; (I) adaxial (upper) side of leaf; (J) abaxial (lower) side of leaf; (K) longitudinal section of tuberous root.

**Source.** Fernández, E. 2005.

### 2.4.1 Stem

In a plant is possible to find three main stems (Figure 4) but sometimes the number of main stems reaches up to five. There are between 4 and 12 secondary stems growing from the primary stems, not in case of the main stem. The branching of the stems normally starts from 4 to 5 months after planting.

Three types of branching are identified:

- Basal → occurs in the main stem
- Overall → branches form along the whole stem
- Top branch → occurs in the upper third of the plant

The number of secondary stems its from 2 to 10 (Lebeda et al., 2011).



**Figure 4.** Yacon stem

**Source.** Author

### 2.4.2 Leaves

Leaves (Figure 5) are opposite and narrow, growing out of aerial nodes. There are two types of leaves:

- Upper leaves: ovate-lanceolate, without lobes and hastate base.
- Lower leaves: broadly ovate and hastate or subhastate, connate and auriculate at the base of the plant.



**Figure 5.** Leaves of yacon

**Source.** Author

The surfaces of both of them, are densely pubescent. The epidermis of both is covered by trichomes (from 0.8 to 1.5 mm long, 0.05 mm diameter) and glands that contain terpenoid compounds (Lebeda et al., 2011).

When the plant reaches the flowering state, the yacon plant contains from 13 to 16 pairs of leaves. Finally, at the end of the flowering stage, plants only produce small leaves (Seminario et al., 2003).



**Figure 6.** Detail of yacon leaves

**Source.** Seminario et al., 2003

The chemical composition of the leaves are summarized in Table 1.

**Table 1.** Chemical composition of yacon leaves

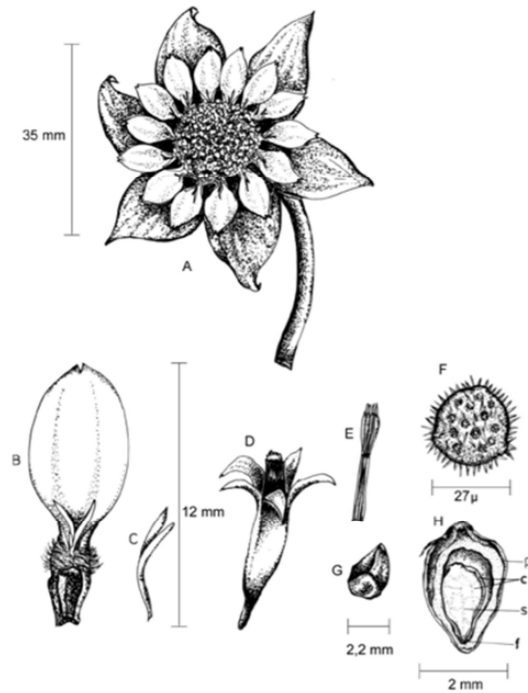
Compunds (%)	Leaves (fresh)	Leaves (dry)
Water	83.2	-
Ash	2.7	12.5-16
Proteins	2.9	17.1-21.2
Lipids	1.2	4.2-7.4
Fiber	1.7	10-11.7
Saccharides	1.4	8.6

**Source.** Lebeda et al., 2011

### 2.4.3 Flowers

The flower (Figure 7(A)) is composed by an inflorescence called “chapter”. Each “chapter” has between 14 and 16 female flowers (zygomorphic) and between 80 and 90 male flowers. The female flowers open before the male flowers (Seminario et al., 2003).

Yacon flowers are two or three toothed ray flowers, with 12 mm long and 7 mm broad, which are pistillates. They are yellow to bright orange and it depends on the clone. Pollen grains are globular, sometimes with 3 poles, bright yellow, glairy at the surface and about 27  $\mu$  in diameter (Lebeda et al., 2011).



**Figure 7.** Inflorescence and flowers of yacon. (A) inflorescence; (B) female flower; (C) pistil; (D) male flower; (E) stamens; (F) pollen grain; (G) fruit; (H) fruit structure

**Source.** Seminario et al., 2003

#### 2.4.4 Fruit

The fruit (Figure 7 (G)) is an achene. The pericarp is thin and dry in the maturity and has external stretch marks. It measures approximately 3.7 mm long and 2.2 mm wide (Seminario et al., 2003).

The endosperm in yacon fruit is not present and the storage components are located in cotyledons. When immature, they are coloured purple. They then turn dark brown to black when matured, with a dry thin pericarp on the outer part of the fruit marked with a longitudinal serration forming parallel groves (Meza, 2001).



#### 2.4.5 Root

Yacon has two types of roots (Figure 11): fibrous, thin ones that set the plant in the soil and absorb the water and nutrients and other thickened stores of white or purple colour (Seminario et al., 2003).

The thick storage tubers are formed at the bottom of rhizomes within 90 to 120 days after the moment of plantation. This storage tuberous roots are responsible of the synthesis of storage substances, which are the valuable part of the crop.

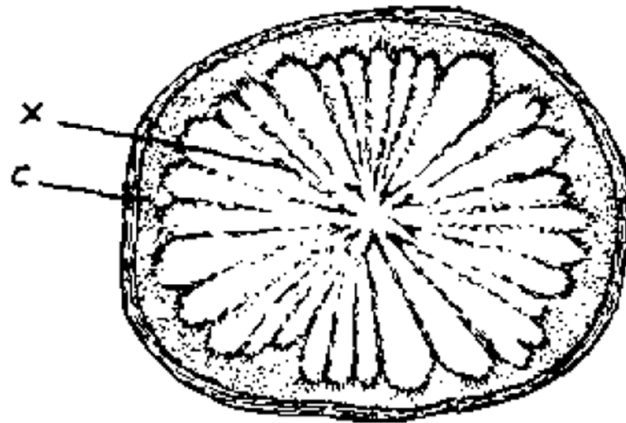
The reason of the tuberous root growth is the proliferation of parenchymatous tissue in the root cortex and focused in the vascular cylinder.



**Figure 8.** Yacon tuberous root

**Source.** Author





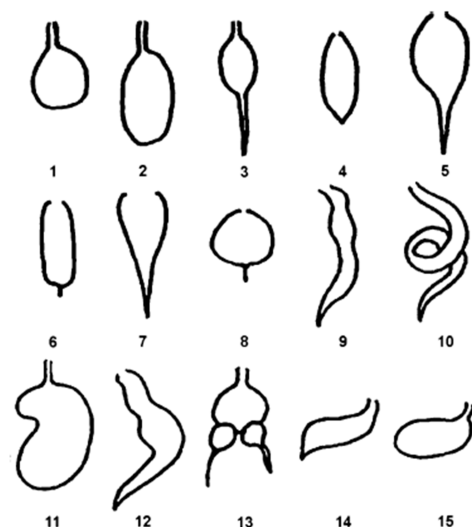
**Figure 9.** Transverse section of the tuberous root (x, xylem; c, cortex tissues)

**Source.** León 1964

At the beginning, tuberous roots are fusiform (almost all of them) and later the shape becomes elliptical or circular. In some cases, this roots acquire irregular shapes due to the stones at the soil or sometimes due to the pressure of neighboring roots (Grau and Rea, 1997; Lebeda et al., 2011).

According to Bredemann (1948) and Calvino (1940) the shapes of tuberous roots is due to tuber development and not by the cultivar (Lebeda et al., 2011).

These are the tuber shapes (Figure 10):



**Figure 10.** Categories of yacon morphotypes

**Source.** J. Frcek 2001

Their size and shape depend on the particular clone (Valentová et al., 2003).

The roots have a thin skin which is firmly attached to the flesh of the rhizome and the tissue of the tuberous root is soft because the flesh contains around 90% of water, making them fragile for harvesting and transporting (Manrique et al., 2005).

The tuberous root bark is thin (1-2 mm) with a cream, purple, pink, brown or ivory white colour. The flesh colour, typical for each genotype, is yellow, pink, cream and white with purple striations (Lebeda et al., 2011).

The factors of the storage roots: variety, type of soil or the country, can influence in the shape or size. The usual weight is between 200 g and 600 g (Seminario et al., 2003).

The stem tubers of yacon called rhizomes, serve for the propagation of the crop. They are branched and have an irregular shape, containing a lot of buds in the upper part, usually white, creamy and purple colours, influenced by the soil type and genotype (Lebeda et al., 2011). These rhizomes named “cepas”, grow directly on the basal part of the main stem. A mature rhizome can be broken from 10 to 20 parts with approximately 3-5 growing buds (Valentová and Ulrichová, 2003; Manrique et al., 2005).

Thin fibrous roots are found in the bottom of the rhizomes, that later becomes bulky and lignified with a weight between 0.5 and 4.5 kg that depends on the size, ecotype, thickness and environmental conditions (Lebeda et al., 2011).



**Figure 11.** Roots of yacon  
**Source.** Author

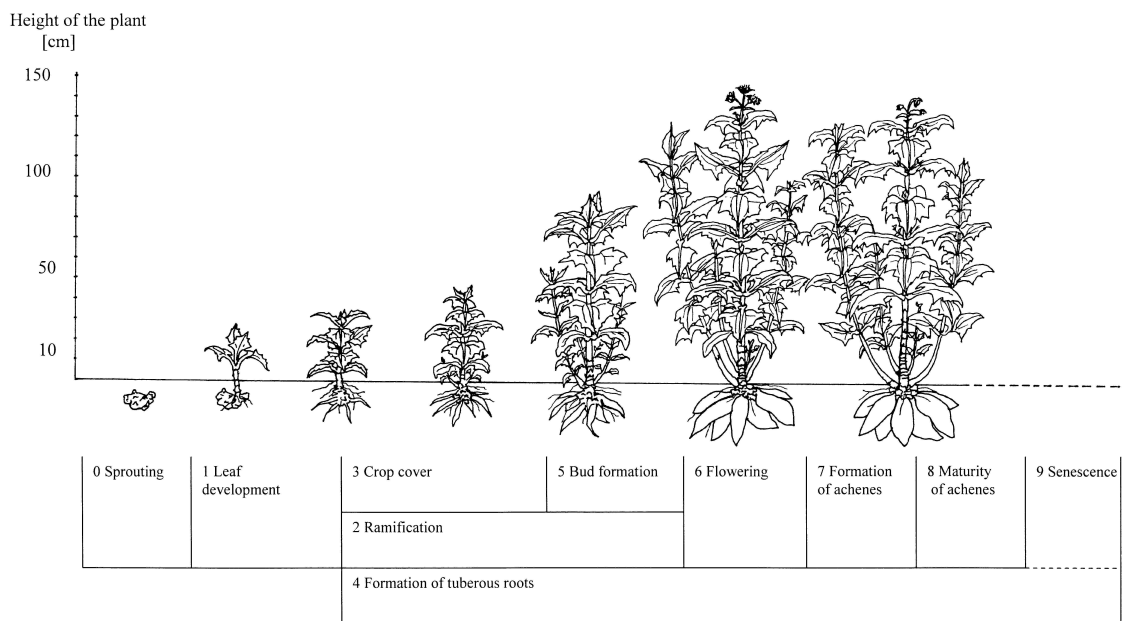
## 2.5 Reproductive biology of *Smallanthus sonchifolius*

According to Grau and Rea (1997) yacon is out-crossing, and for that reason it needs pollinating agents; its pollen has a low level of fertility.

The main argument of this out-crossing is the glairy of the pollen grain and the colours of the female flowers, also producing sweet substances, which makes it attractive to the insects like bees, flies, butterflies, bumblebee (Seminario et al., 2003).

Yacon has a low level of sexual reproduction, although the flowers has normal pistils and stamens (Polanco, 2011).

Yacon has ten principal growth and development stages (according to BBCH scale): 0 Sprouting, 1 Leaf development, 2 Ramification, 3 Crop cover, 4 Formation of tuberous roots, 5 Bud formation, 6 Flowering, 7 Formation of achenes, 8 Maturity of achenes, and 9 Senescence (Figure 12).



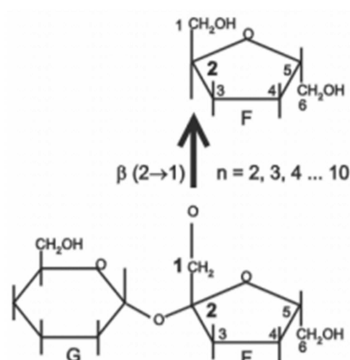
**Figure 12.** Principal growth stages of yacon (according to BBCH scale)

**Source.** Fernández E. et al., 2007

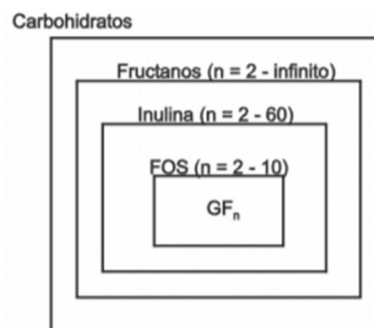
### 3 PROPERTIES OF YACON

#### 3.1 Tuber

Yacon is one of the roots storing food with major content of water (Seminario et al., 2003), that usually exceeds 70% of the fresh weight. Due to high water content, root energy value is low. The tuberous roots contain only 0.3-3.7 % protein, but 70-80 % of the dry matter (dm) is constituted by saccharides, mainly fructooligosaccharides (FOS) (Lachman et al., 2004).



**Figure 13:** Chemical composition of FOS



**Figure 14:** Inuline and FOS

**Source:** Seminario et al., 2003

The roots accumulate also potassium, composed polyphenols, antioxidant substances and other ingredients with fungicidal activity (Seminario et al., 2003).

The consumption of fresh yacon roots has an hypoglycemic effect in healthy people (Mayta et al., 2001).

The acute consumption of the fresh root of yacon reduces the posprandial glycemc response in healthy subjects. So it has an hypoglycemic effect (Mayta et al., 2004).

Yacon consumption, using the fresh roots or the leaves as an infusion, it is a natural excellent choice to the diabetic people because it does not increase the level of glucose in blood (Palacios et al., 2007).

Yacon is a plant valued for having nutritious and medicinal features and those have contributed to its use in treating diabetes once the hypoglycemic action have been attributed (Arnao et al., 2011).

The root is considered prebiotic and hypocaloric, because of its FOS content (Arnao et al., 2011).

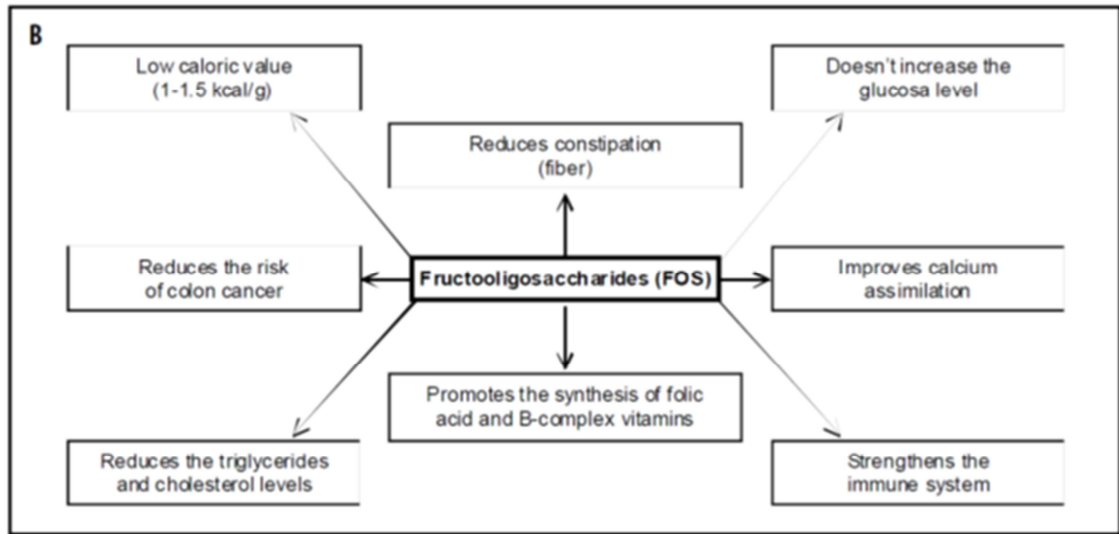
The FOS have various features:

- Intestinal microflora and prebiotic effect: the FOS can not be metabolized in the digestive track but they are fermented in the colon by the group of bacteria of intestinal flora. Those bacteria have benefits for the health; moreover they inhibit the growth of other bacteria that produce toxins. This feature of FOS is called prebiotic effect.
  
- Diabetes, obesity and overweight: the FOS contribute less calories than the majority of carbohydrates, and this is why they are a low calory substitutes of many sugars and can be included in the weight loss diet (Manrique et al., 2003).

Other favorable health effects have also been associated with the fermentation of FOS in the colon. These include:

- Cholesterol and triglycerides: reduction of level
- Calcium assimilation
- Colorectal cancer: inhibition of the production of toxins
- Strengthening the immune system
- (Seminario et al., 2003).

These three last features have been investigated on laboratory rats, but the results of the studies realized on humans show at occasions the contrary.



**Figure 15.** Some health effects associated with the consumption of FOS

**Source.** Manrique et al., 2005

To summarize, the roots of yacon are a source of little calories for human, have a low glycemic index (level of glucose in the blood) and can be considered an alternative product in diet of diabetics, as well as those who try to fight overweight or obesity (Manrique et al., 2005).

Its high water concentration and fiber contribute to reduce the hunger and can prevent and control constipation (Manrique 2003).

### 3.2 Leaves

As oppose to the roots of yacon, where the FOS is a main ingredient, the plant's leaves have a very little amount of water and its chemical components are still little-known. So far, it has been stated that they contain among others sesquiterpenes, lactones and flavonoids. Some of those components have a fungicide and antioxidant activity, for which in the future bio insecticides and new products of the pharmacy industry can be developed (Seminario et al., 2003).

Lachman et al. (2007) measured the content of total polyphenols (TP) in rhizomes, tuberous roots and leaves of five genotypes of yacon and also the inulin and fructose, observing the presence of phenolic compounds (chlorogenic, ferulic, protocatechuic , gallic, rosmarinic, gentisic, and caffeic acids and their derivatives).

According to the obtained results the approximate proportion of the content of TP in the tuberous roots (tr=1), leaves (le), and rhizomes of yacon (rh) is 1 : 1.4 : 3.3 (tr:le:rh).

It has been shown that the provision of infusions elaborated on the base of leaves to the diabetic rats during 30 days reduced the levels of glucose in their blood. Although the principal asset is unknown, it has been proved that the infusion improves the concentration of the insulin in the blood (Aybar et al., 2001).

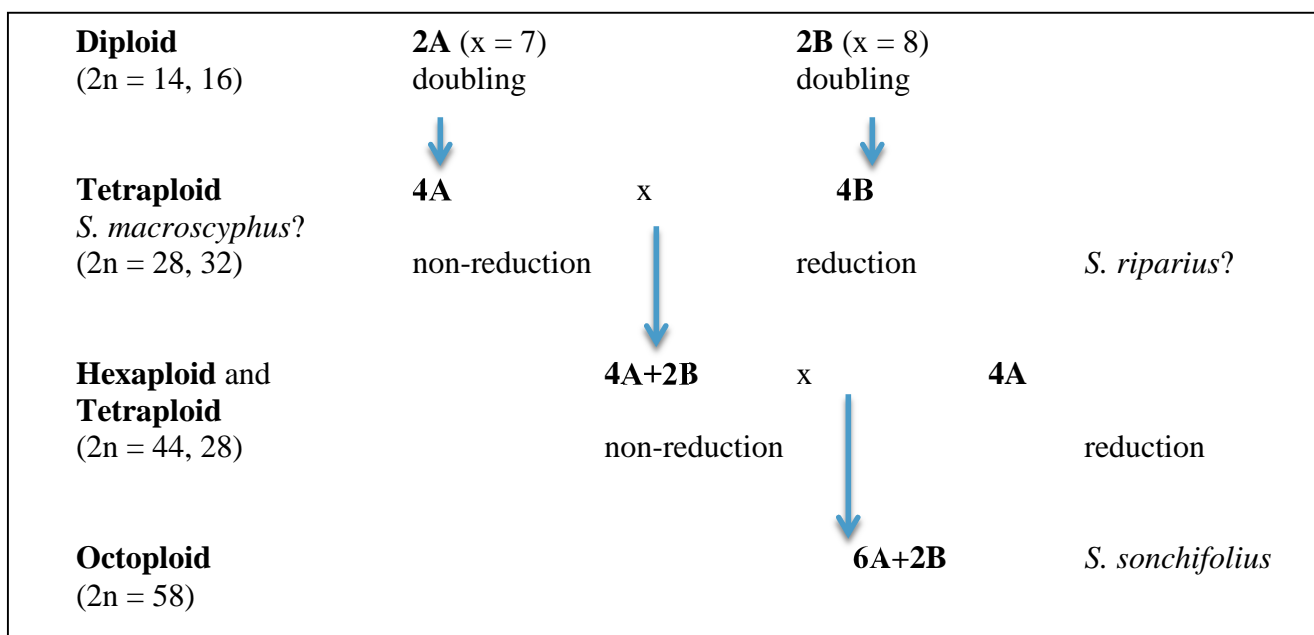
In spite of nonexistence of clear scientific proof, it is recommended to consume the infusions of this plant species as a natural product having a beneficial impact on the health of those suffering from diabetes or metabolic disorders (Sánchez and Genta, 2007).

#### **4 POLYPLOIDY**

Polyploidy consists of the increased genome size, caused by the presence of three or more sets of chromosomes in an organism's somatic cells. For many years, when choosing a specimen of cereal, or other type of crops for their height or larger fruits, humans accidentally chose polyploid specimens. After this situation, the cultivation of polyploid plants has brought a lot of advantages, such as better yield, seedless fruits, larger size. This selection of plants was done in other crops by means of different levels of polyploidy (Alcántar, 2014).

In modern plant breeding polyploidy plants might be of value since they are often more productive and vigorous. Also it might also expected that plants of cassava, with a number of chromosomes greater than 36 may produce roots with an increased starch content besides a greater total volume (Granner et al., 1941)

The studies of Ishiki et al. (1997) are consistent with allopolyploidy, suggesting a yacon caryotype composed by two genomes. The propose an octoploid 6A+2B structure would explain the  $2n=87$ . The Figure 16 shows the hypothetical crossings that occurred during the evolution of yacon.



**Figure 16.** Hypothetical evolution of the yacon genome

**Source.** Grau and Rea 1997; Ishiki et al. 1997

## 5 PHOTOSYNTHETIC PIGMENTS

The chlorophyll content is directly related with the concentration of nitrogen. The nitrogen is necessary to the synthesis of chlorophyll so it is important to the process of photosynthesis (Rincón et al., 2010).

The photosynthetic pigments are substances able to catch solar energy and to transform it into chemical energy using the photosynthesis.

There are two types of photosynthetic organisms: Procariotes (bacteriochlorophyll a); Eukaryotes (chlorophyll a) (Manrique, E., 2003).

The biosphere receive solar radiation between 290 and 3000 nm of wavelength, but basically the major part of the energy is between 380 and 710 nm, where it is possible to find the chlorophylls and the secondary pigments (Larcher, 1995).



	Primary pigment	Secondary pigment	Absorbed wavelength (nm)
Red algae	Chl a	Ficocianine Ficoeritrine Aloficocianine	From purple-blue to oranjge-red (415-670 nm)
Brown algae	Chl a	Chl c	From purple-blue to red
Green algae, moss and vascular plants	Chl a	Chl b Carotenes Xanthophylls	From purple-blue to orange-red (454-670 nm)

**Table 2.** Most important photosynthetic pigments at the different group of organisms (eukaryote) with the range of solar absorption

**Source.** Lawlor, 1993

To talk about photosynthesis is the same as assimilation of CO<sub>2</sub>. These one is related to the solar radiation (Manrique, E., 2003).

## 6 CHLOROPHYLL IN PLANTS

Using the resources available in the right manner, one can achieve the best efficiency in terms of number of seeds and bigger percentage of oil (sunflower). These resources are: humidity and the temperature of soil, the genetic influence, the density and distribution of plants, the absorption of water and nutrients and the interception of the luminous radiation.

The energy that the plants use to grow and support themselves comes from the luminous energy transformed in the process of photosynthesis. This explains the lineal relation existing between the amounts of luminous radiation absorbed by the plant and its growth in dry weight (Aguirrezábal et al., 2001). The availability of photosynthesis which depends on the photosynthetic pigments affects the plant growth (Farghali et al., 2014).

The photosynthesis depends equally on the surface area of the crop able to absorb the energy (leaves), and on the activity of the photosynthetic apparatus for the unity of the green area of the crop (converting the radiation into a chemical energy) (Aguirrezábal et al., 2001).

In the moment of growth, the higher leaves of sunflower have lower level of photosynthesis because of the small size that have the medium leaves, that are the ones that give the maximum of assimilation; in exchange, the basal leaves show numbers including negatives, the reason behind it being the higher respiration than the photosynthetic activity. A mean that the plant grows older, the maximum capacity of photosynthesis that moves upward and then the flowering were shown in the higher placed leaves that are younger (Juan Valero, 1992).

The leaf area index (LAI) is a useful variable for the measurement of the surface of leaves. It characterizes the dynamics and productivity of the forestry ecosystems. The canopy cover (COB) regulates the amount of light that penetrates into the forestry system (Aguirre-Salado et al., 2011).

## **7 HYPOTHESES**

The main hypotheses of the thesis were as follows:

- The content of photosynthetic pigments change across the vegetation period of yacon crop.
- The ploidy level of the different yacon landraces influences the content of chlorophylls (a, b) and carotene.
- The morphology of the plant could be related to the contents of photosynthetic pigments and tuberous root mass.

## 8 OBJECTIVES

The main objective of the present thesis was to determine the content of photosynthetic pigments during the vegetation period of yacon landraces (*Smallanthus sonchifolius*) and evaluate their relationships with plant characteristics.

The specific objectives of the thesis were as follows:

- To determine the possible changes in the photosynthetic pigments of the plants during the vegetation period.
- To evaluate whether the ploidy level of the different yacon landraces are related with the content of photosynthetic pigments.
- To assess if some plant characteristic (e.g., plant morphology) could be used as a predictor of the content of photosynthetic pigments and tuberous root mass.

The assessment of photosynthetic pigments during the vegetation period of yacon could represent another way for a better commercial exploitation of the crop, for example, in order to determine the suitable period to harvest the leaves for its medicinal use.

## **9 MATERIAL AND METHODS**

The average daily temperature during the vegetation was 15.8 °C and sum of rainfall was of 355.5 mm (Meteostation of the Czech University of Agriculture Prague).

The growing season in the Central Europe is influenced by spring frosts (May) and the harvest season depends on first autumn frosts (October).

### **9.1 Germplasm**

The plant material has been acquired from different parts of the world since 1993. It has been maintained at the Faculty of Tropical AgriSciences (FTA, former Institute of Tropics and Subtropics), Czech University of Life Sciences Prague (CULS). Almost all Peruvian accessions were obtained from the University of Cusco (Universidad Nacional de San Antonio Abad del Cusco, UNSAAC). They were brought to Czech Republic in 1993 and have been experimentally cultivated ever since.

The biological material for the analysis was obtained from the plants cultivated under the field conditions of trial plots of FTA CULS (50°05' N, 14°27' E; 286 masl). To ensure the fresh biological material also in the winter, small parts of rhizomes of all accessions were taken from each plant during the harvesting period, planted into the pots and kept in the greenhouses of Botanic Garden of FTA, CULS. In the spring, when there was no more risk of morning frosts, the plants were planted in the field, in the rows 70 cm apart and in the distance of 70 cm between two plants.

There were 24 samples: five clones from Bolivia (BOL20, BOL21, BOL22, BOL23, BOL24), four clones from Ecuador (ECU40, ECU41, ECU42, ECU45) and fifteen clones from Peru (PER01, PER02, PER03, PER04, PER05, PER06, PER07, PER08, PER09, PER10, PER11, PER12, PER13, PER14, PER15). All the clones from Bolivia and Ecuador were octoploids ( $2n=58$ ) and those from Peru contained octoploids ( $2n=58$ ) and dodecaploids ( $2n=87$ ).

## **9.2 Assessment of plant morphology**

Was analyzed some morphological aspects of the plants from different countries (Ecuador, Peru and Bolivia) with two types of ploidy, octoploids clones ( $2n=58$ ) and dodecaploids ( $2n=87$ ). Only the plants from Peru contains octoploids and dodecaploids.

### **9.2.1 Diameter and height**

During the experiment, the diameter of the stem in three different parts was measured using an electronic caliper. The first was the lower part of the plant (highest diameter), 20 cm from the ground; the second was at the third level pair of the leaf (starting from the top) and the last one was under its bud. In order to simplify the data comparison, the data used is the mean of the three before mentioned measurements.

The height of the plants was measured three times, once a month. At the end of July, August and September 2015.

### **9.2.2 Leaves**

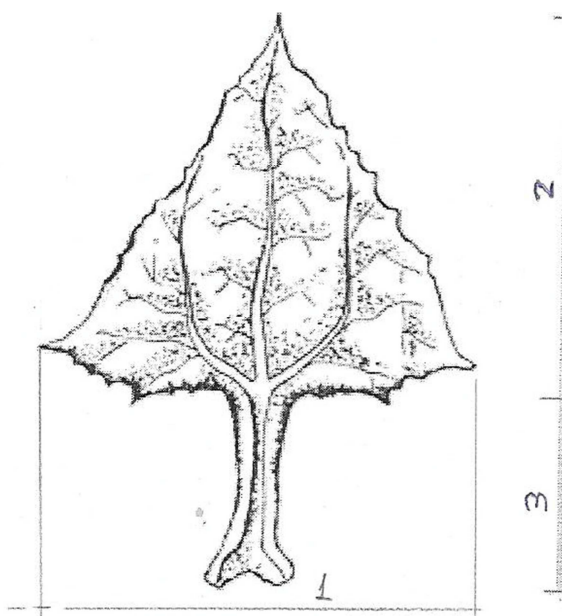
The leaf area ( $\text{cm}^2$ ) and morphology of leaves was measured. Firstly the photo of the leaves was taken with a camera (Nikon d90), then, using the Image J program, the leaf area in squared centimeters was measured.



**Figure 17.** Leaf on the graph paper

**Source.** Author

Three more parts of the leaf were an object of the measurements.



**Figure 18.** Measures of the leaf: Measure 1 (wide of the leaf); Measure 2 (length of the leaf from the beginning of the petiole); Measure 3 (size of the petiole).

**Source.** Author

### **9.3 Determination of chlorophyll content:**

#### **9.3.1 SPAD measurements**

The chlorophyll content was measured with the Chlorophyll Meter SPAD. Three plants per clon were randomly selected for the chlorophyll measurements. Ten measurements per plants in the leaves were taken, which gives a total of 30 measurements per clone. This samples were taken in three months: July, August and September of 2015.

In the last month, the extraction of chlorophyll in those clones was made in the laboratory.

#### **9.3.2 Chlorophyll extraction**

Some samples of the leaves were taken to extract the chlorophyll values in the laboratory.

At the end of the experimental period, photosynthetic pigments were extracted from fully expanded leaves of plants grown under each treatment, using 200 mg of fresh plant material in a mortar with 80% aqueous acetone.

The homogenized will be filtered and completed to 25 ml with the same acetone. Chlorophyll a (Chl a), chlorophyll b (Chl b) contents will be determined with a spectrophotometer, using two wavelengths (663 and 645 nm). Pigment concentrations ( $\mu\text{g g}^{-1}$  FW) will be calculated following the method of Lichtenthaler & Wellburn (1983).

### **9.4 Harvest**

The above ground parts of the 24 plants were harvested by cutting the main stem shoots at 10 cm above the trunk during the last week of October 2015.

The tuberous roots were harvested by hand on the same day and it was separated from the rhizomes. After, both of them were washed. The mass of tuberous roots were evaluated.



## 9.5 Statistical analysis

All statistical analysis were performed using the SPSS 20 statistical software package (SPSS Inc, Chicago, IL, USA). The Shapiro-Wilk and Levene tests were used to check the normal distribution of the data and the homogeneity of variance, respectively. To check for changes in the content of photosynthetic pigments during the three months evaluated, repeated-measures ANOVA test was used. To check for differences in plant morphology as well as in photosynthetic pigment contents among the different countries and ploidy level, student-t and one-way ANOVA tests were used. When variables were not normal distributed, the Mann-Whitney U and Kruskal Wallis tests were used. Differences were considered statistically significant when  $p < 0.05$ . The data are expressed as mean  $\pm$  standard deviation.

## 10 RESULTS

### 10.1 Chlorophyll and carotene contents

#### 10.1.1 Changes in chlorophyll content during plant growth

There were no significant difference among July and August in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ). However, in September the chlorophyll a, chlorophyll b and carotene values were significant lower than in July and August ( $p < 0.05$ ). The values are shown in Table 3.

**Table 3.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September

Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,107.18 $\pm$ 317.48 a	337.45 $\pm$ 115.90 a	358.41 $\pm$ 98.83 a
August	1,092.05 $\pm$ 301.46 a	332.41 $\pm$ 110.40 a	353.44 $\pm$ 92.81 a
September	1,005.29 $\pm$ 285.02 b	305.81 $\pm$ 103.53 b	325.32 $\pm$ 87.14 b

(Chl a: Chlorophyll a; Chl b: Chlorophyll b). The values of each column followed by the different letters show statistically significant differences at  $p < 0.05$ .

### 10.1.2 Changes in chlorophyll content during plant growth, for octoploids plants (2n=58)

There were no significant difference among July and August in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ) for octoploids plants (2n=58). However, in September the chlorophyll a, chlorophyll b and carotene values were significant lower than in July and August ( $p < 0.05$ ) for octoploids plants (2n=58). The values are shown in Table 4.

**Table 4.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September, for octoploids plants (2n=58)

Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,071.43 $\pm$ 294.85 a	329.94 $\pm$ 114.86 a	349.03 $\pm$ 94.94 a
August	1,051.72 $\pm$ 273.66 a	323.40 $\pm$ 107.90 a	342.65 $\pm$ 87.64 a
September	970.62 $\pm$ 265.78 b	298.12 $\pm$ 102.51 b	315.93 $\pm$ 83.05 b

Chl a: Chlorophyll a; Chl b: Chlorophyll b) The values of each column followed by the different letters show statistically significant differences at  $p < 0.05$

### 10.1.3 Changes in chlorophyll content during plant growth, for dodecaploids plants (2n=87)

There were no significant difference among July, August and September in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ) for dodecaploids plants (2n=87). The values are shown in Table 5.

**Table 5.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September, for dodecaploids plants (2n=87)

Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,243.04 $\pm$ 399.02 a	366.02 $\pm$ 128.75 a	394.05 $\pm$ 116.57 a
August	1,245.30 $\pm$ 385.33 a	366.66 $\pm$ 125.83 a	394.45 $\pm$ 110.94 a
September	1,137.04 $\pm$ 349.04 a	335.02 $\pm$ 114.00 a	361.00 $\pm$ 103.06 a

Chl a: Chlorophyll a; Chl b: Chlorophyll b) The values of each column followed by the different letters show statistically significant differences at  $p < 0.05$ .

## 10.2 Chlorophyll and carotene contents per countries

### 10.2.1 Changes in chlorophyll content during plant growth in the Bolivian clones

There were no significant differences among July and August in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ) and between July and September in Bolivia. However, in August and September the chlorophyll a, chlorophyll b and carotene values were significant lower than in July ( $p < 0.05$ ) in Bolivian clones. The values are shown in Table 6.

**Table 6.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September, from Bolivia

Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,078.28 $\pm$ 395.49 ab	334.06 $\pm$ 142.61 ab	355.97 $\pm$ 117.89 ab
August	1,052.21 $\pm$ 335.00 a	324.62 $\pm$ 121.87 a	347.93 $\pm$ 97.88 a
September	974.49 $\pm$ 309.67 b	300.74 $\pm$ 112.96 b	322.03 $\pm$ 89.82 b

### 10.2.2 Changes in chlorophyll content during plant growth in Ecuadorian clones

There were no significant difference among July, August and September in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ) in Ecuadorian clones. The values are shown in Table 7.

**Table 7.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September, from Ecuador.

Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,141.04 $\pm$ 263.54 a	373.50 $\pm$ 87.62 a	361.61 $\pm$ 79.82 a
August	1,095.81 $\pm$ 201.66 a	357.99 $\pm$ 66.08 a	348.13 $\pm$ 67.23 a
September	991.99 $\pm$ 274.30 a	323.01 $\pm$ 82.72 a	314.12 $\pm$ 82.53 a

### 10.2.3 Changes in chlorophyll content during plant growth in Peruvian clones

There were no significant difference among July and August in chlorophyll a, chlorophyll b and carotene content ( $p > 0.05$ ) in Peruvian clones. However, in September the chlorophyll a, chlorophyll b and carotene values were significant lower than in July and August ( $p < 0.05$ ) in Peru. The values are shown in Table 8.

**Table 8.** Descriptive statistics (mean values  $\pm$  SD) of chlorophyll a, chlorophyll b and carotene content in July, August and September, in Peruvian clones

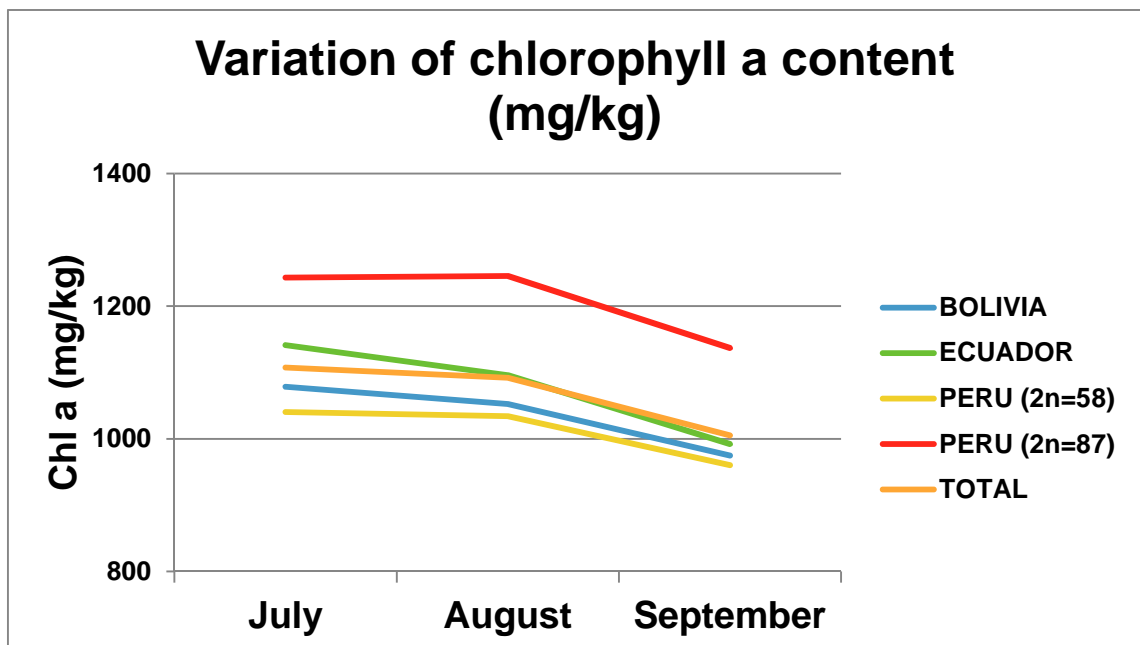
Month	Chl a (mg·kg <sup>-1</sup> )	Chl b (mg·kg <sup>-1</sup> )	Carotene (mg·kg <sup>-1</sup> )
July	1,107.79 $\pm$ 324.65 a	328.98 $\pm$ 119.00 a	358.37 $\pm$ 103.46 a
August	1,104.32 $\pm$ 328.32 a	328.19 $\pm$ 120.89 a	356.69 $\pm$ 102.05 a
September	1,019.10 $\pm$ 298.90 b	302.91 $\pm$ 111.33 b	329.41 $\pm$ 93.02 b

#### 10.2.4 Variation of chloropylls and carotene content, per countries

The values of Chl a of octoploids, during July, August and September, was lower than the average value (3.23 %, 3.69 %, 3.45 %, respectively).

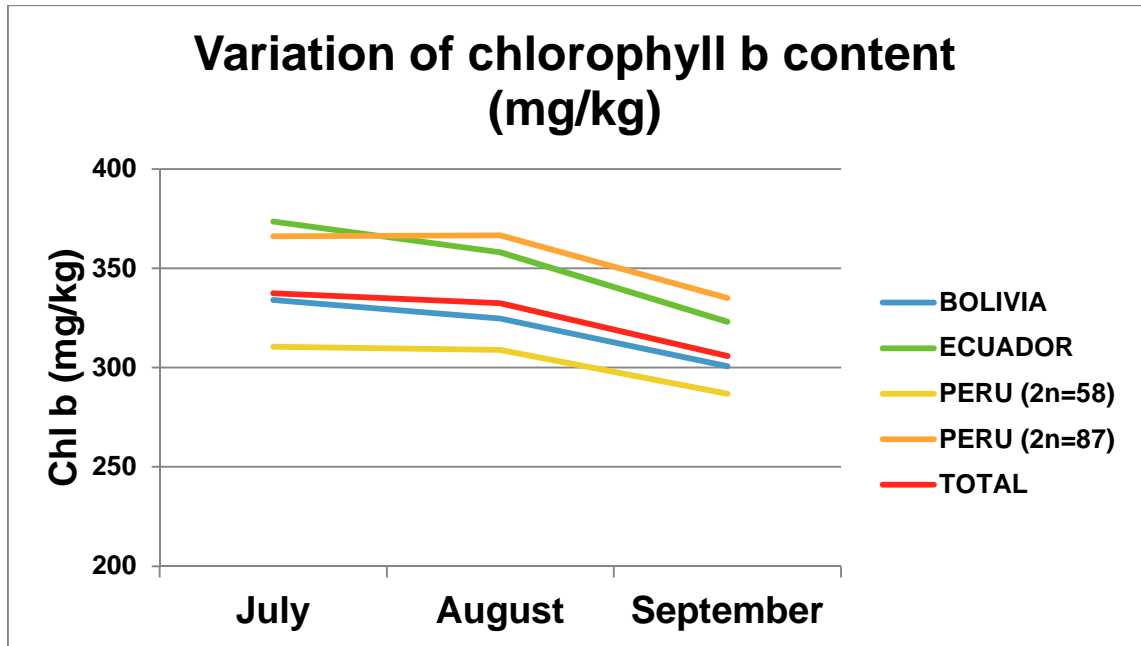
The values of Chl a of dodecaploids, during July, August and September, was higher than the average value (12.27 %, 14.03 %, 13.11 %, respectively). Similar values were observed in Chl b and Carotene.

The variation of chloropylls and carotene content, in the clones from the three countries is shown in Figures 19, 20, and 21.



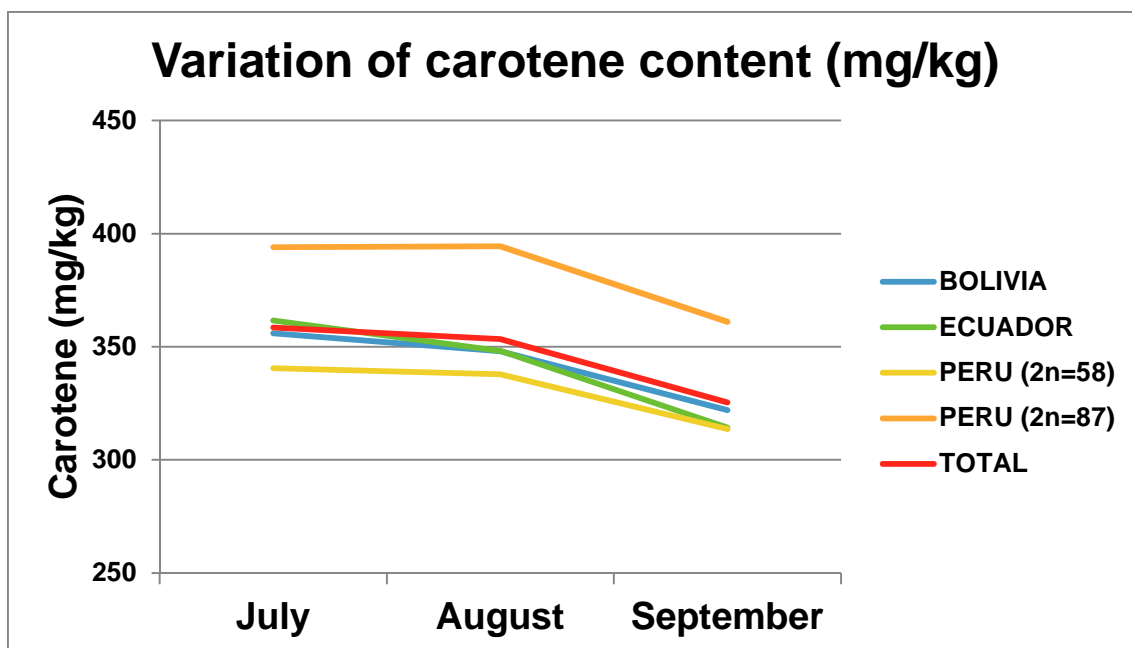
**Figure 19.** Variation of chlorophyll a content during July, August and September

Peru (dodecaploid clones) was 12,27 %, 14,03 %, and 13,11 % higher than the mean during July, August and September, respectively.



**Figure 20.** Variation of chlorophyll b content during July, August and September

Peru (dodecaploid clones) was 8,47 %, 10,3 %, and 9,55 % higher than the mean during July, August and September, respectively.

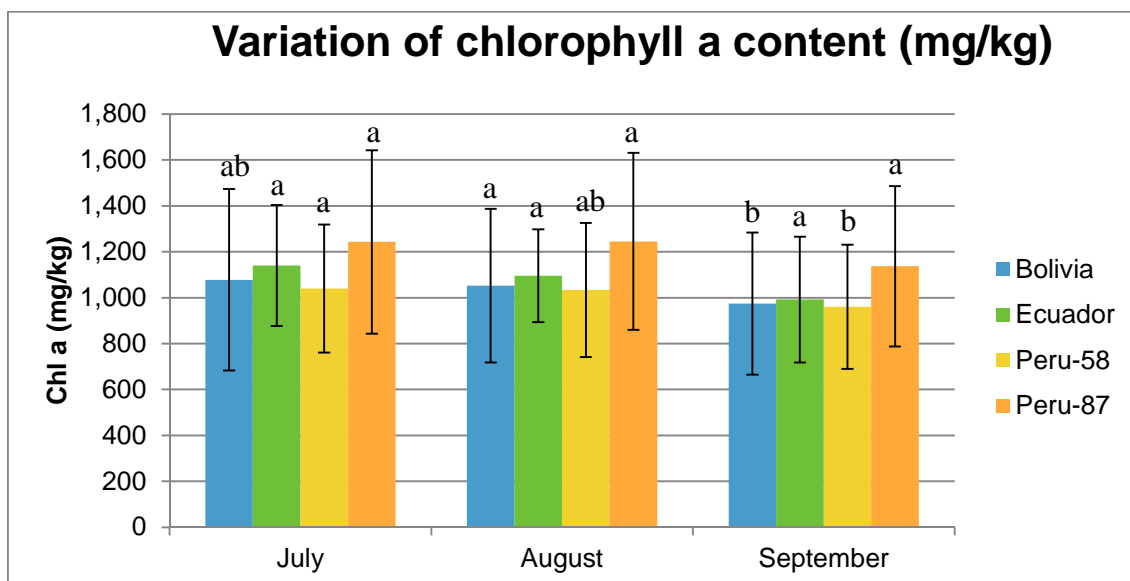


**Figure 21.** Variation of carotene b content during July, August and September.

Peru (dodecaploid clones) was 9,95 %, 11,6 %, and 10,97 % higher than the mean during July, August and September, respectively.

### 10.2.5 Measurement of chlorophyll and carotene content per months

Observing the ploidy level of the clones from Bolivia, Ecuador and Peru, it is possible to appreciate the different average values of chlorophyll a content (Figure 22).



**Figure 22.** Variation of chlorophyll a content ( $\text{mg kg}^{-1}$ )

The total chlorophyll a content in July (1,107.18 mg·kg<sup>-1</sup>) decreased 1.37 % in August and 7.94 % in September.

The total chlorophyll a content in Bolivia in July (1,078.28 mg·kg<sup>-1</sup>) decreased 2.42 % in August and 7.39 % in September.

The total chlorophyll a content in Ecuador in July (1,141.04 mg·kg<sup>-1</sup>) decreased 3.96 % in August and 9.47 % in September.

The total chlorophyll a content in Peru (octoploids) in July (1,040.16 mg·kg<sup>-1</sup>) decreased 0.61 % in August and 7.13 % in September.

The total chlorophyll a content in Peru (dodecaploids) in July (1,243.04 mg·kg<sup>-1</sup>) increased 0.18 % in August and decreased 8.69 % in September.

The total chlorophyll a content in Peru (dodecaploids) and Ecuador in July was a 12.27 % and 3.06 %, respectively higher than the average.

The total chlorophyll a content in Bolivia and Peru (octoploids) in July was a 2.61 % and 6.05 %, respectively lower than the average.

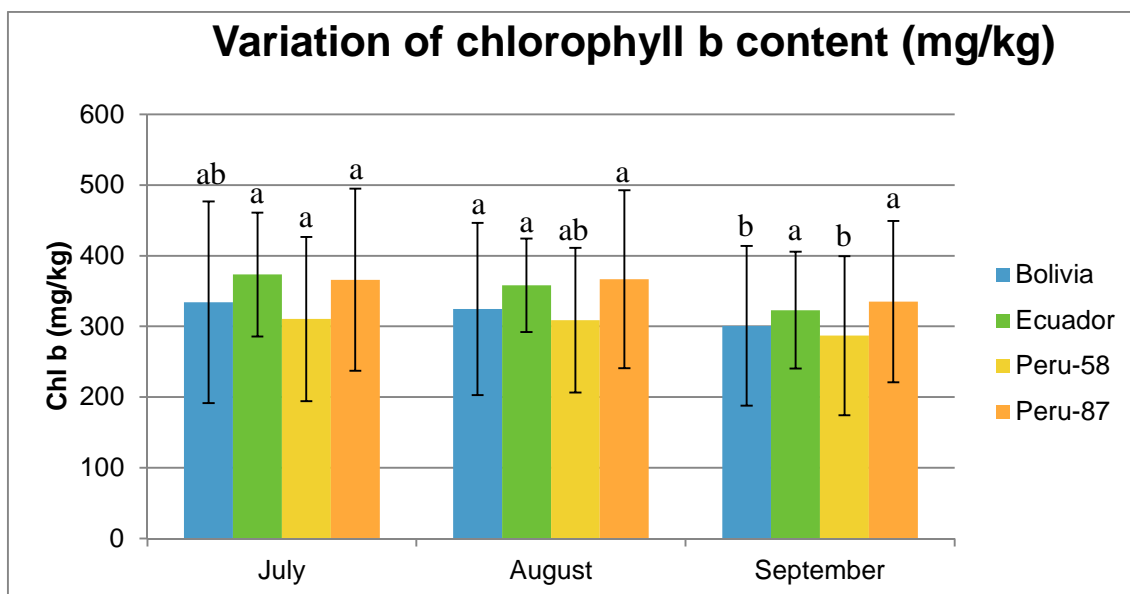
The total chlorophyll a content in Peru (dodecaploids) and Ecuador in August was a 14.03 % and 0.34 %, respectively higher than the average.

The total chlorophyll a content in Bolivia and Peru (octoploids) in August was a 3.65 % and 5.33 %, respectively lower than the average.

The total chlorophyll a content in Peru (dodecaploids) in September was a 13.11 % higher than the average.

The total chlorophyll a content in Ecuador, Bolivia and Peru (octoploids) in September was a 1.32 %, 3.06 % and 4.49 %, respectively lower than the average.

Observing the ploidy level of the clones in Bolivia, Ecuador and Peru, it is possible to appreciate the different average values of chlorophyll b content (Figure 23).



**Figure 23.** Variation of chlorophyll b content ( $\text{mg kg}^{-1}$ )

The total chlorophyll b content in July ( $337.45 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 1.49 % in August and 8 % in September.

The total chlorophyll b content in Bolivia in July ( $334.06 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 2.83 % in August and 7.36 % in September.

The total chlorophyll b content in Ecuador in July ( $373.50 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 4.15 % in August and 9.77 % in September.

The total chlorophyll b content in Peru (octoploids) in July ( $310.45 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 0.48 % in August and 7.15 % in September.

The total chlorophyll b content in Peru (dodecaploids) in July ( $366.02 \text{ mg}\cdot\text{kg}^{-1}$ ) increased 0.17 % in August and decreased 8.63 % in September.

The total chlorophyll b content in Ecuador and Peru (dodecaploids) in July was a 10.68 % and 8.47 %, respectively higher than the average.

The total chlorophyll b content in Bolivia and Peru (octoploids) in July was a 1 % and 8 %, respectively lower than the average.

The total chlorophyll b content in Peru (dodecaploids) and Ecuador in August was a 10.30 % and 7.70 %, respectively higher than the average.

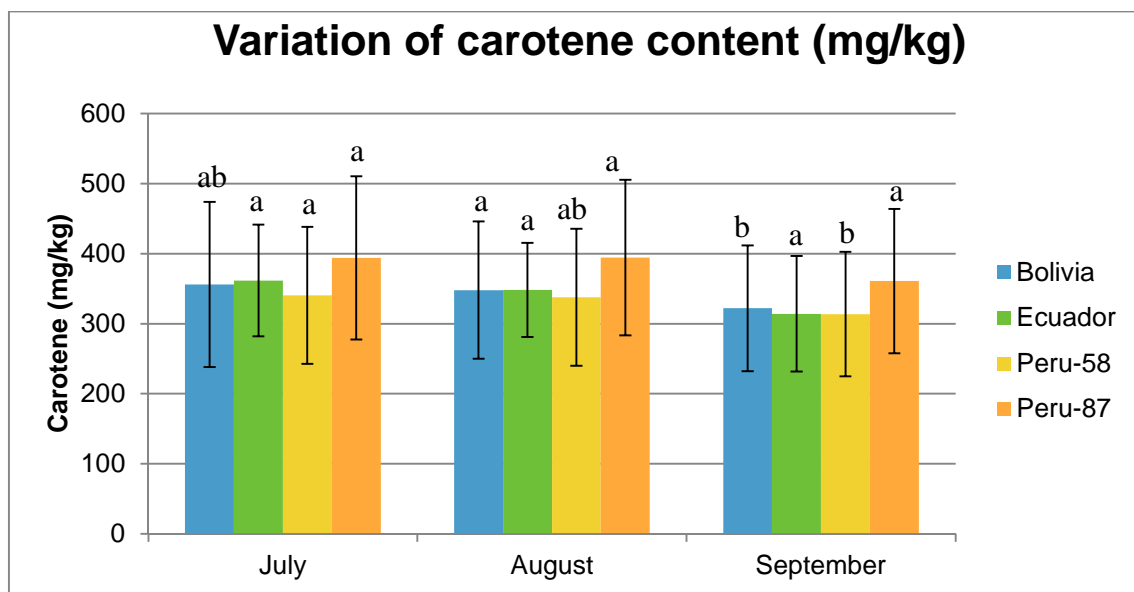
The total chlorophyll b content in Bolivia and Peru (octoploids) in August was a 2.34 % and 7.06 %, respectively lower than the average.



The total chlorophyll b content in Peru (dodecaploids) and Ecuador in September was a 9.55 % and 5.62 %, respectively higher than the average.

The total chlorophyll b content in Bolivia and Peru (octoploids) in September was a 1.66 % and 6.20 %, respectively lower than the average.

Observing the ploidy level of the clones in Bolivia, Ecuador and Peru, it is possible to appreciate the different average values of carotene content (Figure 24).



**Figure 24.** Variation of carotene content ( $\text{mg kg}^{-1}$ )

The total carotene content in July ( $358.41 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 1.39 % in August and 7.96 % in September.

The total carotene content in Bolivia in July ( $355.97 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 2.26 % in August and 7.44 % in September.

The total carotene content in Ecuador in July ( $361.61 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 3.73 % in August and 9.77 % in September.

The total carotene content in Peru (octoploids) in July ( $340.52 \text{ mg}\cdot\text{kg}^{-1}$ ) decreased 0.80 % in August and 7.16 % in September.

The total carotene content in Peru (dodecaploids) in July ( $394.05 \text{ mg}\cdot\text{kg}^{-1}$ ) increased 0.10 % in August and decreased 8.84 % in September.

The total carotene content in Peru (dodecaploids) and Ecuador in July was a 9.94 % and 0.89 %, respectively higher than the average.

The total carotene content in Bolivia and Peru (octoploids) in July was a 0.68 % and 4.99 %, respectively lower than the average.

The total carotene content in Peru (dodecaploids) and Ecuador in August was a 11.60 % higher than the average.

The total carotene content in Ecuador, Bolivia and Peru (octoploids) in August was a 1.50 %, 1.56 % and 4.42 %, respectively lower than the average.

The total carotene content in Peru (dodecaploids) in September was a 10.97 % higher than the average.

The total carotene content in Bolivia, Ecuador and Peru (octoploids) in September was a 1.01 %, 3.44 % and 3.60 %, respectively lower than the average.

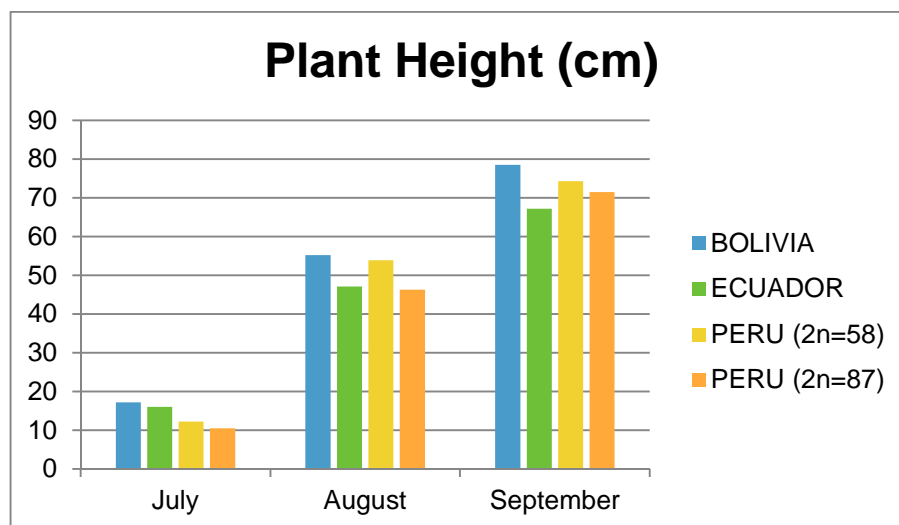
### 10.3 Plant morphology

Plant height (cm) showed highly significant differences ( $p < 0.01$ ) in July, August and September. The values are shown in Table 9.

**Table 9.** Descriptive statistics (mean values  $\pm$  SD) of plant height (cm)

Month	Plant height (cm)
July	13.5 $\pm$ 5.9 a
August	51.4 $\pm$ 10.7 b
September	73.5 $\pm$ 14.3 c

The height of the plants (cm) is represented at Figure 25.



**Figure 25.** Height of the plants (cm)

#### 10.4 Plant morphology. Other traits measured in September

In September was measured also the tuber mass (g), diameter of the stem (mm), leaf area (cm<sup>2</sup>), leaf width (cm), leaf length (cm) and petiole length (cm), which results are shown at Table 10, per countries and ploidy level.

**Table 10.** Tuber mass (g), diameter of the stem (mm), leaf area (cm<sup>2</sup>), leaf width (cm), leaf length (cm), petiole length (cm) and relation among leaf length and leaf width, per countries and ploidy level

		Tuber mass (g)	Diameter (mm)	Leaf area (cm <sup>2</sup> )	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)	Lenght/width
2n=58	BOLIVIA	597.1	24.30	213.34	20.28	16.53	8.49	0.82
	ECUADOR	539.5	23.52	238.56	21.30	17.24	10.40	0.81
	PERU	893.6	24.74	208.00	18.56	16.79	9.07	0.90
	TOTAL	741.0	24.36	215.84	19.59	16.81	9.19	
2n=87	PERU	632.9	23.87	260.52	20.88	18.41	9.73	0.88
TOTAL		718.5	24.26	225.15	19.86	17.15	9.31	0.86

The mean value of leaf area in octoploids (2n=58) is a 4.13 % lower than the total mean value. The mean value of leaf area in dodecaploids (2n=87) is a 15.71 % higher than the total mean value. In conclusion, dodecaploids clones were higher in leaf area (260.52 cm<sup>2</sup>) than octoploid clones (215.84 cm<sup>2</sup>).

#### 10.5 Correlations between plant morphology and photosynthetic pigments

To understand the correlation among all studied variables (Chl a, Chl b, carotene, plant height, tuber mass, diameter of the stem, leaf area, leaf width, leaf length, and petiole length), Pearson's correlation coefficient was calculated. The analysis was conducted using averaged values of each variable and results are reported in Table 11.

**Table 11.** Correlation matrix of the data

		Height	Chl a	Chl b	Carotene	Diameter	Area	Leaf width	Leaf length	Petiole length	Tuber mass
Height	r	1									
	p										
Chl a	r	0.097	1								
	p	0.651									
Chl b	r	0.173	0.959**	1							
	p	0.420	0								
Carotene	r	0.034	0.960**	0.903**	1						
	p	0.806	0	0							
Diameter	r	0.093	-0.476*	-0.580**	-0.526**	1					
	p	0.667	0.019	0.003	0.008						
Area	r	-0.036	-0.417	-0.456	-0.472	0.358	1				
	p	0.868	0.042	0.025	0.020	0.086					
Leaf width	r	-0.010	-0.265	-0.301	-0.291	0.197	0.890**	1			
	p	0.963	0.211	0.153	0.167	0.357	0				
Leaf length	r	0.191	-0.457*	-0.485*	-0.538**	0.435*	0.931**	0.759**	1		
	p	0.371	0.025	0.016	0.007	0.033	0	0			
Petiole length	r	0.195	0.044	0.027	-0.098	-0.165	0.578**	0.485*	0.537**	1	
	p	0.361	0.839	0.902	0.648	0.441	0.003	0.016	0.007		
Tuber mass	r	0.414*	0.140	0.134	0.226	0.060	-0.389	-0.349	-0.287	-0.130	1
	p	0.044	0.513	0.532	0.287	0.780	0.061	0.094	0.174	0.544	

\* The correlation is significant as the level 0.05 (bilateral)  
 \*\* The correlation is significant as the level 0.01 (bilateral)

Data expressed as Chl a and diameter ( $r = -0.476$ ,  $p = 0.019$ ), leaf area ( $r = -0.417$ ,  $p = 0.042$ ), and leaf length ( $r = -0.457$ ,  $p = 0.025$ ), show a negative correlation ( $p < 0.05$ ).

Data expressed as Chl b and diameter ( $r = -0.580$ ,  $p = 0.003$ ), show a negative correlation ( $p < 0.01$ ).

Data expressed as Chl b and leaf area ( $r = -0.456$ ,  $p = 0.025$ ), and leaf length ( $r = -0.485$ ,  $p = 0.016$ ), show a negative correlation ( $p < 0.05$ ).

Data expressed as carotene and diameter ( $r = -0.526$ ,  $p = 0.008$ ), and leaf length ( $r = -0.538$ ,  $p = 0.007$ ), show a negative correlation ( $p < 0.01$ ).

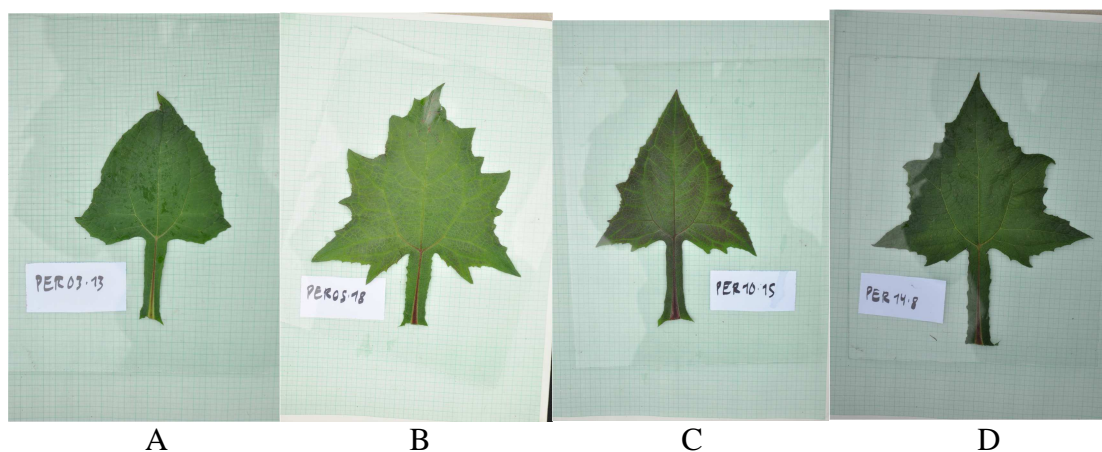
Data expressed as carotene and leaf length ( $r = -0.472$ ,  $p = 0.020$ ), show a negative correlation ( $p < 0.05$ ).

Data expressed as plant height and tuber mass ( $r = 0.414$ ,  $p = 0.044$ ), show a positive correlation ( $p < 0.05$ ).

The highest correlation was observed between plant height and tuber mass ( $r = 0.414$ ).

## 10.6 Morphology of the leaves

The different kind of leaves can be classified in four groups (Figure 26):



**Figure 26.** Groups of leaves classified by the shape: A (ovate); B (hastate); C (lanceolate); D (sub-hastate)

**Source.** Author

- Group A, containing the clones: PER03, PER04, PER06, PER08, BOL21 and BOL23.
- Group B, containing the clones: PER05, PER09, PER11, PER12, PER13 and BOL20.
- Group C, containing the clones: PER10, PER15, BOL22 and ECU42.
- Group D, containing the clones: PER14, BOL24 and ECU41.

Other: the rest of clones.

The results (chlorophyll a, chlorophyll b, carotene, height, tuber weight, leaf area, diameter of the stem and measures of the leaves) were analyzed in September.

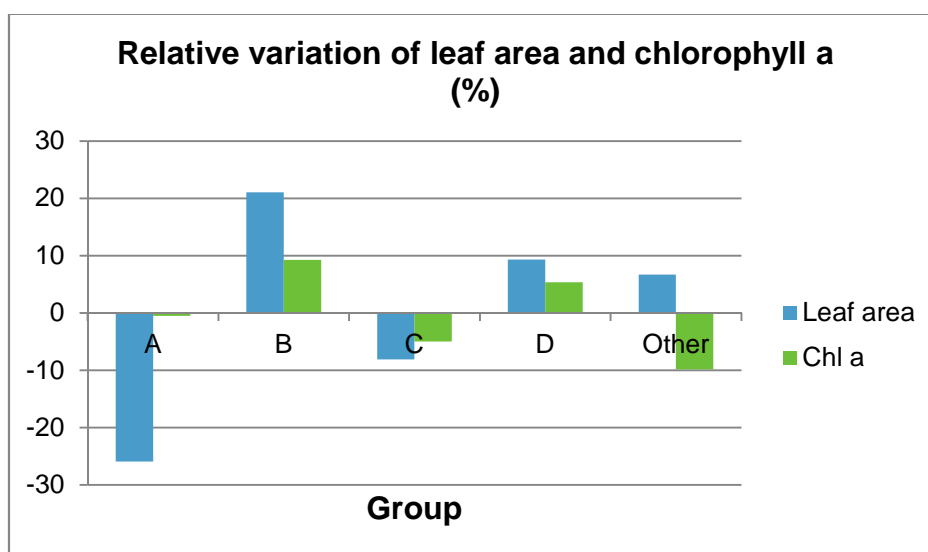
Group A: mean value of chlorophyll a ( $1,000.3 \text{ mg} \cdot \text{kg}^{-1}$ ) was 0.5 % lower than the average ( $1,005.29 \text{ mg} \cdot \text{kg}^{-1}$ ).

Group B: mean value of chlorophyll a ( $1,098.63 \text{ mg} \cdot \text{kg}^{-1}$ ) was 9.28 % higher than the average ( $1,005.29 \text{ mg} \cdot \text{kg}^{-1}$ ).

Group C: mean value of chlorophyll a ( $955.35 \text{ mg} \cdot \text{kg}^{-1}$ ) was 4.97 % lower than the average ( $1,005.29 \text{ mg} \cdot \text{kg}^{-1}$ ).

Group D: mean value of chlorophyll a ( $1,059.41 \text{ mg} \cdot \text{kg}^{-1}$ ) was 5.38 % higher than the average ( $1,005.29 \text{ mg} \cdot \text{kg}^{-1}$ ).

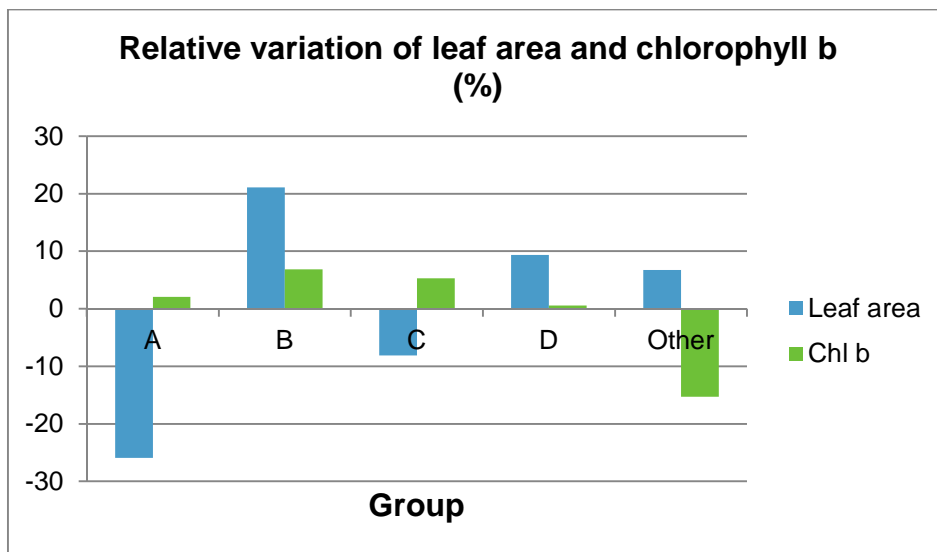
At the Figure 27 it is represented the relative value of chlorophyll a and the leaf area of each group compared to the average value.



**Figure 27.** Leaf area and chlorophyll a (%) compared to the average value

Thus, group B had a leaf area higher in a 21.06 % respect to the average and the chlorophyll a content is a 9.28 % higher respect to the average. Group (D) had a leaf area higher in a 9.34 % respect to the average and the chlorophyll a content is a 5.39 % higher respect to the average. When the leaf area is lower than the average, the chlorophyll a is also lower (group A, -25.93 %, -0.5 %; group C, -8.10 %, -4.97 %).

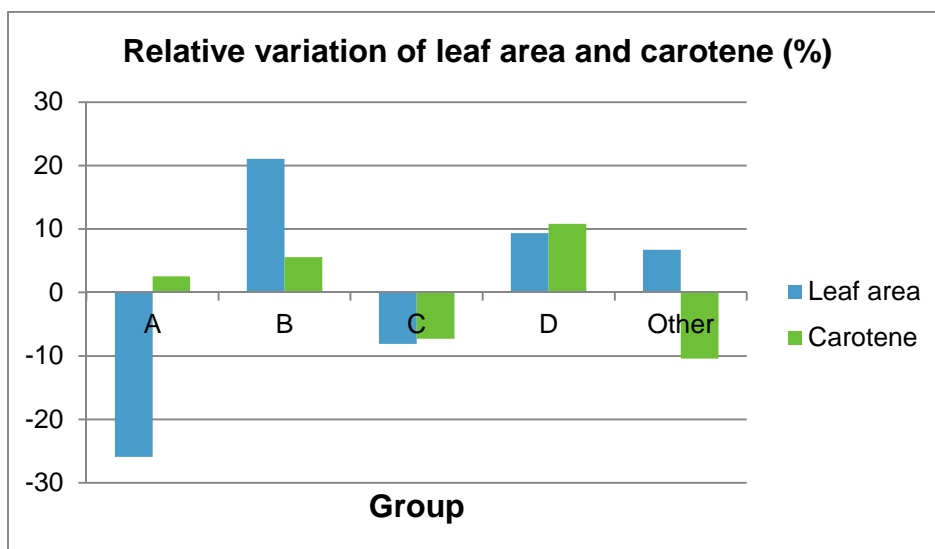
At the Figure 28 it is represented the relative value of chlorophyll b and the leaf area of each group compared to the average value.



**Figure 28.** Leaf area and chlorophyll b (%) compared to the average value

Thus, group B had a leaf area higher in a 21.06 % respect to the average and the chlorophyll b content is a 6.85 % higher respect to the average.

At the Figure 29 it is represented the relative value of carotene and the leaf area of each group compared to the average value.



**Figure 29.** Leaf area and carotene (%) compared to the average value

Thus, group B had a leaf area higher in a 21.06 % respect to the average and the carotene content is a 5.57 % higher respect to the average. Group (D) had a leaf area higher in a 9.34 % respect to the average and the carotene content is a 10.84 % higher respect to the average. When the leaf area is lower than the average, the carotene is also lower (group C, -8.10 %, -7.29 %).

At the Table 12 it is represented the relative variation of chlorophylls, carotene and leaf area (%)

**Table 12.** Relative variation of chlorophylls, carotene and leaf area (%)

Group	Relative leaf area variation (%)	Relative chlorophyll a variation (%)	Relative chlorophyll b variation (%)	Relative carotene variation (%)
A	-25.93	-0.50	+2.07	+2.57
B	+21.06	+9.28	+6.85	+5.57
C	-8.10	-4.97	+5.32	-7.29
D	+9.34	+5.38	+0.55	+10.84
Other	+6.72	-9.80	-15.29	-10.43

To summarize, when the relative variation increase (group B and D) the relative variation of chlorophylls and carotene increase. When the relative variation decrease (group C) the relative variation of chlorophyll a and carotene decrease.



## 11 DISCUSSION

Yacon is one of the most important tuberous plants today, as both the tuber and the leaves are exploited for their multiple properties and uses in food and pharmacology. This is demonstrated by the numerous studies and research studies that are being carried out on yacon. The objective of this study was to find the relationship between the chlorophyll content and the morphological characteristics of the plant.

The data of the 24 clones of Bolivia, Ecuador and Peru, with different ploidy level, during July, August and September 2015 was analyzed.

It is possible that the chlorophyll content change depending on the leaves selected in the plant. The paper of Rincón et al., (2010) showed that the chlorophyll content on the leaves depended at the height of the measurement, selecting the leaves and said that the major chlorophyll content was at the middle part of the plant. If I had selected the leaves at the upper part of the plant, the chlorophyll content could be higher.

In the study of Liao et al., (2016), were compared many vegetative traits, for example: height of the plant, relative chlorophyll content index (CCI), leaf area (LA), the photosynthetic efficiency of the leaves. Thus, this traits were significantly higher in polyploid than the diploid groups. As a conclusions, the polyploid group had advantages in this variables. Compared to my study, the higher ploidy level (dodecaploids,  $2n=87$ ) had a larger leaf area (LA) and chlorophyll content.

In the paper of Van Laere et al., (2011), the diameter of the stalk of some genotypes was higher for tetraploids compared with their diploids. Opposite results were obtained in my study about the diameter. Van Laere et al., (2011) obtained that tetraploids were characterised by a reduced biomass. In comparison to my study, the results were similar, related to the tuber mass.

The leaf area index (LAI) is a useful tool for characterizing the productivity of forestry systems. The study of Aguirre-Salado et al., (2011) shows that the canopy cover is quite related to the LAI according to know the amount of penetrating light, which controls some dependent processes. It means that also the cover could affect to the chlorophyll content in a crop. In my study, the chlorophyll content at the leaves that was analyzed, was in the middle part of the plant so maybe, if it was analyzed at the upper leaves of the crop, this chlorophyll content should be higher.

The leaf area of a plant during the different stages in the growth period is very important for the calibration and adaptability in general for the sunlight radiation application, simulating the different agroclimates (Medina et al., 2013). In my study the chlorophyll content was higher during August and it was also caused for the big size of the leaves. That represents the best moment to harvest yacon leaves in that agroclimatic conditions.

## 12 CONCLUSIONS

The content of chlorophyll (a, b) and carotene change across the vegetation period of yacon. Thus, in September, the content of photosynthetic pigments was significant lower than in July and August. Related with the ploidy level, the dodecaploids plants tend to have a higher content of photosynthetic pigments than the octoploids plants.

In relation with plant morphology, the content of photosynthetic pigments in yacon is negatively related with the leaf size. On the other hand, the dodecaploids plants tend to have a higher leaf size than the octoploids plants. Related with plant production, the tuberous roots mass is positively related with the plant height.

The assessment of photosynthetic pigments in yacon represents a useful tool to improve the commercial exploitation of the crop.

### **13 RECOMMENDATION**

The future research will be conducted to analyse the total polyphenol content and antioxidant capacity in the aqueous extract of yacon leaves to assess possible changes related with the ploidy level and the duration of the vegetation period. The relationships between chlorophyll content, polyphenol content, and antioxidant capacity will be assessed. This approach could be of great importance for a better characterisation of the plant in relation with its antioxidant properties for medicinal purposes.

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