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Department of Crop Sciences and Agroforestry in Tropics and Subtropics

Effect of ploidy levels on biomass yield of yacon (*Smallanthus sonchifolius*)

Author: Štěpán Pokorný

Supervisor: doc. Dr. Ing. Eloy Fernández Cusimamani

Bachelor thesis

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Declaration:

I confirm, that this Bachelor thesis “Effect of ploidy levels on biomass yield of yacon (*Smallanthus sonchifolius)*“ is original result of my own work and that I have used no other resources than referenced. I agree this work to be placed of CULS Prague and was accessible to study purpose.

Prague May 2012

Štěpán Pokorný

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Abstract

Yacon (*Smallanthus sonchifolius*) is a perennial plant from the family *Asteraceae.* Yacon is grown for his edible tuberous roots which contain FOS (fructooligosacharides). Its origin is from the Andean, but for his properties and variability it expanded to the other parts of the world. The aim of this thesis is to study influence of ploidy levels to yield of biomass (tuberous roots, aboveground part) in plants grown in the climatic conditions of Czech Republic. For this work were chosen three clones with different level of ploidy from the collection conserved in ITS- CULS in Prague - 'NZL I' (2n=58); 'PER 65' (2n=87) and 'POLY 3' (2n=116). Plants were cultivated under field conditions on experimental plots of ITS-CULS in Prague. During the vegetation, biometrical data were collected and the macrophenological BBCH scale was elaborated. After the harvest was measured the weight of tuberous roots, rhizomes and aboveground part. Also been recorded the number of tubers per plant (the weight and lenght of the biggest), the height of the plants and number of stems. These measures were made in two consecutive years. All the results were statistically evaluated. In terms of tuberous roots, the results show, that the statistically biggest yields had dodecaploid clones 'PER 65' (year 2010-1.3±0.28kg/plant; year 2011-0.68±0.24kg/plant), then octoploid clones 'NZL I' (0.77±0.29kg/p.; 0.43±0.32kg/p.) and the lowest yield had hexadecaploid clones 'POLY 3' (0.06±0.04kg/p.; 0.05±0.04kg/p.). The yield of aboveground part has the same results- the statistically biggest yields had dodecaploid clones 'PER 65'(1.2±0.28kg/p.; 3.1±0.2kg/p.), after were the octoploid clones 'NZL I' (0.75±0.29kg/p.; 0.56±0.14kg/p.) and then the hexadecaploid clones 'POLY 3' (0.3±0.03kg/p.; 0.42±0.07kg/p.). There is no big statistical significant difference between the clones in the yields of rhizomes, the plant height and number of tuberous roots per plant. There are differences in yields between year 2010 and 2011. Its mean, that besides the influence of ploidy level, also climatic conditions and lenght of vegetation affect the yield. Based on the obtained results can be summarized, that the ploidy level have influence on the yield of biomass (mainly at the tuberous roots and the aboveground part). But it is not so that a plants with higher chromosome number have the biggest yields.

Keywords: biometric evaluation, dodecaploid, hexadecaploid, octoploid, ploidy, yield

Abstrakt

Jakon (*Smallanthus sonchifolius*) je víceletá rostlina z čeleďi *Asteraceae*. Jakon se pěstuje pro své jedlé kořenové hlízy, které obsahují FOS (fruktooligosacharidy). Jeho původ je z And, ale díky svým vlastnostem a přizpůsobivosti se rozšířil do dalších částí světa. Cílem této práce je studium vlivu úrovně ploidie na výnos biomasy (kořenových hlíz a nadzemní části) u rostlin pěstovaných v klimatických podmínkách České Republiky. Pro tuto práci byly vybrány tři klony s různou úrovní ploidie z kolekce uchovávané na ITS-CULS v Praze- ‘NZL I‘ (2n=58); ‘PER 65‘ (2n=87) a ‘POLY 3‘ (2n=116). Rostliny byly pěstovány v polních podmínkách na pokusných pozemcích ITS-CULS v Praze. Během vegetace byly shromažďovány biometrické údaje a byla navržena makrofenologická BBCH stupnice. Po sklizni byla měřena váha kořenových hlíz, rhizomů a nadzemní části. Také byl zaznamenán počet hlíz na rostlinu (váha a délka největší), výška rostlin a počet stonků. Tyto měření byly prováděny ve dvou po sobě jdoucích letech. Všechny výsledky byly statisticky vyhodnoceny. Z hlediska kořenových hlíz, výsledky ukázaly, že statisticky největší výnosy měly dodekaploidní klony ‘PER 65‘ (rok 2010-1.3±0.28kg/rostlina; rok 2011- 0.68±0.24kg/rostlina), poté oktoploidní klony ’NZL I‘ (0.77±0.29kg/r.; 0.43±0.32kg/r.) a nejnižší výnos měly hexadekaploidní klony POLY 3‘ (0.06±0.04kg/r.; 0.05±0.04kg/r.). Výnos nadzemní části měl stejné výsledky- statisticky největší výnosy měly dodekaploidní klony ‘PER 65‘(1.2±0.28kg/r.; 3.1±0.2kg/r.), potom oktoploidní klony ’NZL I‘ (0.75±0.29kg/r.; 0.56±0.14kg/r.) a nakonec hexadekaploidní klony ‘POLY 3‘ (0.3±0.03kg/r.; 0.42±0.07kg/r.). Mezi klony nejsou velké statistické rozdíly ve výnosu rhizomů, výšce rostlin a počtu hlíz na rostlinu. Jsou zde rozdíly ve výnosech mezi lety 2010 a 2011. To znamená, že kromě úrovně ploidy, také klimatické podmínky a délka vegetace ovlivňují výnos. Na základě získaných výsledků lze shrnout, že úroveň ploidie má vliv na výnos biomasy (hlavně u kořenových hlíz a nadzemní části). Ale není to tak, že rostliny s vyšším počtem chromosomů mají větší výnosy.

Klíčová slova: biomerické hodnocení, dodekaploid, hexadekaploid, oktoploid, ploídie , výnos

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1.Introduction

From South America, and especially from Andean region, comes many crops which are important for humans. Between the most famous belongs potatoes, maize and beans, and they are grown all over the world. There is some other crops, which are not so well known, but they also deserve attention for their properties and qualities. One of them is yacon (*Smallanthus sonchifolius)* from the family *Asteraceae*, which is grown by local people for centuries. Locals grown yacon on their gardens, only few plants for family. It is grown for children because of sweet taste, for its medicinal properties or just like a ornamental plant (Grau & Rea, 1997) .

Because yacon was close to disappear, FAO (Food and Agriculture Organization) declared it an endangered species in 1981 and started to support research and development through ICFR (International Council for Fitogenetic Resources). Yacon is grown for his edible tuberous roots and the aboveground part can be used as fodder to animals. Unlike other root crops yacon contains fructooligosacharides (FOS) which is very dietary and can be used in diet for diabetics. In the leaves can be found the phenolic antioxidants with medicinal properties. Yacon can be also used as a industrial crop. Thanks to this properties and adaptability to various climates and altitudes, yacon expanded to the other parts of the world (New Zealand, Japan, Korea, Russia, England, Czech Republic and United States) where it successfully grown (Fernández *et al.*, 2005).

Yacon has many varieties, which are diferent in the colours of tuberous roots and leaves, in the content of FOS (fructooligosaccharides) and in the chromosome number. In addition to genotype of yacon, climatic conditions (rainfall, temperature), lenght of vegetation period also the level of ploidy can affect both the yields of yacon biomass (especially the tuberous roots) and the content of FOS. At yacon landraces occur two levels of ploidy- octoploid (2n=58) and dodecaploid (2n=87) plants. Yields of biomass (tuberous roots) are in the area of its origin states 20-40t/ha and outside the Andes 20-50t/ha (Manrique *et al.*, 2004).

This work is focused upon to influence of ploidy levels to yields of yacon biomass in the climatic conditions of Czech Republic.

2.Literature Review

2.1 Taxonomy

Yacon with almost 30 000 other species belongs to the family Asteraceae (Compositaea). This family contains the largest number of described species, including many important crops, and is distributed in all continents except Antarctica. The family is monophyletic, but there is a great diversity in habitus, habitats, life cycles, pollination mechanisms, and chromosome numbers (Lebeda *et al.*, 2011).

At first yacon and its relatives were placed in polymnia (tribe Heliantheae, subtribe Melampodiinae) a genus founded by Linnaeus in 1751. In 1978 Robinson after more studies separates the species into two genera Smallanthus and Polymnia (Grau & Rea, 1997). Most Central American species and all the South American species were placed in the genus smallanthus, while a few North American species remain in polymnia. In polymnia the achene walls are smooth without striations while in Smallanthus they have shallow grooves on the surface (Lebeda *et al.*, 2011)

According to phylogenetic classification proposed by Cronquist (1955) and Meza (2001), the taxonomy of yacon is:

• Kingdom: Plantae
• Subkingdom: Embryobionta
• Division: Magnoliophyta
• Class: Magnoliopsida
• Subclass: Asteridae
• Order: Asterales
• Family: Asteraceae
• Subfamily: Asteroidae
• Tribe: Helianthae
• Subtribe: Melampodiinae
• Genus: Smallanthus Mackenzie
• Species: Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson.

2.1.1 The other Smallanthus species

Table 1:The other *Smallanthus* species (modified according to Grau & Rea, 1997)

The other *Smallanthus* species			
Species	**Habitus**	**Distribution**	**Chromosome No.**
Smallanthus apus (Blake)		Mexico	32
Smallanthus connatus (Spreng.)	Annual herb	Uruguay, Paraguay, southeastern Brazil and eastern Argentina	32
Smallanthus fruticosus (Benth.)	Shrub or tree	Ecuador, southern Colombia, northern Peru	>50
Smallanthus glabratus (DC.)	Shrub or tree	Peru, Chile, Ecuador	30
Smallanthus jelksii (Hieron.)	Shrub or tree	Peru	58
Smallanthus latisquamos (Blake)	Herb	Costa Rica	16
Smallanthus lundelii (H.Robinson)	Herb	Guatemala	
Smallanthus macroscyphus (Baker ex. Martius)	Perennial herb	Bolivia, northwestern Argentina	32
Smallanthus maculatus (Cav.)	Coarse herb	Mexico, Guatemala, Honduars,Costa Rica, Nicaragua	32,66,68
Smallanthus macvaughii (Wells)	Herb	Mexico	
Smallanthus meridensis (Steyerm.)	Herb	Venezuela, Colombia	
Smallanthus microcephalus (Hieron.)	Shrub or small tree	Ecuador	54, 60
Smallathus oxacanus (Sch.bip. ex Klatt)	Herb	Mexico, Guatemala, Honduars	32
Smallanthus parviceps (Blake)	Shrub or tree	Southern Peru, nortern Bolivia	58
Smallanthus pyramidalis (Triana)	Tree	Venezuela, Ecuador, Colombia	58, 60
Smallanthus quichensis (Coult.)	Herb	Costa Rica, Guatemala	
Smallanthus riparius (H.B.K.)	Herb or shrub	Southern Mexico to northern Bolivia	30,32
Smallanthus siegesbeckius (DC.)	Perennial herb	Peru, Brazil, Bolivia, Paraguay	
Smallanthus suffruticosus (Baker)	Herb or shrub	Venezuelan Amazonia	
Smallanthus uvedalius (L.)	Perennial herb	Eastern United States	32

2.2 Botanical and Morphological Description

Yacon is a perennial herb, but in the cultivation system is considered as an annual (Fernández *et al*., 2007).

The root system is composed of up to twenty tuberous storage roots and an extensive system of thin fibrous roots (see appendix Fig:1). The weight of storage roots is from 100 g up to 1000 g. Their shape, size and color are different and depend on cultivar. The basic colors of tuberous flesh is white, cream, purple, yellow or pink (Grau & Rea, 1997). The root tubers are edible and after the harvest colorless. Have lentgth up to 25 cm (Valentová *et al.*, 2001).

Rhizomes are the main vegetative propagating organs of yacon. The generative reproduction capability was lost during evolution . They are growing directly on the basal part of the main stem and have irregular shape with many eyes on their tops (Grau & Rea, 1997).

The stems can grow up to two meters in height, but depends on natural conditions and ecotype characteristic (Seminario *et al*., 2003) . The aerial stems are cylindrical, hollow at maturity with few branches in most clones or ramified in others. The colours of stem are from green to purplish. The number of internodes depends on ecotypes and size of the plant (Lebeda *et al.* 2011) (see appendix Fig:2).

Leaves grow from every aerial nodes and they grow opposite. The leaves can be varies colors like: green, yellow green, bright green or dark green. Lower leaves are broadly ovate, upper leaves are ovate-lanceolate, without lobes. At flowering stage, the yacon plant has from 13 to 16 pairs of leaves. At post-full flowering stage, plants only produce small leaves (Seminario *et al*., 2003).

Inflorescences are small, 1-3 cm in diameter with color from yellow to orange (Frček & Lojková, 1997), and they are growing only on the top of the stems (see appendix Fig:3). Not every plant reach the flowering stage, depends on environment of the growing area. The production of flowers is limited at yacon . Yacon produces limited number of seeds and germination rate is between 15-30% (Valentová *et al*., 2001).

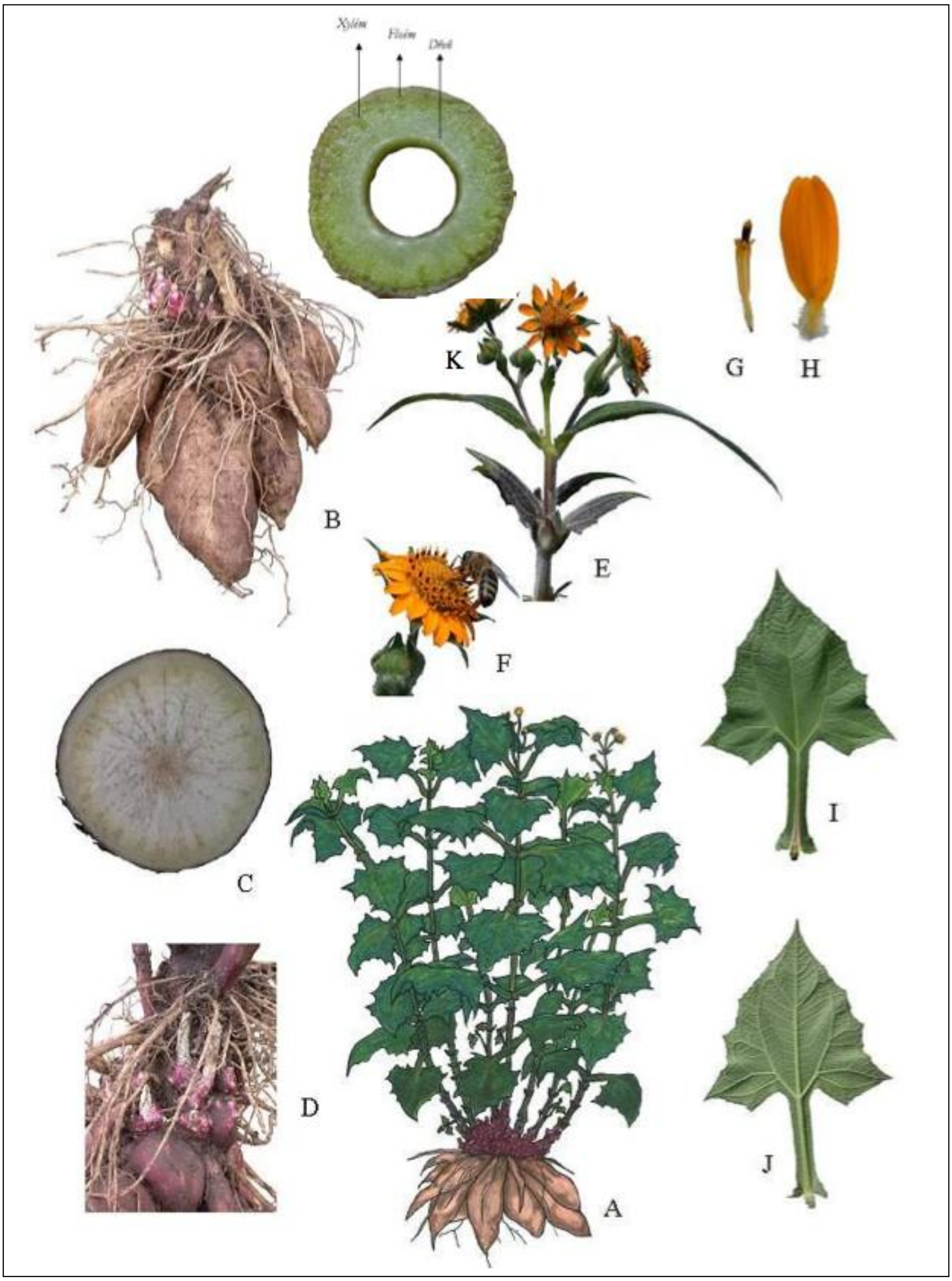

Fig. 1: Morphology and anatomy of yacon plant (Fernández, 2005), A-habitus of yacon; B-tuberous root; C- transverse section of tuberous root; D- rhizomes; E- inflorescence; F-flower head; G- staminate disk flower; H- pistilate ray flower, I- adaxial (upper) side of leaf; J- abaxial (lower) side of leaf; K- section of stem

2.3 Origin and Growing Areas

The properties of yacon were recognized by the farmers before the Incas civilization. The first written mention of yacon comes from the chronicles of Bernabé Cobo (1653) (Muñoz, 2009).

Its original area is the highlands of the Andes, from southern Colombia to northern Argentina (1800-2800 meters above the sea level). But it is also very adaptable to a wide diversity of climates and soils (from sea level up to 3500 meters above the sea level) (Manrique *et al.,* 2004). The best conditions for the production of roots reservants are between 1500-2000 meters above sea level and optimal temperatures during the day time between 18-25°C (Santana & Cardoso, 2008).

The plant is able to tolerate high temperatures with good irrigation, but it is very sensitive to frost, and temperatures below 0°C can damage or destroy the aboveground portion. The roots are damaged after the frost reach them through the soil (Michl, 1995)

Yacon has good water management and can survive long periods of drought. The optimum of rainfall is about 800mm. (Muñoz, 2009).To reach a good yield is recommended equally irrigation through the whole vegetation season. (Frček & Vejvodová 1996).

The plant is adaptable to a wide range variety of soils and can tolerate wide pH range (from acid to weakly alkaline). The suitable soils are rich, moderately deep to deep, light, well-structured and well-drained soils, (Grau & Rea, 1997) with pH 5,5-8 (Muñoz, 2009). In crop rotation has the yacon same place like potatoes, it means after organic fertilization.

Yacon has been described as day-neutral for stem and tuberous formation, but this proces begins very late in the growing season at higher latitude (New Zealand). This may indicate that the plant has a weak short-day response (Grau & Rea, 1997). This explain why its cultivation is limited to a much shorter period in European climates (Valentová & Ulrichová, 2003).

Outside the Andes is yacon grown in New Zealand, to there it was bring from Ecuador in 1982. To Japan was yacon introduced from Korea in the 70‘s and from New Zealand in 1985. To Czech Republic was yacon imported in 1994. There is yacon grown at the experimental farm of Czech University of Life Sciences in Prague and at the Research

Institute in Havlíčkův Brod.(Fernández *et al.* , 2005). Brazil, Russia, Taiwan, England and some places in United States are other countries where yacon is extended. In Italy there was a serious cultivation attempt in the late 1930s, which faded during World War II (Calvino, 1940) (see Fig. 2).

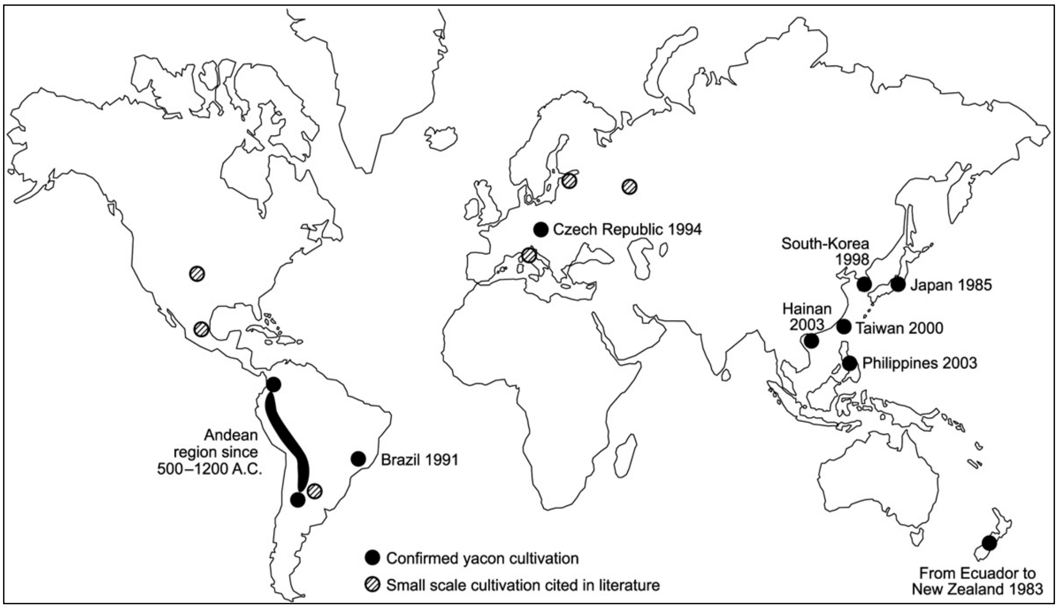

Fig. 2. Cultivation of yacon in different parts of the world (Ojansivu *et al*., 2011)

Yield per hectar for yacon is typically 20-40t/ha. It is strongly affected by location and cultivar, also by agricultural management and by fertilization. In Brazil, Sao Paulo the yield reach after mineral fertilization 50t/ha against the average 30t/ha (Manrique *et al.*, 2004).

In Peru eighteen of twenty departments grow yacon on the area almost 600ha for commercial purposes with yields from 15t/ha up to 50t/ha. (Manrique *et al*., 2004).

In New Zealand yacon production is best suited to warmer northern regions. In the south, the colder temperatures and a shorter growing season limit the production. In the north almost 10 ha of commercial lots are cultivated with using machine planting and harvested with modified potato equipments. Root yields from the commercial planting are 30-40 t/ha. This does not reach the yields from experimental work (90-100t/ha), but it is expected to increase after more research (Douglas *et al.*, 2005).

2.4 Uses and Importance

Fresh roots accumulate mostly water (70%), sacharides (20%) (Lachman *et al*., 2003) and small amounts of fiber, vitamins and minerals (Grau & Rea, 1997). Roots and leaves contain antioxidants like chlorogenic acid, tryptophan and several phenols caffeic acid derivatives (Muñoz, 2009) (see Table 2). The calculated yacon roots energy of fresh matter is very low 619-937 Kj/kg and has similar properties like dietary fibre (Lachman *et al*, 2003).

Table 2:Chemical composition of tuberous roots, leaves and stems (A-Calvino, 1940; B-Bredemann, 1948; C- León, 1964; D- Nieto, 1991; F-Frček *et al.*,1995; cit. Lachman *et al.*,2003)

Compound	Tuberous roots							
	fresh				dry			
	A	B	C	D	A	B	C	D
Water (%)	69.50	92.70	86.6	84.80	–	–	–	–
Ash (%)	2.40	0.26	–	3.50	6.71	3.59	–	23.03
Proteins (%)	2.22	0.44	0.30	3.70	7.31	6.02	2.24	24.34
Lipids (%)	0.13	0.10	0.30	1.50	0.43	1.32	2.24	9.87
Fibre (%)	1.75	0.28	0.50	3.40	5.73	3.88	3.73	22.37
Saccharides (%)	19.67	–	–	–	67.53	–	–	–

Compound	leaves			stem			leaves and stem	
	A		F	A		F	B	
	fresh	dry	dry	fresh	dry	dry	fresh	dry
Water (%)	83.20	–	–	86.70	–	–	92.00	–
Ash (%)	2.68	15.98	12.52	1.35	10.23	9.60	1.03	14.49
Proteins (%)	2.87	17.12	21.18	1.51	11.37	9.73	1.13	15.97
Lipids (%)	1.24	7.40	4.20	6.30	2.26	1.98	0.22	3.04
Fibre (%)	1.68	10.04	11.63	3.57	26.85	23.82	1.11	15.69
Saccharides (%)	1.44	8.58	–	1.55	11.70	–	–	–

The local people in Andes classified yacon as a fruit, it is because yacon tuberous roots possess an agreeable sweet flavour (Grea & Rea, 1997). The roots are consume usually raw and striped. Before eating, the roots are exposed to the sun for 4-7 days to increase their sweetness. Enyzmatic processes in tuberous roots ferments a content of fructose nearly ten times, glucose three time, while saving content of saccharose similarly to freshly harvested roots and lowering the water content by 8 % (Fernández *et al.*, 2005). This procedure is called *ckochoascca* (Herrera, 1943). They are eaten peeled because of the skin taste like resin. Tuberous roots can be also stewed, retaining in part their crispiness, or grated and squeezed through a cloth to obtain a sweet refreshing drink. Yacon is also tasty chopped up in fruit salads mixed with bananas, oranges, pawpaws, etc (Grea & Rea, 1997).

Also young stems and leaves can be used as vegetable (to the salads and etc). In Japan yacon tubers are processed into juices, bakery products, fermented beverages, lyophilized powder or pulp. The leaves and stems are mixed with tea leaves. In Brazil, medicinal properties have been ascribed to yacon leaves, and they are used to prepare a medicinal tea. A methanol extract of yacon leaves contained ent-kaurenoic acid and related diterpenoid substances. These compounds probably play a certain physiological role in the defense mechanism of this plant and it is highly pest resistant (Valentová & Ulrichová, 2003).

Yacon aboveground parts contain large amounts of proteins and this can be used as green stuff for livestock (Valentová & Ulrichová, 2003). Also tuberous roots can be used to feed ruminants, because they can metabolized all yacon carbohydrates including oligofructans (Grea & Rea, 1997).

One of the problems in using yacon, is that the fresh tuberous roots have low durability and are very fragile. They can be easily damaged at the harvest and thereafter attacked by fungi and rot symptoms. There are some ways how to preserve yacon after harvest. One of the promising processing techniques is the production of dry chips. Tuberous roots are peeled and cut in thin slices. These slices are dried in a plastic tunnel and afterwards in the oven at 60°C (Kakihara *et al.*,1996). The chips can be stored indefinitely. Another way is to made *Chancaca*. It is made from the juice which is boiled and concentrated to produce solid dark-brown blocks, similar to product obtained from concentraiting sugarcane juice (Grea & Rea, 1997). In Japan yacon pickles are produce and sold in the supermarkets.

There is many options how to use yacon as a medicament. Original Peruvian population used for centuries yacon tubers as a traditional folk medicament to treat hyperglycemia, kidney problems and for skin rejuvenation (Valentová & Ulrichová, 2003). In Bolivia yacon is commonly consumed by diabetics and people who suffer from digestive problems (Grea & Rea, 1997). Volpato *et al* (1997) demonstrated hypoglycemic activity by the water extract of dried leaves which were given to rats with induced diabetes.

The consumption of yacon also modifies gut microflora composition (thanks to oligofructans- see Fig: 3) and its metabolic activities. This activities is beneficial to modulate lipid metabolism, calcium absorption and good function of childhood immune system (Valentová & Ulrichová, 2003). The several carbohydrates which are stored in the roots are: fructose, glucose, sucrose, low polymerization degree (DP) oligosaccharides (DP 3 to 10 fructans), and traces of starch and inulin (Asami *et al*., 1989) (see Table 3).

Oligosacharides purified from yacon roots have been identified as beta-(2-1)-fructooligosacharides with terminal sucrose (inulin type oligofructans) (Goto et al.,1995). Simiral low DP fructans have been used as sucrose substitutes because they are considered as dietetic. They have a beneficial influence on the human intestinal flora. Oligofructans have been classified as prebiotics because they are not digested in human gastrointestinal tract (humans have no enzyme capable of hydrolysing the β(2-1) bond) and they are transported to the colon. There the selected species of gut micro-flora, such Bifidobacterium and Lactobacillus, ferment the oligofructans (Valentová & Ulrichová, 2003). The relative proportions of oligofructans and monosacharides fluctuate during the growing cycle and after harvesting (Grau & Rea, 1997).

Table 3: Contents of saccharides in yacon tuberous roots (Valentová et al., 2001)

Saccharide	Content (mg/g dry matter)
Fructose	350.1 ± 42.0
Glucose	158.3 ± 28.6
Sucrose	74.5 ± 19.0
GF_2	60.1 ± 12.6
GF_3	47.4 ± 8.2
GF_4	33.6 ± 9.3
GF_5	20.6 ± 5.2
GF_6	15.8 ± 4.0
GF_7	12.7 ± 4.0
GF_8	9.6 ± 7.2
GF_9	6.6 ± 2.3
Inulin	13.5 ± 0.4

GFx= glucofructans, where index x is a number of fructose units in the molecule

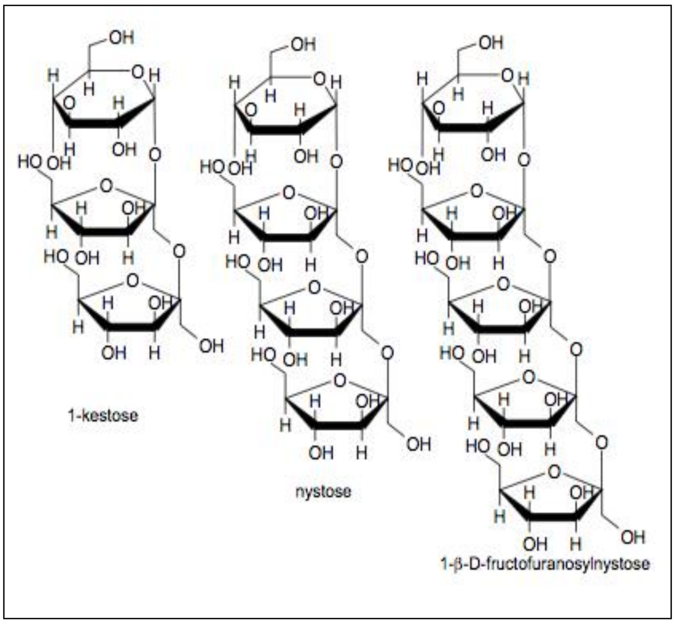

Fig 3: Chemical structure of three main fructooligosaccharides (GF_2 – GF_4) (Lachman *et al.*, 2003)

2.5 Breeding of Yacon

The main way how is yacon propagated is vegetatively. This method maintains genetic stability of the clones, but monoculture produces susceptibility to pests and pathogens. The high degree of ploidy at yacon reduces fertility and restricts the use of conventional breeding techniques. To increase yield or saccharide content, positive selection must be used (Lebeda *et al*., 2011).

Grau & Rea (1997) deduced, based on geographical distribution, growth habit, and morphology of aboveground part, that the following wild species are closely related to yacon: *S. connatus, S. macroscyphus, P. riparius, S. meridensis, S. suffruticosus* and *S. siegesbeckius.* Most of these species have a lower chromosomes number than yacon, because this chromosome number incompatibility, the conventional breeding techniques could not be used. In the varietal improvment of yacon the biotechnology will take a main role (polyploidization), (Lebeda *et al*., 2011).

The main breeding goals in yacon are: higher yields, higher content of inulin-type FOS in tuberous roots, higher content of antioxidants in leaves, shortening of growing period and increased frost tolerance (Lebeda *et al*., 2011).

2.6 Polyploidy

Ploidy in the genetics determines the number of chromosomes in the cell nucleus. Polyploidy is defined as the possesion of three or more complete sets of chromosome. Increasing and decreasing the number of chromosomes is used as a useful method to produce a modified plant genotypes in breeding and in other genetic studies (Ramsey & Schemske, 1998).

Two distinct types of polyploids are described- autopolyploids and allopolyploids. The autopolyploids arise within population of individual species, however the allopolyploids are the product of interspecific hybridization. To induce polyploidy in plants the major cytological mechanism are somatic doubling in mitosis, and nonreduction in meiosis. Somatic doubling in meristem tissue of juvenile or adult sporophytes has been observed to produce mixoploid chimeras. The nonreduction process in meiosis generates unreduced gametes, also referred as 2*n* gametes, which contain the full somatic chromosome number (Ramsey & Schemske, 1998).

The synthetic production of chromosome doubled in vitro plants can be achieved by interfering with the plant's cell cycle. The plant cell cycle has different phase: a G1-phase (post-mitotic interphase), an S-phase (DNA synthesis phase) a G2-phase (pre-mitotic interphase) and an M-phase (mitosis) (Francis, 2007).The Idea is to disrupt a cell cycle when the chromosome complement is doubled (between S-phase and the end of the mitosis phase) and inhibit the separation of chromosomes (see Fig 4). This will result in cells with a doubled chromosome complement. For this is need a chemical antimitotic agent (Dhooghe *et al*., 2010).

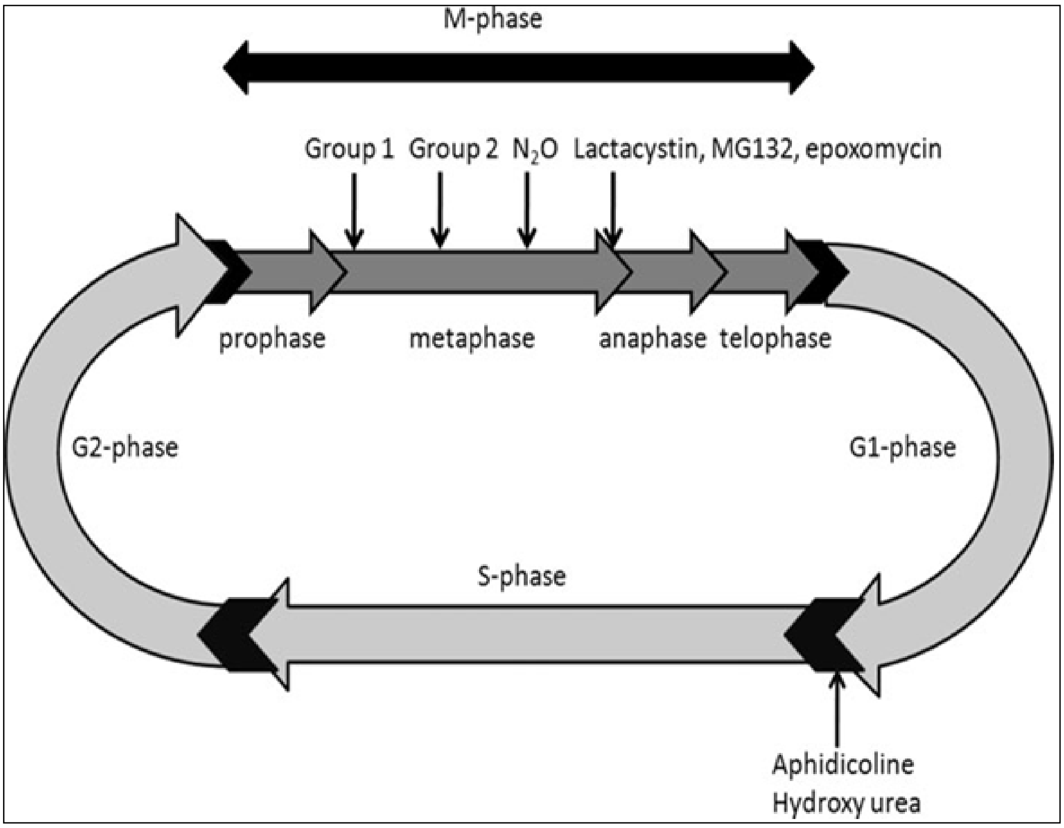

Fig.4 The different action points of antimitotic agents on the plant's cell cycle. Group 1-colchine, orazylin, trifluralin. Group 2-carbamates (Dhooghe et al., 2010).

The most commonly used antimitotic agent is colchine. It is an alkaloid which is extracted from the seed and bulbs of the *Colchicum autumnale* (Eigsti & Dustin, 1955). An advantage of colchin is that the sterilization does not reduce its polyploidizing capacities (Zhang *et al* , 2007). There is also some disadvantages: colchin causes side effects such as sterility, chromosome losses, gene mutation and is very toxic to humans. The alternative antimitotic agent is oryzalin, triflurarin and some herbicides (Dhooghe *et al*., 2010).

The protocol of in vitro chromosome doubling consist of several sub-processes. At first, the plant material (plantlets or shots, buds, callus, somatic or zygotic embryo, seeds, nodal segment or tuberous segments) must be treated wit antimitotic agents. Also, the concentration and time during which the plant material is exposed to antimitotic agents is very important (Dhooghe *et al*., 2010) .

To determinate polyploidy, methods like chromosome counts, flow cytometry and the evaluation of morphological or anatomical parameters, can be used. Chromosome counting is the most concrete method because it determines the effective chromosome number. But it is very laborious, only a limited number of cells can be analyzed and mistakes are easily made when counting many small chromosomes. The flow cytometry can provide a representative picture of a heterogeneous population of cells (Dolezel *et al*., 2007), it is a rapid method for ploidy testing and allows the examination of a large numbers of plants (Dhooghe *et al*., 2010).

The chromosome number in somatic cells of yacon is very diverse depending on the plant material used. In Ecuadorian material (Nieto, 1991) and in Peruvian material (Talledo & Escobar, 1996) was detected chromosome number (2n=60). In other Peruvian material grown at the La Molina University the chromose number was (2n=32) (León, 1964). Another study mentioned (2n=58) chromosomes in clones from Ecuador, Bolivia, Peru and (2n=87) chromosomes from Argentina (Ishiki *et al.*, 1997).In the plants grown in Czech Republic, which are from Bolivia, Ecuador and New Zealand, Fernández & Kučera (1997) determine the chromosome number (2n=58) and Viehmannová (2009) determine (2n =87).

When working with Peruvian material, yacon was described as a tetrapliod (Talledo & Escobar, 1996 ; Grau & Rea, 1997). In the more detailed work yacon is considere as an allopolyploid derived from hybridization between *Smallanthus macroscyphus* and/or similar related wild species (2n=28, A= 7) and *Smallanthus riparius* (2n= 32, B=8 ; A and B= two different genomes) (Grau & Slanis, 1996). The octoploid (2n= 6A + 2B= 58) and dodecaploid (2n = 9A + 3B= 87) organization of yacon genome could be explained by its hybrid origin (see figure 5). The octoploid structure is propose to be dominant in most yacon clones of 2n = 8x = 58, while a dodecaploid structure would explain 2n= 87 (Lebeda *et al* 2011). The effects which may affect the chromosome number is the presence of B chromosomes in other *smallanthus* taxa and fact, that yacon is propagated by clones. (Ishiki *et al*. 1997*)*. But chromosome number in some species remains still an issue (Carr *et al.* 1999).

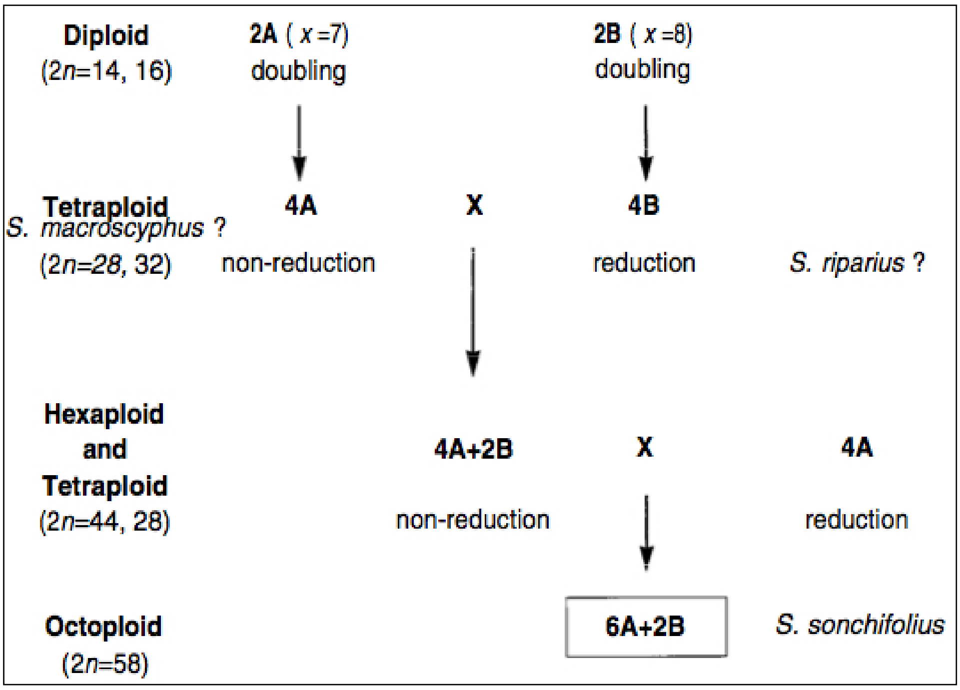

Fig. 5: Hypothetical evolution of yacon (Ishiki *et al.* 1997, modified by A. Grau)

3. Aims of the Thesis

This work study the biomass yields (aboveground part and tuberous roots) of three landraces with different level of ploidy (2n=58; 2n=87; 2n=116) grown in the climatic conditions of Czech Republic.

This thesis is focused on two main aims:

- Biometric evaluation of plants of selected landraces with different chromosome number of *Smallanthus* species
- Study of differences in yields, mainly tuberous roots and aboveground part, between the landraces and its statistical evaluation

Hypothesis:

I. Level of ploidy will have influence on the yields of tuberous roots and aboveground parts.

II. Level of ploidy will have no influence on the yields of tuberous roots and aboveground parts.

4. Materials and Methods

4.1 Plant Material

For experiments was used landraces 'NZL 1' ; ‘POLY 3' and 'PER 65' from the collection conserved in ITS- CULS in Prague.

The 'NZL 1' is a clone bought from Aucland in New Zealand in 1993, but is orinally from Andes. It is octoploid clone with chromosome number (2n = 58). The ' PER 65' is from organization CICA (Centro Internacional de Cultivos Andinos) from Peru, and was brought to Czech Republic in year 2005. It is a dodecaploid clone (2n = 87) The 'POLY 3' was experimentally obtained by Viehmannová (2009) by means of *in vitro* polyploidization from octoploid clone 'NZL 1'. It is a hexadecaploid clone (2n= 116).

4.2 Methods

4.2.1 Cultivation of Yacon on Trial Field

During the winter, clones are harboring in the Botanical garden of ITS- CULS in Prague. Yacon can be planted after the last frost in spring (mostly the second half of May) (Fernández *et al.* 2006). Yacon was planted manually in the ridges with the same sowing preparation like potatoes. The field was fertilized by manure before planting (cattle manure, 20t/ha). The spacing between ridges was 0.7 x 0.7 m. The trial field of ITS- CULS in Prague lies in elevation of 285 meters above sea level (50° 05‘ North Latitude, 14° 27‘ East Longitude) .The climatic area is mildly warm and mildly dry. The average annual temperature is 9,0°C.

Table 4: Meteorological data for year 2010-2011 (MSCULS,2012)

Year	Mean air temperature during the growing season (°C)	Total percipitation during the growing season (mm)	Lenght of growing season (days)
2010	15,6	328,4	132
2011	16,4	303,9	144

The meteorological data is from Meteorologic station of CULS. The station lies in elevation of 280 meters above sea level(50°08´ North Latitude, 14°22´ East Longitude).

During the vegetation the field was regularly irrigated (once a weak) and twice weeded by hand. Once the growth is fully evolved there is no need for the other cultivation actions as yacon is resistant to weeds (Fernández *et al.* 2006).

It is advisible to shift the harvest as late as possible, but the aboveground part and the tuberous roots must not be damaged by the first autumn frosts (October) (Fernández *et al.* 2006). At first, the stems was cut off approximately ten centimeters from the ground. Then it was weighted all together (from one plant), afterwards the leaves were cut off from the stems and weighted separately. This measures were made by lifting weight (see appendix Fig:4). After that the underground part was digged, also manually, it is very fragile so it must be done carefully. The roots was cleaned up from the ground, then the tuberous roots were separeted from the rhizomes and this was measured on the digital table weight KPZ 2-03-5 with readability from 1,0 g.

4.2.2 Biometric Evaluation

During the vegetation were measured: the height of plants, number of pairs of leaves, ramification of stem, width of plants in row and the time of discovering first tuberous roots. The measures were made thirteen times:14th, 18th , 24th , 30th ,36 th , 45th , 54th , 64th , 75th , 84th , 96th , 105 th and in 132 th day of vegetation.This measures were made in year 2010

In the end of vegetation during the harvest were measured: the plant height, number of stems, the ramification on main stem, the weight of the leaves, the weight of the stems, the weight of the tuberous roots, number of tuberous roots per plant (and the lenght and weight of the bigest) and the weight of the rhizomes. This measures were made in year 2010 and year 2011. From every landrace ten clones were measured.

4.2.3 Statistical Evaluation

The data analyzed using t-test for independent samples or analysis of variance (ANOVA) in program StatSoft STATISTICA 7.0. The statistical evaluation was made for all observed objects-the plant height, number of stems, the ramification on main stem, the weight of the leaves, the weight of the stems, the weight of the tuberous roots, number of tuberous roots per plant (and the lenght and weight of the bigest) and the weight of the rhizomes. Also were made the correlations between the aboveground part, weight of tubers and weight of rhizomes at all three clones.

5. Results and Discussions

5.1. Growth During the Vegetation

During the observation it was evident that the landraces have different development, altough have the same growing conditions. At the first sight the landrace 'PER 65' grown faster, look vitaly and have massive development of leaves. The second two landraces look the same at the beggining than the 'NZL I' starts growning little faster then ‘POLY 3‘ (see Fig 6.). This confirm the results of Viehmannová (2009), that the hexadecaploid plants were typically growing slowly with small height and a small spread between plants (see Table 5). Also the beginning of formation tuberous roots were different among the clones. First started formation of tubers at ‘PER 65‘, then at ‘NZL I‘ and finally at ‘POLY 3‘. None of these three clones start bud formation or flowering (see Table 5). This confirm the results of Fernández *et al.* (2006), that in the climatic conditions of Czech Republic due to a short growing period there is no bud formation or flowering.

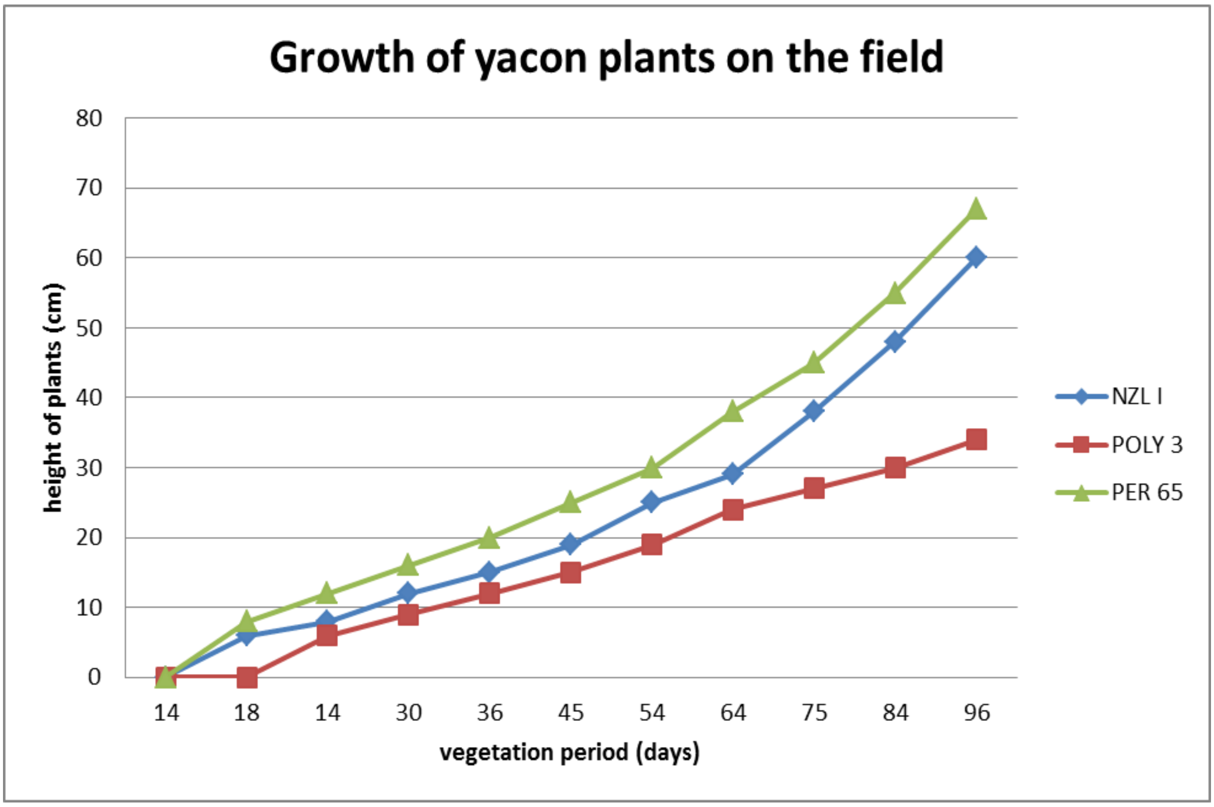

Fig 6: The average high of plants during vegetation under the field conditions

After the last measures at 132th day of the vegetation (day of harvest) according to Fernández *et al.* (2007) macrophenological scale for the three different clones was made (see Table 5)

Table 5:BBCH scale for describing the phenological growth of clones with different level of ploidy

decimal code	description	NZL I	PER 65	POLY 3
0	Germination/sprouting	0-14	0-14	0-24
1	Leaf development	14-64	14-53	18-64
10	Cotyledons completely unfolded	14	14	18
11	1. pair of true leaves unfolded	18	14	24
12	2. pair of true leaves unfolded	24	18	30
15	3. pair of true leaves unfolded	30	24	36
17	4. pair of true leaves unfolded	45	36	45
19	5. pair of true leaves unfolded	64	54	64
2	Formation of side shoots(ramification)	64-110	64-110	75-110
21	Beginning of ramification	64	64	81
25	Ramified half of stem	84	75	105
29	All stem ramified	105	96	114
3	Crop cover	54-84	45-75	64-105
31	Beginning of crop cover: 10%of plant meet between rows	54	45	64
32	20% of plants meet between rows	54	45	64
33	30% of plants meet between rows	64	54	75
34	40% of plants meet between rows	64	54	75
35	50% of plants meet between rows	75	54	84
36	60% of plants meet between rows	75	64	84
37	70% of plants meet between rows	84	64	96
38	80% of plants meet between rows	84	64	96
39	90% of plants meet between rows	84	75	105
4	Formation of tuberous roots	75-132	64-132	84-132
40	Beginning of tuberous roots formation	75	64	84
49	Maturity of tubrous roots	132	132	132
5	Bud formation			
6	Flowering			
7	Formation of achenes			
8	Maturity of achenes			
9	Senescence			

5.2. Yields of Biomass 2010

All measures were made in the day of the harvest -132th day of vegetation (see Appendix Table 1) on the fresh matter.

From the table 6 can be see that there is statistical significant difference in the yields of aboveground part and tuberous roots between all landraces. The biggest yields reach clones of ‘PER 65‘ , the second is clones of ‘NZL I‘ and the clones of ‘POLY 3‘ have the lowest yield (see Table 6). That proved that in the same climatic conditions the level of ploidy have an influence on the yields. This confirm hypothesis I.

In the weight of rhizomes, it is statistical significant difference only between the ‘PER 65’ which has the biggest yield and ‘POLY 3‘(see Table 6). There is no difference between ‘POLY 3‘ and ‘NZL I’ , although they have differences in yield of tuberous roots. It may confirm the results of Viehmannová (2009), which proved, that the rhizomes of hexadekaploid clones have a higher relative representation in the underground biomass compared to the octoploid clones.

About the other observed characteristics (the plant height, number of tuberous roots per plant and the lenght and weight of the bigest tuber) there is no statistical significant difference between the ‘NZL I‘ and ‘PER 65‘ . The difference is with these two last mentioned and ‘POLY 3‘ (see Table 6) . There is no statistical difference between number of stems and number of side shots on the main stem. Which can indicate that the ‘POLY 3‘ could need a longer vegetation period to grow high and create more tuberous roots. It was suggest by Viehmannová (2009) that the polyploidy causes extension of vegetation period.

In year 2010 the highest plant was one clone from landrace 'NZL I' with 103 cm. One of the plant from 'PER 65' has the most tuberous roots-56 pieces. Also from 'PER 65' was the longest tuberous roots with 23 cm. The heaviest tuberous roots has 322 g and was from 'NZL I'.

At ‘NZL I‘ and ‘PER 65‘was found correlation between yield of aboveground part and rhizomes, which means that these two parts influence each other. Also at ‘NZL I‘ is correlation between tuberous roots and rhizomes (see Table 7).

Table 6:Quantitative characteristics of selected landraces in 2010

2010			
	NZL I	PER 65	POLY 3
weight of aboveground part(g/plant)	751,1 ± 177,4a	1206 ± 280,6b	305 ± 33,5c
weight of tuberous roots (g/plant)	774,7 ± 288,5a	1329,3 ± 283,9b	61,6 ± 40,3c
weight of the rhizomes (g/plant)	652,5 ± 415,1ab	728 ± 260a	507,8 ± 160,9b
plant height (cm)	65,8 ± 16,7a	73,7 ± 8,3a	36,9 ± 5,8b
number of stems on plant	8,2 ± 3,3a	13,1 ±4,8a	6,5 ± 1,6a
number of side shots on main stem	5,1 ± 1,1 a	1,6 ± 1,6a	2,3 ± 1,7a
weight of leaves (g/plant)	348,8 ± 79a	621 ± 125,2b	158,5 ± 16,7c
weight of stems (g/plant)	424,4 ± 90,9a	585 ± 170,3a	146,5 ± 19,5b
number of tuberous roots on plant	19,2 ± 5,8a	30,1 ± 10,1a	2,7 ± 1,6b
lenght of the longest tuberous roots (cm)	14 ± 1a	19,8 ± 4,5a	7 ± 2,7b
weight of the heaviest tuberous roots (g)	134,4 ± 77,8a	155,3 ± 46,8a	27,5 ± 19,5b

ab means in columns denoted by the same letters are not significantly different by LSD at $\alpha = 0.05$

Table 7: Yield correlation coefficients of different yacon plant parts 2010

2010		Aboveground part	Tuberous roots	Rhizomes
Aboveground part	NZL I		0,36	0,64*
	PER 65		0,62	0,74*
	POLY 3		-0,07	0,26
Tuberous roots	NZL I	0,36		0,85*
	PER 65	0,62		0,51
	POLY 3	-0,07		-0,42
Rhizomes	NZL I	0,64*	0,85*	
	PER 65	0,74*	0,51	
	POLY 3	0,26	-0,42	

* marked correlations are significant at the 0.05 level

5.3. Yields of Biomass 2011

All measures were made in the day of the harvest -144th day of vegetation (see Appendix Table 2) on the fresh matter.

In the yields of aboveground part there is statistical significant difference between all landraces, the biggest yield have ‘PER 65‘, then is the ‘NZL I‘ and the lowest yield have the ‘POLY 3‘(see Table 8). It is the same as the year 2010 which may indicate that the ploidy level have bigger influence than the climatic conditions (see appendix Table 1 and Table 2), maybe because of irigation method.

In the yields of tuberous roots there is statistical significant difference between the ‘POLY 3‘ which have the lowest yield and the two rest landraces (see Table 8). It is different from the year 2010. It can be cause by the longer vegetation period or that the ‘PER 65‘ was not on the same part of the field.

In the yields of rhizomes there is statistical significant difference between the ‘PER 65‘ and the two rest landraces (see Table 8). It confirms the results from previous year and from Viehmannová (2009) that the rhizomes of hexadecaploid clones have a higher relative representation in the underground biomass compared to octoploid clones.

The highest plant from year 2011 was clone from 'PER 65' with 152 cm. The plant with the most pieces of tuberous roots was also from 'PER 65'- 23 pcs. The longest and the heaviest tuberous roots was from 'NZL I' with 430 g and 17 cm.

In year 2011 was find the correlation at ‘PER 65‘ between the yield of aboveground part and rhizomes, and at ‘NZL I‘ between the yield of tuberous roots and aboveground part (see Table 9), which means that the aboveground part did show an effect on yield of tuberous roots and rhizomes.

Table 8:Quantitative characteristics of selected landraces in 2011

2011			
	NZL	PER 65	POLY 3
weight of aboveground part (g/plant	558,5 ± 144,6a	3123 ± 206,2b	418 ± 67c
weight of tuberous roots (g/plant)	425,9 ± 329,2a	683 ± 241,5a	45,9 ±43,4b
weight of the rhizomes (g/plant)	568 ± 394,5a	1698,6 ± 233,4b	650,8 ± 386,5a
plant height (cm)	56,5 ± 12,7a	149,8 ±2,1b	44,5 ± 9,4a
number of stems on plant	5,1 ± 1,7a	11,4 ± 1,9a	4,4 ± 1,2a
number of side shots on main stem	6,4 ± 0,8a	4,6 ± 0,6b	5,3 ±1,5ab
weight of leaves (g/plant)	283,3 ± 62,8a	1124 ±55,4b	213 ± 32,6a
weight of stems (g/plant)	277,2 ± 81,8a	1999 ± 165,8b	205 ±39,8 c
number of tuberous roots on plant	5,5 ± 3,8a	15 ± 4,9 b	1,6 ± 0,8c
lenght of the longest tuberous roots (cm)	10,2 ± 4,2a	12,38 ± 3,4a	6,4 ± 2,7a
weight of the heaviest tuberous roots (g)	175,1 ± 123,8a	14,5 ± 1b	32,1 ± 30c

ab means in columns denoted by the same letters are not significantly different by LSD at $\alpha = 0.05$

Table 9: Yield correlation coefficients of different yacon plant parts 2011

2011		Aboveground part	Tuberous roots	Rhizomes
Aboveground part	NZL I		0,8*	0,54
	PER 65		0,41	0,66*
	POLY 3		-0,33	0,62
Tuberous roots	NZL I	0,8*		0,2
	PER 65	0,41		0,75*
	POLY 3	-0,33		0,15
Rhizomes	NZL I	0,54	0,2	
	PER 65	0,66*	0,75*	
	POLY 3	0,62	0,15	

* marked correlations are significant at the 0.05 level

6. Conclusion

In Czech Republic yacon has been grown since 1994. It is grown only in few places like experimental plots and by some people for own consumption. In climatic conditions of Czech Republic yacon reaches relatively good yields (up to 35 t/ha of tuberous roots). Nowadays it is possible to buy rhizomes in some garden centers and grow yacon in the garden. It is becoming very popular especially for diabetics, which discover its medicinal properties.

Based on the results, it can be concluded that the ploidy level of yacon (*Smallanthus sonchifolius*), grown in the climatic conditions of Czech Republic, has influence on yields of biomass (mainly at aboveground part and tuberous roots). But it does not mean that the plants with higher chromosome number have biggest yields. The dodecaploid clone (2n= 87) have bigger yields than octoploid (2n=58) and hexadecaploid (2n=116), which means, that increasing of the chromosome number does not increase the yield.

For future research is recommended to determine the influence of ploidy level on content of FOS (fructooligosaccharides). It would be beneficial to try to grow hexadecaploid clone under other climatic conditions in the place of origin of yacon (longest vegetation period). Yacon can be use in diet of diabetics, as a healthy sweet for children and has beneficial effect on lipid metabolism. Because of this could be recommended to increase the area were is yacon grown, and sell it in the health food stores.

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8.Appendix

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Table 1: Meteorological data for the year 2010 in Suchdol , Prague (MSCULS, 2012)						
	June (5.6.)	July	August	September	October (15.10.)	Total
Days of cultivation per month	25	31	31	30	15	132
Main air temperature (°C)	17,5	21,4	18,2	11,7	9	
Total percipitation (mm)	72,9	90	128,7	35,1	1,7	328,4

Table 2: Meteorological data for the year 2011 in Suchdol, Prague (MSCULS, 2012)							
	May (23.5.)	June	July	August	September	October (15.10.)	total
Days of cultivation per month	7	30	31	31	30	15	144
Main air temperature (°C)	17,1	18,2	17,8	19,1	15,98	10,29	
Total percipitation (mm)	4,5	60	120,3	70	25,5	23,6	303,9

fig 1: Tuberous roots (photo:author)

Fig 2: Yacon plants (photo: author)

Fig 3: Yacon Inflorescences (photo: author)   Fig 4: lifting weight (photo: author)