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Assessment of genetic diversity of *Solanum sessiliflorum* Dunal by application of ISSR marker

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Declaration

I, Sokhour May, declare that I have written the whole master thesis **"Assessment of genetic diversity of *Solanum sessiliflorum* Dunal by application of ISSR marker"** independently and all the literature resources are listed at the end of the thesis.

In Prague

…….

Sokhour May

Abstract

Solanum sessiliflorum Dunal is an Amazonian crop species and one of the valuable crops in Solanaceae family serving main roles in Latin American's cuisine, soft drink, and mainly in ethnomedicine. This species contains variations in fruit shape and size; however, the research on genetics is limited. Among the molecular markers, Inter Simple Sequence Repeat (ISSR) provides simple technique, produces more polymorphic loci, and its cost is inexpensive. It has been used on many *Solanum spp.* such as *S. tuborosum, S. nigrum,* and *Lycopersicon esculentum* etc. A total number of 248 samples divided into 40 populations were collected from 3 regions in Peru. Based on fruit shape, it was divided into five: trangular, heart, oblong, flat, and round. DNA isolation was done by CTAB method and six primers were selected from 30 primers after optimum annealing temperature of PCR. They provided several clearly reproducible bands. In total, they generated 6143 bands with 133 amplified loci, while 131 were polymorphic bands. Shannon Index within populations was 0.1398 and Nei's genetic diversity was 0.0905 in average. Among populations, Shannon Index showed higher value to 0.2783 accompanying with Nei's Genetic distance which was 0.1672, especially gene flow acquired high number 0.5871 and G_{ST} got 0.7277. The analysis of molecular variance (AMOVA) illustrated 72% of genetic diversity within populations, 21% among populations and only 7% among regions. The hierarchical clustering (UPGMA) of populations tend to distinguish LP11, LP12, LP13, and LP14 from the rest. Another dendrogram of all the samples differentiated neither according to the populations nor regions. The factorial analysis strongly supported this dendrogram except samples from LP1 and LP3, which were almost together. Even though the evaluation of phenotypes founded only on fruit shape, ISSR helped to obviously investigate the genetic variation within this species. This present study will stand as the basic part for further research in genetics, particularly in breeding program.

Keywords: *Solanum sessiliflorum*, ISSR, genetic diversity, fruit shape

Abstrakt

Solanum sessiliflorum Dunal je rostlinný druh z čeledi Solanaceae původem z Peruánské Amazonie využívaný k přípravě tradičních pokrmů, nealkoholických nápojů, a hlavně v tradičním léčitelství. U tohoto druhu je známa velká morfologická variabilita, především tvaru a velikosti plodů. O genetické variabilitě tohoto druhu jsou dostupné pouze velmi limitované informace. Mezi dostupnou techniku molekulárních markerů patří technika Inter Simple Sequence Repeat (ISSR), která byla aplikována u mnoha druhů rodu *Solanum* spp. jako je například *S. tuborosum*, *S. nigrum* a *Lycopersicon esculentum* atd. Celkový počet testovaných 248 vzorků představuje 40 populací, které byly nasbírány ve třech regionech Peru. U tvaru plodů bylo hodnoceno pět typů- trojúhleníkový, srdčitý, podlouhlý, plochý a kulatý. Izolace DNA byla provedena metodou CTAB a z celkového počtu 30 ISSR primerů, bylo vybráno šest primerů pro finální hodnocení. Celkový počet bandů byl 6143 při počtu lokusů 133, z toho 131 lokusů bylo polymorfních. Průměrná hodnota pro Shannon Index mezi populací byla 0,1398 a Nei index k porovnání genetické variability mezi vzorky byl 0,0905. Mezi populacemi Shannon Index ukázal vyšší hodnotu (0,2783) společně s Nei indexem (0,1672), genetickým tokem (0,5871) a G_{ST} (0,7277). Analýza molekulární variance (AMOVA) ukázala 72% genetické variability uvnitř populací, 21% mezi populacemi a pouze 7% mezi regiony. Hierarchické shlukování (UPGMA) ukázalo tendenci odlišení populací LP11, LP12, LP13 a LP14 od ostatních. Dendrogram neukázal jasné shlukování dle lokalit sběru jednotlivých populací ani regionů. Přesto je patrná tendence u vzorků některých populací se shlukovat do jedné skupiny, i přesto že i zde je patrná promíchanost vzorků mezi jednotlivými populacemi. Podobné výsledky ukázala také faktorová analýza vzorků z populací, kde populace LP1 a LP3 vytvořily oddělenou skupinu. I přesto, že morfologické hodnocení bylo založeno pouze na tvaru plodů společně s informacemi o variabilitě ISSR markerů poskytlo prvotní informace o genetické variabilitě druhu *Solanum sessiliflorum*. Tato studie bude základ pro další výzkum a šlechtitelský program.

Keywords: *Solanum sessiliflorum*, ISSR, genetická diverzita, tvar plodu

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List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
CTAB	Cetyl Trimethyl Ammonium Bromide
DAF	DNA Amplification Fingerprinting
IRAP	Inter Retroelement Amplified Polymorphism
ISSR	Inter Simple Sequence Repeat
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
PVP	Polyvinylpyrrolidone
REMAP	Retrotransposon-Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SCAR	Sequence Characterized Amplified Regions
SEAP	Secreted Alkaline Phosphatase
SNP	Single Nucleotide Polymorphism
SSAP	Sequence-Specific Amplification Polymorphism
SSR	Simple Sequence Repeat
STS	Sequence-Tagged Site
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

1. Introduction

Solanaceae family is one of the most important domesticated crops which have been cultivated for many years. *Solanum* is the largest angiosperm genera, with approximately 1500 species distributed worldwide and plays a key role in food supply and medicine (Peralta *et al.*, 2008; Chiarini and Barboza, 2007; Kandimalla *et al*., 2015). In total, there are 115 genera, 8,400 scientific plant names of species rank for the family Solanaceae, and only 2,678 are accepted species names (The Plant List, 2003). This family consists of herb, small tree, shrub, woody and spine; moreover, ornamental, poisonous, and medicinal plants are included, which mostly originated in Central and South America (Lim, 2013). The genus includes many important economic plants such as the tomato, potato and brinjal, as well as a number of lesser-known cultivated species, such as the pepino (*S. muricatum* Aiton.), naranjilla (*S. quitoense* Lam.), cocona (*S. sessiliflorum* Dunal.) and tree tomato (*S. bataceum* Cav.).

Among the plants listed above, there are still underutilized crops having economic benefits, one of which is *Solanum sessiliflorum* Dunal. According to (Salick, 1989), it is used in ethno medicine and is seldom grown or planted for only small scale farming in Peru. Domestication of this species has been investigated by (Salick, 1992). *S. sessliflorum* is common in the upper Amazon Basin and cultivated sporadically along the Amazon and Orinoco river systems. It is an important genetic resource for the Amazonian people, because its fruit is traditionally used for food, medicine and cosmetics (Silva Filho et al., 2005). Because of high variation in fruits according to different shapes and sizes, this leads to many research on characterization of morphological variation and chemical composition (Marx et al., 1998; Jiliana et al, 2013), biological activities (Goncalves et al., 2013), phytoremediation for the deleterious effects of methylmercury on the reproductive system of rats (Silva et al., 2014). Anyway, there is no research conducting on the DNA of *Solanum sessiliflorum*, so I decided to work on genetic diversity on *S. sessiliflorum* to clarify about the variation in morphology.

2. Literature review

2.1 Taxonomy and relative species

S. sessiliflorum was known in the previous time as *S. hyporrhdium* then was called as *S. topiru* (Duarte, 2011).

Sciencetific Name: *Solanum sessiliflorum* Dunal

Synonyms: *S. alibile, S. arecunarum* Pittier, *S. topiro* Dunal

Kingdom Plantae – Plants

- Subkingdom : Tracheobionta
- Superdivision : Spermatophyta
- Division : Magnoliophyta
- Class : Magnoliopsida
- Subclass : Asteridae
- Order : Solanales
- Family : Solanaceae
- Genus : Solanum L.
- Species : *Solanum sessiliflorum* Dunal

Vascular name:

Brazil : Maná-cubiu, Maná, Topiro and Tomate de índio (Portuguses)
Colombia : Cocona, Coconilla or Naranjilla, Lulo, Tupiru,
French : Tomate Chauve Souris
Guyana : Orinoco apple
Hungarian : Orinoco-Paradicsom
Peru : Cocona, Kukush
Spanish : Topiro
Swedish : Topiro
Venezuela : Tupiru (Lim, 2013)

2.2 Origin and Distribution

Solanum sessiliflorum is originated in western Amazon and was domesticated by pre-Columbian Amerindians (Lopes and Pereira, 2005). Lacking of real origin, *S. sessiliflorum* might be originated in eastern slope of Peruvian, Columbian and Equatorial Andes or in the Amazon basin (Schuelter, 2009; Guimarães et al., 2012), but the spineless one is unknown while it has been detected only in the field from Peru and Colombia to Venezuela and bordering regions of Brazil. Apolinar Diez de la Fuente, Humboldt and Bonpland found it in Orinoco river (Morton, 1987) and sparsely grown along the lowland of Amazon and the upper Amazon basin to 1219 m in altitude in the Andean hills (Goldsmith, 2006). They are also found in forest gaps, but not a lot in dense primary forest as weeds around artificial clearings (Cappelletti et al., 2000). The areas of cultivation are Ecuador, Venezuela, Brazil and Peru (Melgarejo et al., 2003). 100 years ago, immigrant introduced new economic plants to the amazon region which badly influenced the cultivation of *Solanum sessiliflorum;* therefore, most Amazonian people are not familiar to it (Marx et al., 1998).

Figure 1: Distribution map of *S. sessiliflorum* (GIS@SU, 2012)

2.3 Botanical description of *Solanum sessiliflorum*

Habitat and ecological changes with the domestication process create variability in morphology. The key parameters are the content of the chemical composition: glucose and sucrose play major role in fruit ripening, citric, malic, and succinic acids are important in fruit growth and maturation and the level of fructose and glucose provoke the morphotype differences (Jaramillo, 2011).

2.3.1 Stem

This plant is an herbaceous shrub with highly branched and soft stem that its height can be as tall as 2 m (Melgarejo et al., 2003). The twigs are densely white-hairy. *S. sessiliflorum* Dunal var. *georgicum*, the wild variety, has spines on stem, branches and leaves (Morton, 1987).

2.3.2 Leaves

The leaves are huge, simple, entire, alternate and asymmetrical at the leaf base, which covered with white hair on the both side, especially on the upper side (Duarte, 2011). The shape is ovate about 45 cm long and 38 cm wide which are oblique at the base, scalloped on the margins, downy on the upper surface, prominently veined beneath (Quijano and Jorge, 2006). There are rusty-hairy on the underside of the new shoots (Morton, 1987). The color of the leaves depend heavily on the varieties from dark to pale green.

Figure 2: Leaf shape of *Solanum sessiliflorum*

2.3.3 Flowers

The flowers are bisexual, greenish-yellow petals with 5 yellow stamens and dark green pointed calyx (Goldsmith, 2006). They are in clusters of 2 or more in the leaf axils (Morton, 1987). It looks like a star during anthesis with 4 to 5cm in diameter and has short peduncle about 0.3 to 0.5 cm long. If the temperature is preferable, the flowers are keeping blossom for whole year, yet the rate of setting fruit is only 5% of 1000 flowers (Duarte, 2011).

2.3.4 Fruits

It has emerged the high genetic diversity (biotypes) according to shape, size, color, flavor and fragrance of the cocona fruits while there are at least 25 biotypes in Peru and 35 in Brazil (Goldsmith, 2006). The fruits are borne singly or in compact clusters on very short peduncles, and capped with the persistent calyx. The shapes are round, oblate, oblong or conical-oval, with bluntly rounded apex: 2.5 cm to 10 cm long, and up to 6 cm wide at the base (Morton, 1987). Most fruits are globose with 31-92 mm long, 33-77 mm in longitudinal diameter and 1.5-19.5 mm of pulp thickness (Silva et al., 2011). The thin, tough skin is covered of short thin soft hairs until the fruit turns fully ripe, then it becomes smooth with golden to orange-yellow, burnt-orange, red, red-brown or deep purple-red in color (Quijano and Jorge, 2006). However, this soft hair can be removed easily by rubbing with your hand (Pereira & Filho, 2010). The yellow jelly like pulp, acidic but acceptable, are found inside which differs in thickness (6 to 10 mm). The fragrance is similar to tomato and the pulp has a pleasant sour (Goldsmith, 2006). The weight of the fruit is also limited by genotype varying between approximately 30 and 400g (Marx et al., 1998) contains 500-2000 glabrous yellow seeds whose length is from 3.2 mm to 4.0 mm with hard endosperm and ovate cotyledons. The loci number differs among genotypes according to the shape of the fruit. For cylindrical fruits, there are generally 4 loci and the heart-shaped, round and flat fruits have 6 to 8 loci (Pereira and Filho, 2010).

The fruits are divided into prickly and non-prickly forms, with large or small fruits:

- *S. sessiliflorum* Dunal var. *sessiliflorum*: –non-prickly plants
- *S. sessiliflorum* Dunal var. *georgicum* : – prickly plants (Cappelletti et al., 2000)

The classification of the fruits hinge on each country. In Perú, the classification is due to fruit size, color and shape, which is divided into 4 types:

- Small-size fruit, purple-red

- Medium-size fruit, yellow
- Round like an apple, yellow
- Pear-shaped, yellow (Goldsmith, 2006)

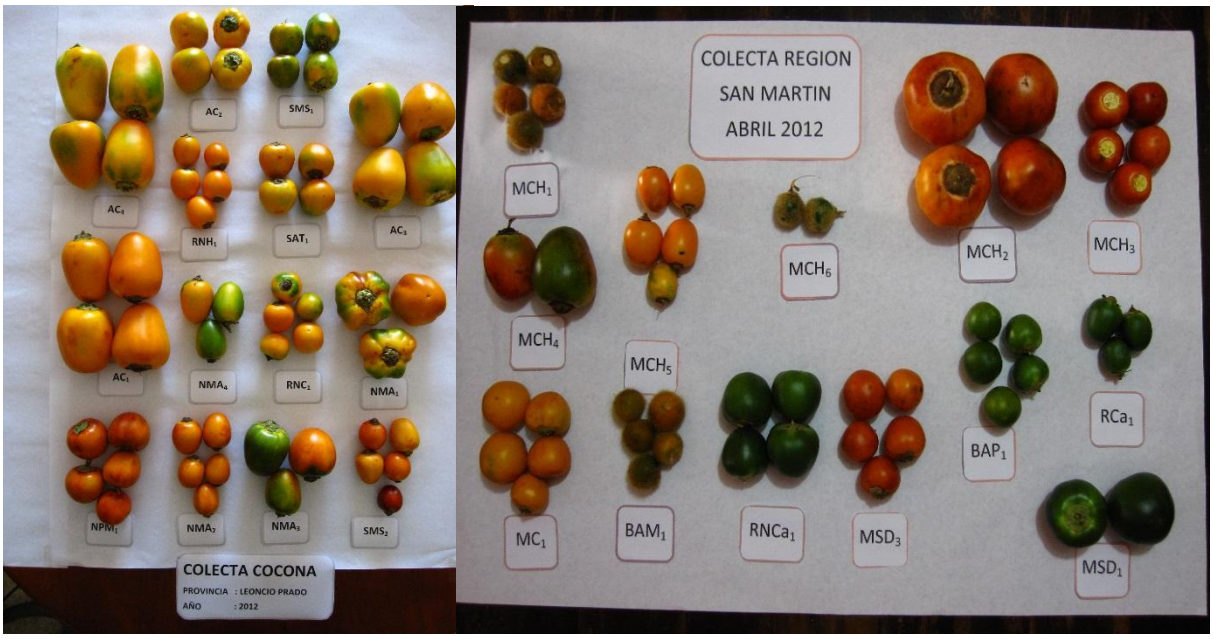

Figure 3: The variation of *Solanum sessiliflorum* fruits

The fruits contain the dietary fiber at the green, turning, and ripe stages; glucose level reach apex in turning stage, but sucrose is low at all stages making the fruit very acidic (Andrade Junior and Andrade, 2012). According to phytochemical analysis of cocona fruit, it contains the useful compounds such as carotenoid, phenolic, and vitamins (Hernandes, 2013).

2.4 Importances of *Solanum sessiliflorum*

Solanum sessiliflorum is famous for its properties which can be used for several ways to serve as food, raw materials for medicine, and cosmetic production. Regional people take the advantage of cocona to use as ethnomedicine and prepare juice from the tasty fruit and sometime replace it with the lime (Silva Filho et al., 2005). Some of them just go to the forest and collect the fruits from wild or half domesticated ecotypes for food (Melgarejo et al, 2003). It is rich in pectin (Caceres et al., 2012). Nevertheless, it is indicated that the fruit is not cytotoxic to human, so the consumption is not harmful but is thought to have genotoxic effects (Hernandes et al., 2014).

- *Medicinal purpose*
    - Fruit: contains high amount of niacin that can be used in hypoglycemic and/or hypocholesterolemic agent (Silva et al., 2014). This vitamin helps to release cholesterol and high triglyceride levels, anemia, diabetes, high blood pressure, migraine and depression (Salick, 1989).
    - Leaves and Roots: used as medicine (Guimarães et al., 2012)
    - Ethnomedicine: antidiabetic, antivenom, scabicide, hypertension, useful scabicide for treating burn (Selina, 2014)
- *Nutrition:* Cocona friut is rich in iron and vitamin A, C and niacin, which is essential mainly for women and children. It contains vitamin A with antioxidant action (β-carotene) and lycopene. Zinc and selenium help in the proper diet (Silva et al., 2014). Phosphorus and calcium are bountiful in fruit (Melgarejo et al., 2004). It contains low amount of calory, so it is considered for dietary products (Pizzinato et al., 2008). Nutritive composition of *S. Sessiliflorum* extracted from fruit powder has been done by (Jiliana et al., 2013) and has been described in the table. The amount of caffeine and theobromine is limited that is not enough to be an essential source of metabolite, which is only 0.97±0.05 and 0.138±0.14 mg/100 g pulp, respectively. Anyway, epicatechin showed the significant levels 1.31±0.11 mg/100g dry weight (d.w.).

Table 1: Nutrient composition extracted from fruit powder (mean ±SE (Standard error)), (Jiliana et al., 2013)

Organic Acid contents (mg/100 g)	
Oxalic	41.6 ± 2.7
Citric	6579.2 ± 42.1
Malic	164 ± 11
Succinic	356 ± 23
Carbohydrate and total polyphenol contents (mg/100 g)	
Sucrose	14617 ± 93
Glucose	4541 ± 29
Fructose	2631 ± 17
Total polyphenols	54±11
Antioxidant capacity	
Antioxidant capacity (g trolox / g powder)	0.044 ± 0.00
DPPH antioxidant capacity (g DPPH / g powder)	0.176 ± 0.01

- *Cuisine*
    - Fruit: is edible, delicious and tasty which can be peeled and comsumed directly when it is ripe to make dessert and many types of food with salad or fish (Morton, 1987). Fruit is used in juice, candies, jams, ice creams, sauces, jam, marmalade, paste, and jelly. In order way, it is a cult cold beverage when it is sweetened with sugar (Quijano and Jorge 2006).
    - Leaves: is boiled and eaten by the Brazilian (Duarte, 2011)
- *Antioxidant properties:* iron (Fe), selenium (Se), manganese (Mn), zinc (Zn), citric acid, carotenoids, and phenolic compounds, hydrophilic extracts, and carotenoids, which are capable of scavenging reactive species of oxygen and nitrogen (Silva et al., 2014)
- Based on research of (Rodrigues et al., 2013) on carotenoid and phenolic compound, they found seventeen carotenoid and three types of phenolic compound in the cocona fruit. The major carotenoids were (all-E)-β-carotene (7.15 μg/g of d. w.) and (all-E)-lutein (2.41 μg/g of d, w.) and the major phenolic compound consisting of 78% was 5-caffeoylquinic acid (1351 μg/g of d. w.). Carotenoid is an effective scavenger of peroxyl radical and hydrophilic extract is a scavenger of hydrogen peroxide and hypochlorous acid.
- *Toxicity:* rural people use it to get rid of the lice (Morton, 1987) and it is probably used as potential source of insect-deterring chemical compounds (Cappelletti et al., 2000).

Because there are several types of fruits, the selection for daily food is made according to the preference. In Peru, some villagers love eating the small berries from the prickly forms as a refreshing tropical fruit juice and comsume the large berries from the unarmed forms on the ground that the furits are tasty with exceptional flavor (peach-tomato). In opposition to this preference, some GuajIbo villagers living in Coromoto near Puerto Ayacucuho prefer the fruits from the spiny form (Salick, 1989).

2.5 Ecological Requirement

S. sessiliflorum requires low elevation (up to 500-600 m) but high temperature up to 24^{o} C. That is why it never grows in closed rainforests. It spreads along river banks and in forest clearings, and is less frequent in dense primary forests (Rascio et al., 2002).

2.5.1 Soil

The best soils for the cultivation of *Solanum sessiliflorum* is well-drained soil (Rubatzky and Yamaguchi, 1997) and it can be grown on acid and infertile soil while normally it shows good condition in humid tropic (Salick, 1989). It has capacity to grow in lowland and upland areas (Andrade Junior and Andrade, 2012). Because it is native to Amazonia, it is able to grow well on floodplain soil without any more input or require only a little amount of fertilizer (Silva Filho et al., 2005).

2.5.2 Rainfall

The optimum rainfall ranges between 2,000 and 8,000 mm (Cappelletti et al., 2000; Rascio et al. 2002).

2.5.3 Temperature

Solanum sessiliflorum is one of the tropical crops requiring average annual temperature higher than 24°C (Cappelletti et al., 2000), starting from 18°C to 30°C in accordance with average relative humidity in the atmosphere 85% (Marx et al., 1998). It is reported that *Solanum sessiliflorum* is impossible to cultivate in temperate regions and tolerant of higher light intensity because it is capable to adapt well to hot climate areas and high relative humidity (Pizzinato et al., 2008)

2.6 Propagation of *Solanum sessiliflorum*

Cocona is self-polinated and contains from 800 to 2000 seeds per fruit (Morton, 1987), (Toribio and Balcazar de Ruiz) stated high percentage of cross-polination cause by win and insect. Its inheritance of fuit shape and size is strongly predominant female parent. If the mother plant has large fruit, its generation has large fruits as well (Salick, 1989).

The cultivation of *S. sessiliflorum* is similar to the cultivation of the plants from the same family, Solanaceae, such as tomato, eggplant, and pepper. It takes six months for *S. sessiliflorum* to set fruit after transplanting and three months for full ripeness (Pereira and Filho, 2010). A vascular plant, it is normally grown from seed which is ready to transplant to the field when its height is from 20 to 30 cm. Firstly, the seeds need to be kept in the shade for two days after extraction from the ripe fruits for the sake of removing the surrounding

gelatin, then clean it and dry with direct sun (Goldsmith, 2006). The germination starts from 15 to 40 days as a very strong seedling. Another method, it can be propagated vegetative. The plant spacing and row spacing is extremely rely on the soil fertility (Morton, 1987) affecting the small and large fruits (Salick, 1992). The flower starts to bloom 2 to 3 months after transplanting (Morton, 1987). The fruits grow rapidly in the early development, up to 80 days after anthesis. At this stage, they are yellow, changing to orange, around 90 days, to deep orange, around 100 days, and orange-brown, around 110 days after anthesis. The whole cycle of fruit development and ripening on the plant is around 90 days (Silva, 2011).

Fertilizer Application: it is made based on soil fertility and types of fertilizer formula. In usual, *S. sessiliflorum* needs 50-70 g per plant of 10-8-10 NPK fertilizer, which should be applied 6 times a year. For low phosphorus soil type, 10-20-10 NPK fertilizer is highly recommended. Sometime, organic fertilizer is necessary to apply since it helps to improve soil fertility. At Manaus, the soil is very sandy, so organic fertilizer 250 tons/ha has been applied to gain yield with the addition of appropriate amounts of triple super-phosphate, urea and chlorate of potassium (Morton, 1987).

Insects and Diseases:

- ***Diseases****:* Its longevity is from 3 to 5 years. The root is easily to be infested by the soil borne diseases such as *Phytophthora, Rhizoctonia, Pythium* if soil is in bad condition. Hence, the life cycle will be shorten to only one year, and ultimately lead to die. This problem can be avoided by crop rotation, proper irrigation and good drainage. The leaves have problem with *Phytophthora infestans* and *Septoria solanicola,* but copper based fungicide is potentially to deal with it. Another problem is Anthracnosis caused by *Colletotrichum gloeosporioides* happening on leaves and fruits and the best solution is to use Mancozeb or Penconazol (Duarte, 2011).
- ***Insects***: cause poor quality to the fruits, one of which is fruit borer, *Neoleucinodes elegantalis*. It mostly occurs every time and reach the highest point from September to December and during high level of rainfall. Its larvae live inside the fruits and feed on them from one to another (Anteparra et al., 2010).

Yield: Yield of *S. sessiliflorum* varies accordingly to different locations and soil fertility. In Columbia, the average annual yield ranges from 10 to 20 kg per plant, while there is increase in yield from 18 to 27 kg per plant in Costa Rica, and the lowest yield occurred at Manaus 2.5-14 kg. Based on soil fertility, the yield is decreased dramatically on unfertilized plantation providing only 20 to 30 fruits per plant or 29 t/ha. On fertilized plantation with a

high-yielding variety, *S. sessiliflorum* is able to produce 136 fruits per plant or 146 t/ha. Cocona fruit remains fresh for a short duration 5 to 10 days at normal temperature. In food processing, 10 kg of fruit provides 3 l of preserved flesh and 1.5 of jelly, or 7.5 l of juice (Morton, 1987).

2.7 Production of *Solanum sessiliflorum*

The production of *Solanum sessiliflorum* is still small scale farming and normally is grown by rural people in the countryside. For the economic reason, the production of cocona is very simple, and highly productive in relation to which genotypes are chosen for cultivation. The yield can be 100 t/ha. With low input of production, the market price of cocona is quite good (Silva Filho et al., 2005).

In Brazil, the cultivation of *S. sessiliflorum* has been practiced for five years in the Southeast of Brazil mainly in rural areas of the São Paulo. In the countryside of metropolitan area of Manaus, the conventional farming of *S. sessiliflorum* has being done by farmers in order to get more income, or else to get a job in horticulture in Amazonas state (Souza et al., 2012). The local Amazonian people cultivate small and medium fruit varieties in order to use in juice production or use as a lime (Silva Filho et al., 2005).

In Peru, the cultivation of *S. sessiliflorum* is grown by indigenous people, Amuesha Ameridians, in the Palcazu valley where they grow all forms of cocona. Small scale production is found at Chanchamayo, Junin, where they produce for selling to canning factory and Lima market. There is also small production of cocona in Iquitos, Peru. However, in Lima, farmers cultivate it seasonally for selling it in summer to cocona shop where there is gigantic wholesaler fruit market. Moreover, one canning factory which is cooperated with French and Peruvian located in San Ramon, Peru make a business in cococa canning of juice for selling and shipping to not only in local market, Lima but also to international market, which is in Italy. Sometimes, cocona can be processed to juices, ices, and marmalade for local consumption because farmers are able to sell it in these forms (Salick, 1992).

In Amazonas (Venezuela), most Amerindian groups (Paaroa, Guajibo, Makiritare, Curipaco) plant it in their swiddens, eat it, and sell it in the local markets (Salick, 1989).

2.8 Breeding of *Solanum sessiliflorum*

Cocona was used to breed with naranjilla (*Solanum quitoense*) a shrubby perennial plant in Colombia for the purpose of disease and pest resistance. The most prominent one is root knot nematode. Because naranjilla fruit is tastier and cocona is quite similar in morphology, they have been bred together. The breeding was successful while the hybrid looked much more like cocona, but the fruit was smaller than naranjilla. Anyway, it was quite resistant to diseases and pests (Heiser, 1993). Another breeding was occurred between two varieties of cocona (Santa Luzia and Thaís) from crop station of São Paulo to evaluate the effective methods of artificial pollination technique for economically important traits. These two varieties (with and without protection with organza cloth) were bred in natural crosses and crosses with emasculation. The result showed that the use of emasculation without organza cover was exceptional for fruit yield while Santa Luzia was a female parent (Pizzinato et al., 2008). Inasmuch as sexual reproduction provokes high genetic variability in cocona whose agronomic characteristic does not meet the commercial viewpoint, the research in micropropagation was done by (Machado, 2005) to get the desirable characteristic clones mainly high yield, biotic and abiotic stress tolerance. By adding BAP (6-Benzylaminopurine, benzyl adenine) and IAA (Indole-3-Acetic acid) growth regulators to the MS medium, there was a larger number of sprouting with well-developed root system and ideal height for acclimatization.

2.9 Molecular Markers for Analyzing Genetic Diversity

Genetic Diversity: "The genetic variability within a population or a species. The formation of individuals differing in genotype, or the presence of genotypically different individuals, in contrast to environmentally induced differences which, as a rule, cause only temporary, nonheritable changes to the phenotype" (FAO, 2003).

Molecular markers have been emerged for 30 years to be used in development program of biochemistry and molecular biology. Nowadays, it holds vital roles in all aspects of plant breeding in order to observe the desired genes for the development of backcrossing programs, and to study the genetic variability and diversity (Schulman, 2007). Genetic diversity is commonly measured by two principle methods: Phenotype and Genotype by using *Genetic marker*. Assessment of phenotypic variation uses the morphological traits such as shape and appearance to identify the individual. It can be deemed as genetic if it is not affected by the environmental condition and inheritant to the next generation, while the assessment of genotypic variation focuses on the level of the DNA molecule, consisting of

nucleotides bounded in double helix structure in increasing levels of complexity up to the chromosomal units, responsible for transmitting genetic information.

Inasmuch as no genetic marker is perfect, different genetic markers have different functions in terms of benefits and drawbacks. Anyway, the ideal properties of genetic markers is still required at least some to conform the results.

Genetic markers: desirable properties: highly polymorphic, reproducible, codominant, evenly distributed throughout the genome, discriminating, not subject to environmental influences, neutral, inexpensive, and easy to measure (Acquaah, 2006)

There are three types of Genetic Marker:

- Morphological traits
- Protein (biochemical) markers
- DNA (molecular) markers

2.9.1 Morphological Traits

Genetic diversity based-morphological traits need very simple equipment to handle, but requires expert to identify the plants or species and it is easily affected by environmental factors that can make the variation in phenotype and is limited in the number of species. (Vicente and Fulton, 2003).

2.9.2 Protein-based technology

Unlike the morphological traits, the protein based-technique analyses the plant genetic diversity by the migrational property of proteins, which allow separation by electrophoresis and detected by specific histochemical assays. Although simple equipment is applied for this method with strong assessment of morphological diversity and co-dominant, limitation number of markers and environmental influences are considered as the barriers of this technique.

Application: Gene flow and/or introgression, Genetics of populations, Strategies for ex-situ conservation, Crop evolution, Germplasm evaluation and characterization, Genetic erosion, and Genetic stability of conserved material (Vicente and Fulton, 2003).

2.9.3 DNA-based technology

The most precise and reliable method is DNA markers, which is able to detect the DNA sequences of the nucleus and organelles, not subject to environmental conditions, observe unlimited number, and measure the variation. DNA molecule has three sequence organizations: single copies (protein coding gene), multi copies (sequences with known function: coding and non-coding and sequences with unknown function: repeat and Transposons), and spacer DNA in which a lot of repeats are found with variation in size, number and distribution in genome, which is good enough for studying genetic diversity as a molecular marker. It is found in chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA). DNA technology involves the concepts of: restriction enzymes, nucleic acid electrophoresis, and DNA polymorphism (Vicente and Fulton, 2003). The summary of characteristics of markers is shown in (Table 2), while the evolution of markers is described in (Figure 4).

Table 2: Characterization of markers (Vijayan, 2005)

No	Markers	PCR-based	Polymorphism	Nature	Development Cost	Running Cost
1	AFLP	Yes	High	Dominant	Medium	Medium
2	CAPS	Yes	High	Co-dominant	High	Medium
3	EST	Yes	High	Co-dominant	High	Medium
4	Isozyme	No	Low	Co-dominant	High	Medium
5	ISSR	Yes	High	Dominant	Low	Low
6	RAPD	Yes	High	Dominant	Low	Low
7	RFLP	No	Medium	Co-dominant	High	High
8	SCAR	Yes	High	Co-dominant	High	Medium
9	SNP	Yes	Very High	Co-dominant	High	Medium
10	SSR	Yes	High	Co-dominant	High	Medium
11	STS	Yes	High	Dominant	High	Medium

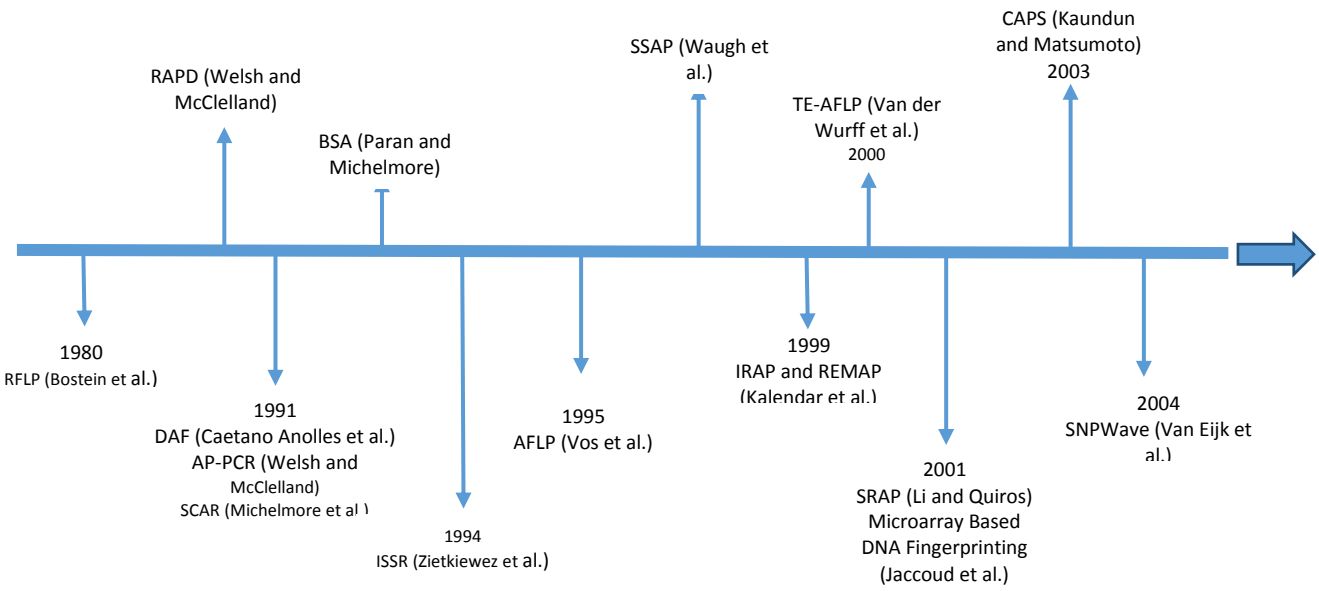

Figure 4: Timeclock of DNA-based markers (Sarwat, 2012)

Application of Molecular markers: phylogenetic and systematics, conservation biology, molecular ecology and developmental biology, numerous uses in forensics, disease testing and paternity assessment (Poczai et al., 2013), assessment of genetic variability and characterization of germplasm, identification and fingerprinting of genotypes, estimation of genetic distances between population, inbreeds, and breeding materials, detection of monogenic and quantitative trait loci (QTL), marker-assisted selection, and identification of sequences of useful candidate genes (ISAAA, 2015)

2.10 Inter-simple sequence repeat (ISSR)

ISSR PCR-based method has been used since 1994 (Zietkiewicz et al., 1994). It amplifies the DNA segment present at an amplifiable distance located between two identical microsatellite repeat regions that are used as the primers (Bornet and Branchard, 2001; Reddy et al., 2002), normally repeated with di- nucleotides, tri-nucleotides, tetra- nucleotides, and penta- nucleotides ranging from 16-25 bp long in opposite direction, to amplify the inter-SSR sequence size. The primers used can be either unanchored or more usually anchored at 3'or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Vijayan, 2005; Guasmi, 2013; Bassil et al., 2013; Adhikari et al., 2015). ISSR-PCR, a simple, quick, and efficient technique, provide high reproducibility and no need to use radio-activity. The primers are long and not proprietary, yet amplify 200–2000 bp long, which is able to see the result by either agarose or polyacrylamide gel electrophoresis. Variations in primer length,

motif and anchor are possible. This technique demands low concentration of DNA while 10 ng of DNA creates the same result as 25 or 50ng of DNA (Reddy et al., 2002). The primers show polymorphism in case of there sequence repeat or deletion or can be insertion or sometime translocation in the genome to affect the distance between the repeats. The primers with di-nucleotide repeats anchored either at 3' or 5' end provide high polymorphism, but the primers anchored at 3' end reveal clearer banding pattern. Di-nucleotide repeats with different codons A, T, C, G influence the polymorphism. AG, GA, CT, TC, AC, and CA reveal better polymorphism than other di, tri, tetra-nucleotides and AT because AT is abundant yet is self-annealed and not amplified (Vijayan, 2005). AG and GA are significant in wheat, trifoliate orange, Douglas fir and sugi, and chickpea. AC provides good result in wheat and potato. ISSR is naturally dominant marker which shows only one allele. Incidentally, it is considered as co-dominant marker as well in some cases which is able to separate the homozygotes and heterozygotes (Reddy et al., 2002).

The detection methods have influence on the rate of polymorphism. There are two methods having been used worldwide are polyacrylamide gel electrophoresis (PAGE) with amalgam of radioactivity followed by PAGE with silver staining and then agarose-ethidium bromide system of detection and agarose gel.

Since ISSR marker has problems with reproducibility and interpretation, most of breeding program use SSR and SNPs instead on account of better polymorphism and reproducibility in nature (Mittal and Dubey, 2010). Compared to SSR, ISSR is good to be selected for study the species without genetic information and sequence information (Bornet et al., 2004; Dhanorkar et al., 2005). ISSR is more reproducible and polymorphic than RAPD owning to using SSR primers (Adhikari et al., 2015), but the cost of development is the same as RAPD even though ISSR primer requires higher annealing temperature and the sequences are non-random designed from microsatellite regions (Kurane et al., 2009). ISSR is from large part of noncoding regions of the genome; therefore, it is more reproducible than isozymes technique because this part contains shortage amount of mutational constraints in the inter-simple sequence repeats and isozymes technique is from the coding regions of the genome. In comparison with mitochondrial and chloroplast DNA marker evolution, ISSR marker is faster since mitochondrial genome's structural organization is wide rearrangement. The slow evolution made mitochondrial gene less significant for genome analysis; however, it is important to reach the conclusion of ancient phylogenetic relationships, and time and mode of diversifications. Both chloroplast and mitochondrial are haploid, maternally inherited, lack recombination and show gametic disequilibrium. Moreover, ISSR is reported to be a highly

variable, reproducible, identified closely related cultivars method and higher percentage of polymorphism, which is good for genetic study (Shipha et al., 2013).

ISSRs have been used to detect many species such as cultivar identification for potatoes, wheat, bean, and barley (Guasmi, 2013), yams (Zhou et al., 2008, Velasco-Ramírez et al., 2014), invasive plant species study (Gui et al., 2007), medicinal plants (Kurane et al., 2009). ISSR was also used to detect the genetic diversity of insect: plant hopper (Liu et al., 2010)

Application: DNA fingerprinting for taxonomic and phylogenetic comparison, gene mapping (Zietkiewicz et al., 1994), determining SSR motif frequency, preliminary study of population diversity, gene tagging and marker assisted selection, evolutionary biology (Reddy et al., 2002).

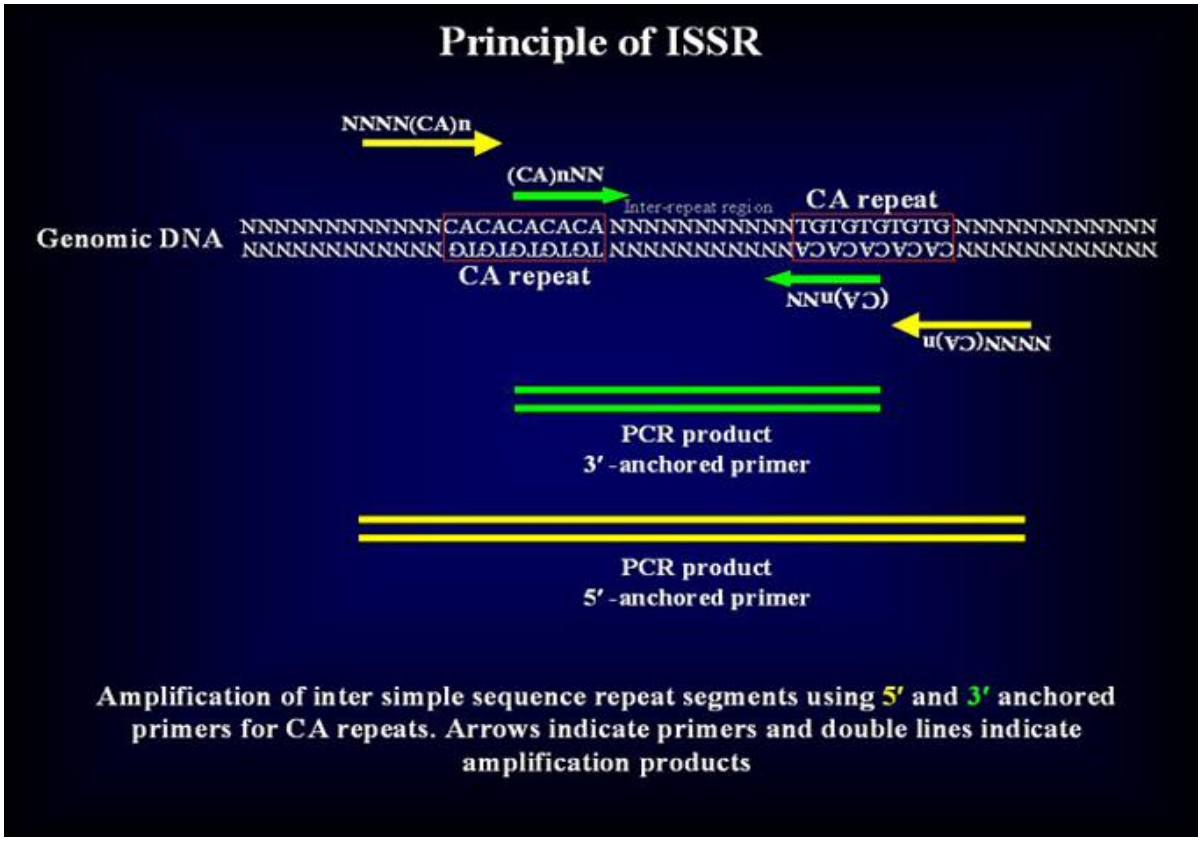

Figure 5: Principle of ISSR (SilkSatDb, 2004)

2.11Genetic study of Solanum spp. by molecular markers

Molecular markers have been preferably used for genetic study for many years including species from *Solanum* genus. One of all is microsatellite, for instance, brinjal (*Solanum melongena* L.*)*, the name called in India, was studied on genetic purity by selecting six out of thirty SSR primers (Arun Kumar et al., 2014). Genetic diversity of *S. melongena* L. was also

done by SSR marker for the purpose of phylogeny and mapping study of 44 accessions while 38 samples are cultivated species and the rest from wild species (Stàgel et al., 2008). There was a recent research on population genetics of eggplant in six stated of Nigeria, Tropical West Africa, using four RAPD primers; as the result, all these four primers produced high percentage of polymorphic bands, which were enough for genetic study (Aguoru, 2015). Another research by RAPD marker was made by (Cernák et al., 2008) focusing on resistance gene diagnosis (Rysto) in wild potato, *S. stoloniferum* via only three primers. Wild potato species and cultivated species once were studied on mitochondrial DNA variation, which was specified on subsections of potato species by practicing PCR markers to find out mtDNA variability in this subsection if there were correlation with series classification, geographical origin, ploidy, and endosperm balance number (EBN) (Scotti et al., 2007). Related to tomato, *S. lycopersicum* and wild relatives for genetic diversity analyze, overall overview of this species by using many types of molecular markers was reported by (Bauchet and Causse, 2012). *S. nigrum*, one of the most important species in the family Solanaceae, was interested in genetic study based on SSR on five species and their progeny (Biljon et al., 2010).

An important review article of pepper, tomato, and eggplant based on genetic resources and advances in breeding by using various molecular techniques was reviewed by (Bebeli and Mazzucato, 2008).

Table 3: Markers selected for genetic study of *Solanum sp.*

Solanum	Markers	Authors	Year
Solanum lycopersicum	SSR	Mazzucato et al.	2007
	SSR	Benor et al.	2008
	ISSR	Aguilera et al.	2011
	SSR	El-Awady	2012
	SNP	Corrado et al.	2013
Solanum tuberosum L	ISSR	Bornet et al.	2002
	SSR, RAPD	Rocha et al.	2010
	ISSR	Salano et al.	2013
	RAPD	Hoque et al.	2013
	SSR	Sharma and Nandineni	2014
Solanum melongena L	ISSR	Isshiki et al.	2008
	ISSR	Shailesh et al.	2009
	ISSR	Ali et al.	2011
	SSR	Caguiat and Hautea	2014
Solanum sp.	RAPD	Poczai et al.	2008
	AFLP, SCAR	Nunziata et al.	2010
Solanum scabrum Mill. and *Solanum nigrum* L.	AFLP	Manoko et al.	2008
Solanum trilobatum	ISSR	Shilpha et al.	2013
Solanum caripense	RFLP	Joy	2007

3. Hypothesis and Objectives

In scientific papers there is no available information about genetic variability of Solanum sessiliflorum. Because application of ISSR markers is revealed to be the suitable marker for genetic study, for it is reproducible, inexpensive, and polymorphic. Moreover, it has been selected to investigate the genetic diversity among *Solanum* species such as *Solanum tuberosum,* and *Solanum lycopericum.* In this study, use of ISSR markers was optimized and used for detection of genetic variability of 248 samples of cocona. Hypothesis was that *Solanum sessiliflorum* Dunal show high variation in fruit shape and size from one place to another as reported by some authors and estimated high morphological variability mainly in shape and size of fruit Therefore, my hypothesis was that *S. sessiliflorum* contained high genetic diversity within and among population.

The aims of the research were metioned as below:

- To optimize the most appropriate DNA extraction methods, selection of suitable primers and conditions for PCR reaction.
- To assess the genetic variability in *Solanum sessiliflorum* (cocona) by applications of Inter Simple Sequence Repeat (ISSR).
- To estimate the genetic variability within and among population of *S. sessiliflorum* originated in different regions of Peru.

4. Methodology

4.1 Collection and preparation of plant material

The plant material analysed in this study was collected from 248 individual plants, 40 populations, (Table 4) in four regions of Peru (Figure 6)]. Collected leafy material of each individual plant was stored in silica gel (P-Lab, Czech Republic). Samples were then carried to Czech University of Life Sciences Prague (CULS Prague), for further DNA analysis in the Laboratory of Molecular Biology at the Faculty of Tropical Agrisciences.

Figure 6: Map of samples collection in Peru

Table 4: Information about samples collected in Peru

Population /Region		Population /subpopulation	Name of subgroup	Name of sample	No. of Samples	Geographical position		Altitude	Type of cultivation	Locality	Village	District
						S	W					
Huanuco 1	Leoncio Prado (LP)	LP1	AC1	AC1-1	22	08°42´33,4“	076°07´44,4“	626 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-2		08°42´33,4“	076°07´44,4“	627 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-3		08°42´33,4“	076°07´44,4“	628 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-4		08°42´33,4“	076°07´44,4“	629 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-5		08°42´33,4“	076°07´44,4“	630 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-6		08°42´33,4“	076°07´44,4“	631 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-7		08°42´33,4“	076°07´44,4“	632 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-9		08°42´33,4“	076°07´44,4“	633 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-11		08°42´33,4“	076°07´44,4“	634 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-12		08°42´33,4“	076°07´44,4“	635 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-13		08°42´33,4“	076°07´44,4“	636 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-14		08°42´33,4“	076°07´44,4“	637 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-15		08°42´33,4“	076°07´44,4“	638 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-17		08°42´33,4“	076°07´44,4“	639 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-18		08°42´33,4“	076°07´44,4“	640 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-19		08°42´33,4“	076°07´44,4“	641 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-20		08°42´33,4“	076°07´44,4“	642 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-21		08°42´33,4“	076°07´44,4“	643 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-22		08°42´33,4“	076°07´44,4“	644 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-23		08°42´33,4“	076°07´44,4“	645 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-24		08°42´33,4“	076°07´44,4“	646 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
				AC1-25		08°42´33,4“	076°07´44,4“	647 m	Cultivated	Caymito	Aucayacu	José crespo y Castillo
		LP2	AC2	AC2-1	7	08°42´28,7“	076°07´33,0“	610 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-2		08°42´28,7“	076°07´33,0“	611 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-4		08°42´28,7“	076°07´33,0“	612 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-5		08°42´28,7“	076°07´33,0“	613 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-6		08°42´28,7“	076°07´33,0“	614 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-7		08°42´28,7“	076°07´33,0“	615 m	Wild	Caymito	Aucayacu	José crespo y Castillo
				AC2-8		08°42´28,7“	076°07´33,0“	616 m	Wild	Caymito	Aucayacu	José crespo y Castillo
		LP3	AC3	AC3-1	21	08°50´45,6“	076°08´20,0“	577 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-2		08°50´45,6“	076°08´20,0“	578 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-3		08°50´45,6“	076°08´20,0“	579 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo

				AC3-4		08°50´45,6“	076°08´20,0“	580 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-5		08°50´45,6“	076°08´20,0“	581 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-6		08°50´45,6“	076°08´20,0“	582 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-7		08°50´45,6“	076°08´20,0“	583 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-8		08°50´45,6“	076°08´20,0“	584 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-9		08°50´45,6“	076°08´20,0“	585 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-10		08°50´45,6“	076°08´20,0“	586 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-11		08°50´45,6“	076°08´20,0“	587 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-12		08°50´45,6“	076°08´20,0“	588 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-13		08°50´45,6“	076°08´20,0“	589 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-14		08°50´45,6“	076°08´20,0“	590 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-15		08°50´45,6“	076°08´20,0“	591 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-16		08°50´45,6“	076°08´20,0“	592 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-17		08°50´45,6“	076°08´20,0“	593 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-18		08°50´45,6“	076°08´20,0“	594 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-19		08°50´45,6“	076°08´20,0“	595 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-20		08°50´45,6“	076°08´20,0“	596 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
				AC3-21		08°50´45,6“	076°08´20,0“	597 m	Cultivated	Cotomonillo	Aucayacu	José crespo y Castillo
		LP4	AC4	AC4		08°42´28,7“	076°07´33,0“	610 m		Caymito	Aucayacu	José crespo y Castillo
				AC4-2	3	08°42´28,7“	076°07´33,0“	611 m		Caymito	Aucayacu	José crespo y Castillo
				AC4-3		08°42´28,7“	076°07´33,0“	612 m		Caymito	Aucayacu	José crespo y Castillo
			AC5	AC5	1	08°42´31,0“	076°07´30,2“	600 m		Caymito	Aucayacu	José crespo y Castillo
		LP5	SAT1	SAT1-1		09°16´56,2“	075°56´55,2“	633 m	Wild	Atahuallpa	Supte	Rupa rupa
				SAT1-2		09°16´56,2“	075°56´55,2“	634 m	Wild	Atahuallpa	Supte	Rupa rupa
				SAT1-3	5	09°16´56,2“	075°56´55,2“	635 m	Wild	Atahuallpa	Supte	Rupa rupa
				SAT1-4		09°16´56,2“	075°56´55,2“	636 m	Wild	Atahuallpa	Supte	Rupa rupa
				SAT1-5		09°16´56,2“	075°56´55,2“	637 m	Wild	Atahuallpa	Supte	Rupa rupa
		LP6	RNH1	RNH1-1		09°17´05,3“	075°55´10,5“	699 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-2		09°17´05,3“	075°55´10,5“	700 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-3		09°17´05,3“	075°55´10,5“	701 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-4		09°17´05,3“	075°55´10,5“	702 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-5	9	09°17´05,3“	075°55´10,5“	703 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-6		09°17´05,3“	075°55´10,5“	704 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-7		09°17´05,3“	075°55´10,5“	705 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-8		09°17´05,3“	075°55´10,5“	706 m	Wild	Huáscar	Supte	Rupa rupa
				RNH1-9		09°17´05,3“	075°55´10,5“	707 m	Wild	Huáscar	Supte	Rupa rupa
		LP7	RNC1	RNC1-1	6	09°17´58,4“	075°54´37,8“	717 m	Wild	Capitan Arellana	Supte	Rupa rupa
				RNC1-3		09°17´58,4“	075°54´37,8“	718 m	Wild	Capitan Arellana	Supte	Rupa rupa

				RNC1-4		09°17´58,4“	075°54´37,8“	719 m	Wild	Capitan Arellana	Supte	Rupa rupa
				RNC1-5		09°17´58,4“	075°54´37,8“	720 m	Wild	Capitan Arellana	Supte	Rupa rupa
				RNC1-6		09°17´58,4“	075°54´37,8“	721 m	Wild	Capitan Arellana	Supte	Rupa rupa
				RNC1-7		09°17´58,4“	075°54´37,8“	722 m	Wild	Capitan Arellana	Supte	Rupa rupa
		LP8	NPM1	NPM1-1	3	09°15´07,1“	075°55´26,6“	690 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
				NPM1-2		09°15´07,1“	075°55´26,6“	691 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
				NPM1-3		09°15´07,1“	075°55´26,6“	692 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
		LP9	NMA1	NMA1-1	2	09°14´21,1“	075°56´28,5“	679 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
				NMA1-2		09°14´21,1“	075°56´28,5“	680 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
			NMA2	NMA2	1	09°14´21,1“	075°56´28,5“	679 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
			NMA3	NMA3	1	09°14´21,1“	075°56´28,5“	679 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
			NMA4	NMA4	1	09°14´21,1“	075°56´28,5“	679 m	Wild	Playa Marona	Naranjillo	José crespo y Castillo
		LP10	SRN9*	SRN9-1	25	09°07´54,6“	076°02´37,0“	609 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-2		09°07´54,6“	076°02´37,0“	610 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-3		09°07´54,6“	076°02´37,0“	611 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-4		09°07´54,6“	076°02´37,0“	612 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-5		09°07´54,6“	076°02´37,0“	613 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-6		09°07´54,6“	076°02´37,0“	614 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-7		09°07´54,6“	076°02´37,0“	615 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-8		09°07´54,6“	076°02´37,0“	616 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-9		09°07´54,6“	076°02´37,0“	617 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-10		09°07´54,6“	076°02´37,0“	618 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-11		09°07´54,6“	076°02´37,0“	619 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-12		09°07´54,6“	076°02´37,0“	620 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-13		09°07´54,6“	076°02´37,0“	621 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-14		09°07´54,6“	076°02´37,0“	622 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-15		09°07´54,6“	076°02´37,0“	623 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-16		09°07´54,6“	076°02´37,0“	624 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-17		09°07´54,6“	076°02´37,0“	625 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-18		09°07´54,6“	076°02´37,0“	626 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-19		09°07´54,6“	076°02´37,0“	627 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-20		09°07´54,6“	076°02´37,0“	628 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-21		09°07´54,6“	076°02´37,0“	629 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-22		09°07´54,6“	076°02´37,0“	630 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-23		09°07´54,6“	076°02´37,0“	631 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-24		09°07´54,6“	076°02´37,0“	632 m	Cultivated	Río negro	Supte	José crespo y Castillo
				SRN9-25		09°07´54,6“	076°02´37,0“	633 m	Cultivated	Río negro	Supte	José crespo y Castillo
		LP11	SMS1	SMS1	3	09°07´35,3“	076°01´00,9“	610 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo

				SMS1-2		09°07´35,3“	076°01´00,9“	611 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo
				SMS1-3		09°07´35,3“	076°01´00,9“	612 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo
		LP12	SMS2	SMS2	3	09°07´37,5“	076°01´02,7“	609 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo
				SMS2-2		09°07´37,5“	076°01´02,7“	610 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo
				SMS2-3		09°07´37,5“	076°01´02,7“	611 m	Silvestre	Monte sacro	SaiPai	José crespo y Castillo
	Leoncio Prado-Tingo	LP13	LCTR1	LCTR1	2	09°08´59,6“	075°59´42,7“	614 m		Los Milagros		
				LCTR1-2		09°08´59,6“	075°59´42,7“	615 m		Los Milagros		
		LP14	LBA2	LBA2	6	09°19´27,2“	076°02´10,1“	690 m		Bella Alta		
				LBA2-2		09°19´27,2“	076°02´10,1“	691 m		Bella Alta		
				LBA2-4		09°19´27,2“	076°02´10,1“	692 m		Bella Alta		
				LBA2-9		09°19´27,2“	076°02´10,1“	693 m		Bella Alta		
				LBA2-12		09°19´27,2“	076°02´10,1“	694 m		Bella Alta		
				LBA2-14		09°19´27,2“	076°02´10,1“	695 m		Bella Alta		
San Martin 2	Tocache (T)	T1	TS1	TS1	1	08°10´22,4“	076°34´21,1“	551 m				
			TS2	TS2	5	08°10´23,2“	076°34´21,9“	529 m				
				TS2-2		08°10´23,2“	076°34´21,9“	530 m				
				TS2-3		08°10´23,2“	076°34´21,9“	531 m				
				TS2-4		08°10´23,2“	076°34´21,9“	532 m				
				TS2-5		08°10´23,2“	076°34´21,9“	533 m				
		T2	TF1	TF1	3	08°09´38,2“	076°33´39,0“	511 m		Filadelfia		
				TF1-2		08°09´38,2“	076°33´39,0“	512 m		Filadelfia		
				TF1-5		08°09´38,2“	076°33´39,0“	513 m		Filadelfia		
		T3	TF2	TF2	1	08°09´00,4“	076°33´15,9“	514 m		Filadelfia		
			TF3	TF3	5	08°08´58,6“	076°33´11,8“	507 m		Filadelfia		
				TF3-2		08°08´58,6“	076°33´11,8“	508 m		Filadelfia		
				TF3-3		08°08´58,6“	076°33´11,8“	509 m		Filadelfia		
				TF3-4		08°08´58,6“	076°33´11,8“	510 m		Filadelfia		
				TF3-5		08°08´58,6“	076°33´11,8“	511 m		Filadelfia		
		T4	TSH1	TSH1	2	08°14´32,2“	076°26´41,3“	485 m		Shishiyacu		
				TSH1-5		08°14´32,2“	076°26´41,3“	486 m		Shishiyacu		
			TSH2	TSH2	2	08°14´32,0“	076°26´42,9“	488 m		Shishiyacu		
				TSH2-2		08°14´32,0“	076°26´42,9“	489 m		Shishiyacu		
			TSH4	TSH4	3	08°14´26,3“	076°26´37,2“	493 m		Shishiyacu		
				TSH4-2		08°14´26,3“	076°26´37,2“	494 m		Shishiyacu		
				TSH4-3		08°14´26,3“	076°26´37,2“	495 m		Shishiyacu		
			TSH5	TSH5	1	08°14´26,5“	076°26´36,3“	484 m		Shishiyacu		
	Moyobamba (M)	M1	MSD1	MSD1	1	06°04´04,1“	077°06´49,9“	848 m		Santo Domingo		
			MSD2	MSD2	1	06°04´04,1“	077°06´49,9“	848 m		Santo Domingo		

			MSD3	MSD3-1	4	06°04´05,8“	077°06´50,3“	848 m		Santo Domingo		
				MSD3-2		06°04´05,8“	077°06´50,3“	849 m		Santo Domingo		
				MSD3-3		06°04´05,8“	077°06´50,3“	850 m		Santo Domingo		
				MSD3-4		06°04´05,8“	077°06´50,3“	851 m		Santo Domingo		
			MCD6	MCD6-1	2	06°04´05,8“	077°06´50,3“	852 m				
				MCD6-2		06°04´05,8“	077°06´50,3“	853 m				
	Rioja	R1	RCA1	RCA1	1	06°03´11,9“	077°09´29,3“	831 m		Capironal		
	Moyobamba		MC1/CM1	MC1-1	6	06°02´18,6“	077°04´54,5“	851 m		Calzada		
				MC1-2		06°02´18,6“	077°04´54,5“	852 m		Calzada		
				MC1-3		06°02´18,6“	077°04´54,5“	853 m		Calzada		
				MC1-4		06°02´18,6“	077°04´54,5“	854 m		Calzada		
				MC1-5		06°02´18,6“	077°04´54,5“	855 m		Calzada		
				MC1-6		06°02´18,6“	077°04´54,5“	856 m		Calzada		
		M2	RNCA1	RNCA1-1	3	05°57´52,4“	077°17´44,1“	847 m				
				RNCA1-2		05°57´52,4“	077°17´44,1“	848 m				
				RNCA1-3		05°57´52,4“	077°17´44,1“	849 m				
		M3	MCH1/MSH1	MSH1-1	3	05°49´02,7“	077°11´25,9“	848 m		Huascayacu		
				MSH1-2		05°49´02,7“	077°11´25,9“	849 m		Huascayacu		
				MSH1-3		05°49´02,7“	077°11´25,9“	850 m		Huascayacu		
		M4	MCH5/MSH5	MSH5-1	2	05°48´28,9“	077°11´01,7“	841 m		Huascayacu		
				MSH5-2		05°48´28,9“	077°11´01,7“	842 m		Huascayacu		
			MCH6/MSH6	MSH6	1	05°51´36,1“	077°11´04,7“	828 m		Huascayacu		
		M5	BAM1	BAM1	5	05°24´56,3“	078°26´21,6“	499 m		Aramango		
				BAM1-3		05°24´56,3“	078°26´21,6“	500 m		Aramango		
				BAM1-4		05°24´56,3“	078°26´21,6“	501 m		Aramango		
				BAM1-5		05°24´56,3“	078°26´21,6“	502 m		Aramango		
				BAM1-6		05°24´56,3“	078°26´21,6“	503 m		Aramango		
		M6	BAP1	BAP1-1	2	05°22´26,2“	078°27´10,0“	373 m		Aramango		
				BAP1-2		05°22´26,2“	078°27´10,0“	374 m		Aramango		
	Tarapoto	TA1	SLQ1	SLQ1-1	2	06°25´17,7“	076°31´19,6“	744 m		Lamas		
				SLQ1-2		06°25´17,7“	076°31´19,6“	745 m		Lamas		
		TA2	SLQ2	SLQ2-1	5	06°25´14,5“	076°31´32,2“	803 m		Lamas		
				SLQ2-2		06°25´14,5“	076°31´32,2“	804 m		Lamas		
				SLQ2-3		06°25´14,5“	076°31´32,2“	805 m		Lamas		
				SLQ2-4		06°25´14,5“	076°31´32,2“	806 m		Lamas		
				SLQ2-5		06°25´14,5“	076°31´32,2“	807 m		Lamas		
Satipo 3	Rio Negro	RN1	SRY1	SRY1	1	11°05´13,3“	074°42´26,3“	621 m		Villa Kapiri		
			SRY2	SRY2-1	2	11°05´13,3“	074°42´26,3“	621 m		Villa Kapiri		

	(RN)			SRY2-2		11°05´13,3“	074°42´26,3“	622 m		Villa Kapiri		
			SRY3	SRY3	1	11°05´13,3“	074°42´26,3“	621 m		Villa Kapiri		
			SRY4	SRY4	1	11°05´13,3“	074°42´26,3“	621 m		Villa Kapiri		
		RN2	SRVK1	SRVK1-1	2	11°07´52,8“	074°40´23,4“	747 m		Villa Kapiri		
				SRVK1-2		11°07´52,8“	074°40´23,4“	748 m		Villa Kapiri		
		RN3	SRN1	SRN1-1	2	11°12´15,9“	074°39´46,7“	659 m		Villa Kapiri		
				SRN1-2		11°12´15,9“	074°39´46,7“	660 m		Villa Kapiri		
Ucayali 4	Padre Abad (PA)	PA	UPR1	UPR1-1	10	09°05´47,5“	075°44´31,5“	598 m		Previsto		
				UPR1-2		09°05´47,5“	075°44´31,5“	599 m		Previsto		
				UPR1-3		09°05´47,5“	075°44´31,5“	600 m		Previsto		
				UPR1-4		09°05´47,5“	075°44´31,5“	601 m		Previsto		
				UPR1-5		09°05´47,5“	075°44´31,5“	602 m		Previsto		
				UPR1-7		09°05´47,5“	075°44´31,5“	603 m		Previsto		
				UPR1-8		09°05´47,5“	075°44´31,5“	604 m		Previsto		
				UPR1-9		09°05´47,5“	075°44´31,5“	605 m		Previsto		
				UPR1-10		09°05´47,5“	075°44´31,5“	606 m		Previsto		
				UPR1-11		09°05´47,5“	075°44´31,5“	607 m		Previsto		
	Alto Shiringal (AS)	AS	UAS1	UAS1-1	4	08°52´03,2“	075°17´54,8“	278 m		AltoShiringal		
				UAS1-2		08°52´03,2“	075°17´54,8“	279 m		AltoShiringal		
				UAS1-3		08°52´03,2“	075°17´54,8“	280 m		AltoShiringal		
				UAS1-5		08°52´03,2“	075°17´54,8“	281 m		AltoShiringal		
	Aguaytia (A)	AS	UAA1	UAA1-1	6	08°50´52,4“	075°06´50,6“	264 m		Asunción del Aguaytío		
				UAA1-2		08°50´52,4“	075°06´50,6“	265 m		Asunción del Aguaytío		
				UAA1-3		08°50´52,4“	075°06´50,6“	266 m		Asunción del Aguaytío		
				UAA1-4		08°50´52,4“	075°06´50,6“	267 m		Asunción del Aguaytío		
				UAA1-5		08°50´52,4“	075°06´50,6“	268 m		Asunción del Aguaytío		
				UAA1-6		08°50´52,4“	075°06´50,6“	269 m		Asunción del Aguaytío		
	Irasola (I)		UAA2	UAA2-2	2	08°50´52,4“	075°06´50,6“	264 m		Asunción del Aguaytío		
				UAA2-3		08°50´52,4“	075°06´50,6“	265 m		Asunción del Aguaytío		
	Curimana ©	C1	UNT1	UNT1-1	2	08°37´01,5“	074°59´14,2“	180 m		Tahuallo		
				UNT1-2		08°37´01,5“	074°59´14,2“	181 m		Tahuallo		
	Curimana		UNT2	UNT2	1	08°37´01,5“	074°59´14,2“	180 m		Tahuallo		
	Neshuya	N	UNM1	UNM1-1	6	08°36´28,9“	074°59´35,9“	195 m		Monte Los Olivos		

	(N)			UNM1-2		08°36´28,9“	074°59´35,9“	196 m		Monte Los Olivos		
				UNM1-3		08°36´28,9“	074°59´35,9“	197 m		Monte Los Olivos		
				UNM1-4		08°36´28,9“	074°59´35,9“	198 m		Monte Los Olivos		
				UNM1-5		08°36´28,9“	074°59´35,9“	199 m		Monte Los Olivos		
				UNM1-6		08°36´28,9“	074°59´35,9“	200 m		Monte Los Olivos		
	Curimana	C2	UCM1/UCN1	UCN1-1	6	08°23´47,1“	075°05´17,9“	188 m		Las Malvinas		
				UCN1-2		08°23´47,1“	075°05´17,9“	189 m		Las Malvinas		
				UCN1-3		08°23´47,1“	075°05´17,9“	190 m		Las Malvinas		
				UCN1-4		08°23´47,1“	075°05´17,9“	191 m		Las Malvinas		
				UCN1-5		08°23´47,1“	075°05´17,9“	192 m		Las Malvinas		
				UCN1-6		08°23´47,1“	075°05´17,9“	193 m		Las Malvinas		
			UCM2	UCM2	1	08°24´07,8“	075°05´21,6“	181 m		Las Malvinas		
	Campo verde (CV)	CV	UCV1	UCV1-1	4	08°28´47,7“	074°48´24,5“	186 m		Campo Verde		
				UCV1-2		08°28´47,7“	074°48´24,5“	187 m		Campo Verde		
				UCV1-4		08°28´47,7“	074°48´24,5“	188 m		Campo Verde		
				UCV1-5		08°28´47,7“	074°48´24,5“	189 m		Campo Verde		
	Ucayali (U)	U1	UBE1	UBE1-1	3	09°02´50,7“	075°36´59,1“	362 m				
				UBE1-3		09°02´50,7“	075°36´59,1“	363 m				
				UBE1-4		09°02´50,7“	075°36´59,1“	364 m				
			UBE2	UBE2	1	09°02´50,7“	075°36´59,1“	362 m				
			UBE3	UBE3-1	2	09°02´50,7“	075°36´59,1“	362 m				
				UBE3-2		09°02´50,7“	075°36´59,1“	363 m				
			UBE4	UBE4-1	2	09°02´50,7“	075°36´59,1“	362 m				
				UBE4-2		09°02´50,7“	075°36´59,1“	363 m				
		U2	UBE5	UBE5-1	2	09°03´12,8“	075°37´08,4“	385 m				
				UBE5-2		09°03´12,8“	075°37´08,4“	386 m				
		U3	UBA1	UBA1-1	8	09°01´38,9“	075°38´07,3“	369 m				
				UBA1-2		09°01´38,9“	075°38´07,3“	370 m				
				UBA1-3		09°01´38,9“	075°38´07,3“	371 m				
				UBA1-4		09°01´38,9“	075°38´07,3“	372 m				
				UBA1-5		09°01´38,9“	075°38´07,3“	373 m				
				UBA1-6		09°01´38,9“	075°38´07,3“	374 m				
				UBA1-7		09°01´38,9“	075°38´07,3“	375 m				
				UBA1-8		09°01´38,9“	075°38´07,3“	376 m				
			Grand Total	**248**								

4.2 Extraction of DNA and ISSR analysis

Dry leaves of *Solanum sessiliflorum* was weighted to 200 mg then inserted into 2 ml microtube. To the microtube, liquid nitrogen was added to freeze the dry leaves and put 2 or 3 glass balls into the microtube when crushing until become powder. After crushing, glass balls was removed and added trace amount of PVP (Polyvinylpyrrolidone), 700 µl of CTAB solution (686 µl of 2% CTAB and 14 µl of mercaptoethanol), and 5 µl of RNase A to the mirotube. Suddenly, close the microtube and briefly vortex 1400rpm for 1min in every 10min on thermoblock at 60^{o}C for 30 min. Afterthat, centrifuge the microtube for 6 min at 14000 rpm then supernatant pipette to new 2 ml microtube. Add 500 µl of chloroform:isoamylalcohol (24:1) and turn over closed microtube 2 to 3 times then let it stand for 5min. Again, centrifuge 6 min at 14000 rpm. Supernatant pipette carefully to take only the transparent supernatant to the new microtube (1.5 ml) and remove contaminated tips and microtubes to a special container (work in the digestor) then add 500 µl of frozen isopropanol, turn it over 1 to 2 times and let it stand for 30 min at -20^{o}C. Centrifuge 3 min at 14000 rpm. White pellet of DNA was stick on the microtube wall, so very carefully outpour supernatant to the glass beaker, and flip upside down the open microtube on filter paper. To the open microtube, add 400 µl of cold 96% of ethanol and incubate 3min on the thermoblock at 37^{o}C. Again, centrifuge for 5min at 14000 rpm. Cautiously outpour the supernatent to the glass beaker, then flip upside the open microtube on the filter paper. Dry pellet in open microtube on the thermoblock at 65^{o}C for 6 to 10 min until the DNA pellet is separated from the microtube wall, then close the microtube. If the DNA pellet has still not yet separated from microtube wall, keep it drying for 1 or 2 min more. To the dried pellet, dissolve it with 50 µl of TE buffer on the thermoblock at 60^{o}C for 10 min and brefly vortex at 6000 rpm then centrifuge for 1 min at 14000 rpm. The DNA quality was determined by 0.8% agarose gel electrophoresis and using a Nanodrop Spectrophotometer (Thermo Scientific, USA). The final concentration of all DNA samples was adjusted to 20 ηg/µl for PCR (Polymerase Chain Reaction), and stored at -20°C.

For the optimization of annealing temperature, 30 ISSR primers (Integrated DNA Technologies, Belgium) were tested. PCR amplification reactions were carried out in a total volume of 20 µl containing 0.5 µl of each primer at a concentration of 0.1 mM, 10 µl PPP Master Mix (Top-Bio, Czech Republic), 0.2 µl of BSA (Bovine Serum Albumine) at a concentration 20 mg/mL (Thermoscientific, Lithuania), 7.3 µl PCR Water (Top-Bio, Czech Republic), and 2 µl of DNA. PCR amplification was performed in T100TM thermal Cycler (Bio-Rad Laboratories, USA) and the annealing temperatures in PCR were optimized for each

primer (Table 5). The cycling conditions were as follows: initial denaturation for 5 min at 94°C, followed by 40 cycles of denaturation for 1 min at 95°C, 1 min at specific annealing temperature in a range from 48°C to 55°C, 2 min at 72°C (extension), these 40 cycles were afterwards followed by a final extension step for 10 min at 72°C. The PCR products were resolved in 1.8% agarose gels in 1xTBE buffer (P-Lab, Czech Republic) using the following program: 120 min at 60 V and 120 mA. The gels were stained with ethidium bromide staining (Carl Roth GmbH, Germany) and the bands were visualized and acquired under UV light (Cleaver Scientific, UK). The size of the amplified products was estimated using 100 bp ladder (Thermoscientific, Lithuania). Ultimately, only 6 primers (UBC810, UBC824, UBC834, UBC835, UBC836, and UBC841) have been used for studying genetic diversity of *Solanum sessiliflorum* (Table 6).

Table 5: Primers selected for testing annealing temperature

Y= Pyrimidines: cytosine or thymine; R= Purine: adenine or guanine (Zietkiewicz et al., 1994)

Primer code (UBC)	Sequence 5‘ – 3‘	Annealing Temperature (°C)
UBC807	AG AG AG AG AG AG AG AG T	48
UBC809	AG AG AG AG AG AG AG AG G	55
UBC810	GA GA GA GA GA GA GA GA T	55
UBC812	GA GA GA GA GA GA GA GA A	52
UBC813	CT CT CT CT CT CT CT CT T	48
UBC814	CT CT CT CT CT CT CT CT A	48
UBC823	TC TC TC TC TC TC TC TC C	52
UBC824	AG AG AG AG AG AG AG AG G	55
UBC826	AC AC AC AC AC AC AC AC C	55
UBC828	TG TG TG TG TG TG TG TG A	48
UBC829	TG TG TG TG TG TG TG TG C	48
UBC834	AG AG AG AG AG AG AG AG YT	48
UBC835	AG AG AG AG AG AG AG AG YC	48
UBC836	AG AG AG AG AG AG AG AG YA	48
UBC840	GA GA GA GA GA GA GA GA YT	52
UBC841	GA GA GA GA GA GA GA GA YC	48
UBC843	CT CT CT CT CT CT CT CT RA	48
UBC844	CT CT CT CT CT CT CT CT RC	55
UBC845	CT CT CT CT CT CT CT CT RG	55
UBC846	CA CA CA CA CA CA CA CA RT	48
UBC847	CA CA CA CA CA CA CA CA RC	52
UBC848	CA CA CA CA CA CA CA CA RG	48

UBC851	GT GT GT GT GT GT GT GT CT G	52
UBC854	TC TC TC TC TC TC TC TC RG	52
UBC855	AC AC AC AC AC AC AC AC YT	52
UBC856	AC AC AC AC AC AC AC AC YA	52
UBC859	TG TG TG TG TG TG TG TG RC	50
UBC866	CTC CTC CTC CTC CTC CTC	48
UBC873	GACA GACA GACA GACA	52
UBC876	GATA GATA GACA GACA	52

Table 6: Primers selected for genotyping

Primer code (UBC)	Sequence 5‘ – 3‘	Annealing Temperature (°C)
UBC810	GA GA GA GA GA GA GA GA T	48.5
UBC824	AG AG AG AG AG AG AG A GG	50.7
UBC834	AG AG AG AG AG AG AG AG YT	51.6
UBC835	AG AG AG AG AG AG AG AG YC	51.6
UBC836	AG AG AG AG AG AG AG AG YA	47.9
UBC841	GA GA GA GA GA GA GA GA YC	51.6

4.4 Data analysis

All experiments were performed in duplicates, and the consistent and well-resolved fragments obtained through amplification of ISSR markers were manually scored. The scoring of amplified DNA fragments was done on the basis of presence (1) or absence (0) in the gel. Data was analysed by using POPGENE (Population genetic analysis, version 1.32) to study Nei’s genetic diversity and Shanon Index within and among populations, to construct the table of genetic distance among populations and regions by Nei's Unbiased Measures of Genetic Identity and Genetic distance 1978 (University of Albata and Center for International Forestry Research, 1997). The Neighbour Joining dendrogram (NJ) was built in Neighbour Joining in Tree View file by the mean of FAMD software (Fingerprinting Analysis with Missing Data, Version 1.31) (Schlüter and Harris 2006). Another dendrogram of genetic distance among populations based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was constructed by POPGENE 1.32. The factorial analysis was then analyzed by using Darvin 7 software. An Analysis of Molecular Variance (AMOVA) was performed by GenAlex 6.502. The frequency of fruit shape was analysed by SPSS software version 19.

5. Results

5.1 Morphological Data

There were high variation in fruit shape and size of *S. sessiliflorum* Dunal according to the populations where they had been collected. In total, fruit shapes were divided into five noticeable groups such as trangular, heart, oblong, round, and flat shape (Figure 7). The most frequency of fruit shape was trangular then heart, round, oblong, and flat shape, accordingly. Related to the percentage, trangular and heart shape occupied at the same proportion, which was 38.5% each as round shape possessed 12.8%, while flat and oblong shape had the lowest percentage accounted for 5.1% (Figure 8). The shapes of fruit varied within regions; however, flat shape was included only in Haunaco region, belonging to LP2 and LP9 populations whose shape looked like tomato as oblong from LP6 (Haunaco) and one more population RN2 from Junin region. Round shape came from one population in Haunaco with three populations in San Martin and they were LP7, R1, T3, M3, and M5. Remarkably, triangular and heart shapes had theirs places in all the four regions. For trangular shape, they were from Haunaco region (LP1, LP3, LP4, LP12, LP13, and LP14), Ucayali region (PA, I, N, CV, and U2), San Martin (T2, M4, and TA1), and the last one from Junin region (RN3). Ultimately, heart shape conquered in three populations in Haunaco region (LP5, LP8, and LP11), five populations in Ucayali region (AS, C1, C2, U, and U3), six populations in San Martin region (T1, T4, M1, M2, M6, and TA2), and one population in Junin region which was RN1. Strongly based on the morphological variation in fruit shape, we found high morphological diversity within regions, but low among regions on the ground that they borne similar shape in fruit. Most surprisingly for some populations, they possessed golden hair surrounding the fruit and they were TA1, M3, and M5 populations in San Martin region. Unfortunataly, there was no information about fruit shape of LP10.

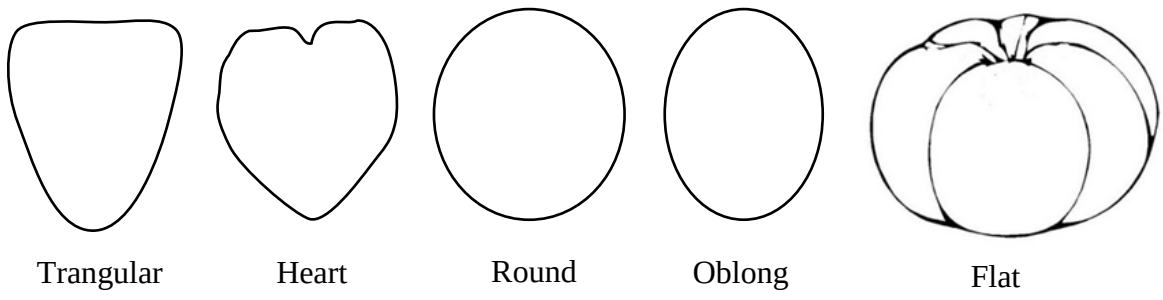

Figure 7: Fruit shapes of *S. sessiliflorum* (Personal care store, 2016)

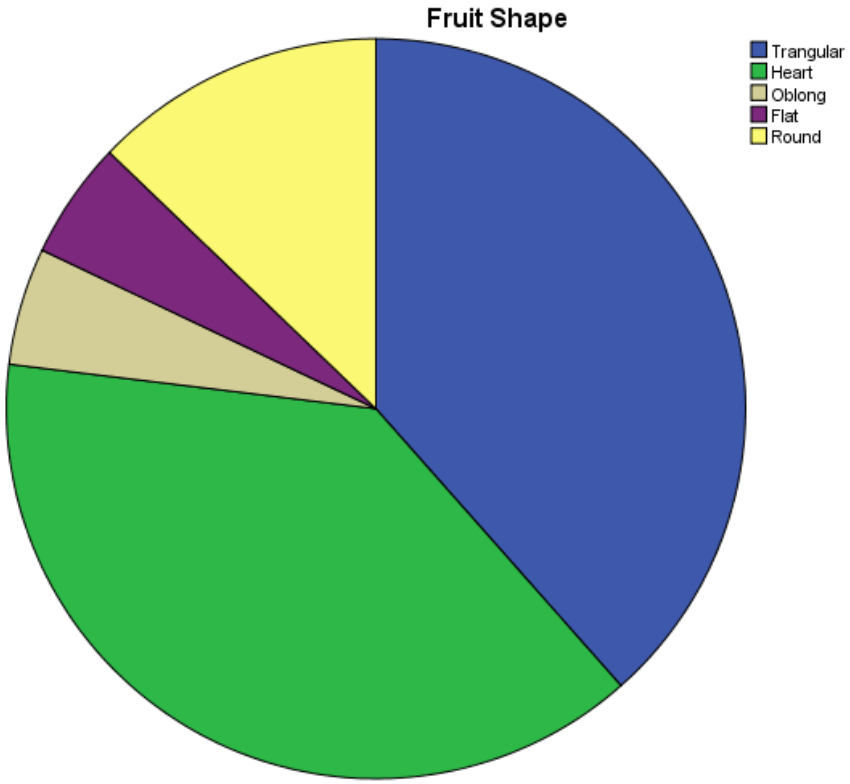

Figure 8: The proportion of fruit shapes of *S. sessiliflorum* from all populations

5.2 Genetic Diversity Analysis

Six primers had been selected from 30 primers to evaluate the genetic diversity of *Solanum sessiliflorum* Dunal because of high polymorphism and reproducibility. Six primers generated133 amplified loci of DNA with a range from 180 (UBC834) - 1500 bp (UBC836) and 131 bands werewepolymorphic loci. Each primer produced several number of polymorphic loci while primer UBC834 obtained the most number of polymorphic bands and UBC824 was the lowest. Primers with absolute polymorphic loci were UBC810, UBC824, UBC835, and UBC841; UBC834 and UBC836 comprised 96% of polymorphic loci. The total number of amplified bands was 6143 while primer UBC841 provided the greatest number, then UBC824, UBC810, UBC834, UBC836, and UBC835, respectively. The summary result of primers was mentioned in the table below (Table 7).

Table 7: Characterization of ISSR markers and their polymorphism

No.	Primers code (UBC)	Sequence 5′- 3′	Annealing temperature (°C)	Total number of bands amplified	Number of scorable bands per primer	No. And frequency of polymorphic bands per primer	Range of amplification (pb)
1	UBC810	$(GA)_8$ T	48.5	1192	24	24 (100%)	400-1300
2	UBC824	$(AG)_8$ G	50.7	1277	16	16 (100%)	280-1100
3	UBC834	$(AG)_8$ YT	51.6	954	26	25 (96.15%)	180-1300
4	UBC835	$(AG)_8$ YC	51.6	482	22	22 (100%)	250-1300
5	UBC836	$(AG)_8$ YA	47.9	830	23	22 (96.65%)	380-1500
6	UBC841	$(GA)_8$ YC	51.6	1408	22	22 (100%)	220-1100
		Total		**6143**	**133**	**131 (98.50%)**	

5.3 Genetic diversity within and among populations

Genetic diversity within populations: Shannon index of all population ranged from 0.0318 to 0.2677 with a mean of 0.1398 while population LP3 had the highest value and population U2 had the lowest number. Along with this, PA, C2, LP10, and LP1 also showed high Shannon Index within population: 0.2279, 0.2255, 0.2183, and 0.2163. The Nei's gene diversity ranged from 0.0218 to 0.1650 with an average of 0.0905. The summary of genetic diversity within population was shown in (Table 8).

Table8: Nei's genetic diversity and Shannon Index within populations

No	Name of Population	No of Sample	Nei ± SD	Shannon Index ± SD	No of Polymorphic Loci	% of Polymorphic Loci
1	LP1	22	0.1340 ± 0.1665	0.2163 ± 0.2383	81	60.9
2	LP2	6	0.1051 ± 0.1632	0.1637 ± 0.2410	47	35.34
3	LP3	21	0.1650 ± 0.1637	0.2677 ± 0.2322	99	74.44
4	LP4	4	0.0756 ± 0.1587	0.1122 ± 0.2305	27	20.3
5	LP5	5	0.0814 ± 0.1505	0.1256 ± 0.2251	34	25.56
6	LP6	9	0.0985 ± 0.1535	0.1568 ± 0.2289	50	37.59
7	LP7	6	0.0719 ± 0.1352	0.1156 ± 0.2042	37	27.82
8	LP8	3	0.0617 ± 0.1403	0.0932 ± 0.2083	23	17.29
9	LP9	5	0.1257 ± 0.1774	0.1920 ± 0.2600	51	38.35
10	LP10	25	0.1350 ± 0.1640	0.2183 ± 0.2368	82	61.65
11	LP11	3	0.0274 ± 0.1048	0.0399 ± 0.1512	9	6.77

12	LP12	3	0.0536 ± 0.1321	0.0809 ± 0.1964	20	15.04
13	LP13	2	0.0374 ± 0.1191	0.0546 ± 0.1739	12	9.02
14	LP14	5	0.0914 ± 0.1668	0.1380 ± 0.2421	36	27.07
15	PA	10	0.1418 ± 0.1617	0.2279 ± 0.2383	75	56.39
16	AS	4	0.1043 ± 0.1628	0.1608 ± 0.2432	43	32.33
17	A	8	0.1324 ± 0.1692	0.2082 ± 0.2490	63	47.37
18	C1	3	0.0818 ± 0.1578	0.1229 ± 0.2331	30	22.56
19	N	6	0.1147 ± 0.1757	0.1748 ± 0.2559	47	35.34
20	C2	7	0.1469 ± 0.1859	0.2255 ± 0.2667	64	48.12
21	CV	4	0.0847 ± 0.1580	0.1287 ± 0.2333	33	24.81
22	U1	8	0.1077 ± 0.1651	0.1683 ± 0.2418	51	38.35
23	U2	2	0.0218 ± 0.0928	0.0318 ± 0.1355	7	5.26
24	U3	8	0.1045 ± 0.1688	0.1612 ± 0.2452	47	35.34
25	T1	6	0.1212 ± 0.1808	0.1833 ± 0.2626	48	36.09
26	T2	3	0.0570 ± 0.1446	0.0835 ± 0.2089	19	14.29
27	T3	6	0.1210 ± 0.1721	0.1863 ± 0.2540	51	38.35
28	T4	7	0.0944 ± 0.1518	0.1497 ± 0.2270	46	34.59
29	M1	8	0.0915 ± 0.1533	0.1437 ± 0.2285	43	32.33
30	R1	7	0.1090 ± 0.1540	0.1740 ± 0.2320	54	40.6
31	M2	3	0.0335 ± 0.1053	0.0512 ± 0.1585	13	9.77
32	M3	3	0.0454 ± 0.1229	0.0686 ± 0.1830	17	12.78
33	M4	3	0.0623 ± 0.1455	0.0927 ± 0.2130	22	16.54
34	M5	5	0.0877 ± 0.1536	0.1361 ± 0.2289	38	28.57
35	M6	2	0.0747 ± 0.1599	0.1091 ± 0.2334	24	18.05
36	TA1	2	0.0436 ± 0.1276	0.0637 ± 0.1863	14	10.53
37	TA2	5	0.0959 ± 0.1598	0.1483 ± 0.2369	41	30.83
38	RN1	5	0.1146 ± 0.1701	0.1765 ± 0.2509	48	36.09
39	RN2	2	0.0934 ± 0.1738	0.1364 ± 0.2537	30	22.56
40	RN3	2	0.0716 ± 0.1572	0.1046 ± 0.2296	23	17.29
	Mean		**0.1398 ± 0.1531**	**0.0905 ± 0.2249**	**39.98**	**30.06**

(Nei= Genetic Diversity, SD= Standard Deviation, LP= Leoncio Prado, PA= Padre Abad, AS= Alto Shiringal, A= Aguaytia, C= Curimana, N= Neshuya, CV= Campo Verde, U= Ucayali, T= Tocache, M= Moyobamba, R= Rioja, TA= Tarapoto, RN= Rio Negro)

Genetic distance among populations: the overall Shannon index of all the samples was 0.2783; the Nei‘s genetic distance was 0.1672 and gene flow presented high among the populations which was 0.5871 and G*st* was 0.7277. The overall genetic distance among the population was clearly illustrated in (Table 10).

Genetic distance among populations in Huanuco region was not so high that the mean was 0.059. The most similar populations were between LP6 and LP7 (0.010), LP5 and LP6, and LP5 and LP7 (0.017), which maybe because they were cultivated in wild and their habitats were in high altitude from 600 to 700 m a.s.l. The most difference populations were between LP1 and LP13 (0.210), and LP4 and LP13 (0.191). Surprisingly, it was clearly distinguished LP11, LP12, LP13, and LP14 from the other populations.

Back to the populations in Ucayali region, the average mean of genetic distance was 0.056. N and C2 populations were almost the same (0.010) on account of altitude position around 190 m where they were collected. Other pairs were between PA & AS, and AS & A population whose value was 0.013. However, U2 population was very dissimilar to all populations in this region; U2 and CV were the most distinguished (0.168). Compared to populations in Huanuco, N and LP2 were the most comparable (0.023), while U2 and LP13 were incomparable.

In San Martin region, the mean of genetic distance was higher compared to those of in Huanuco and Ucayali regions, which was 0.074. The most parallel populations were R1 and M1 (0.012). The reason might be the location of these two populations to stay in high altitude around 850m. The dissimilarity occurred between M2 and T2. It was found to be related to some populations in Huanuco and Ucayali regions as well. For instance, R1 and LP6 populations were somehow similar (0.029). M5 and LP13 populations were very dissimilar (0.235). Moreover, T1 population was not so different to U3 populations, so do R1 and C1 populations in Ucayali region, whereas M6 and U2 were diverse although they were located in similar altitude which was around 300m.

RN1, RN2, and RN3 populations were a bit different to one another. The genetic distance between RN1 & RN2, RN1 & RN3, and RN2 & RN3 were 0.029, 0.036, and 0.049, respectively with an average genetic distance of 0.038. Among the populations in all regions, it was extremely distinguished from populations in Huanuco, mainly to LP11 population, ranging from 0.018 to 0.229. The genetic distance of populations between Junin and Ucayali regions ranged from 0.017 to 0.202. The most similar were between RN1 and PA; the most dissimilar were between R3 and U2. They were quite different from populations in San

Martin regions. Actually, RN1 was analogous to TA2 population (0.014), and RN3 was atypical of T2.

The dendrogram of genetic distance, helped to understand clearly the similarity and dissimilarity of genetic distance among the populations. It divided the overall populations into two clusters, one of which consisted of 4 populations such as LP11, LP12, LP13, and LP14 and the rest gathered in the second cluster. LP11 & LP12 populations and LP13 & LP14 populations were somehow similar on account of the location and altitude from where they were collected. The big cluster revealed the close relationship between LP6 and LP7, N & C2, M1 & R1, A & AS, TA2 & RN1, LP1 & LP3, U3 & T1, RN2 & RN3 populations (**Figure 9**).

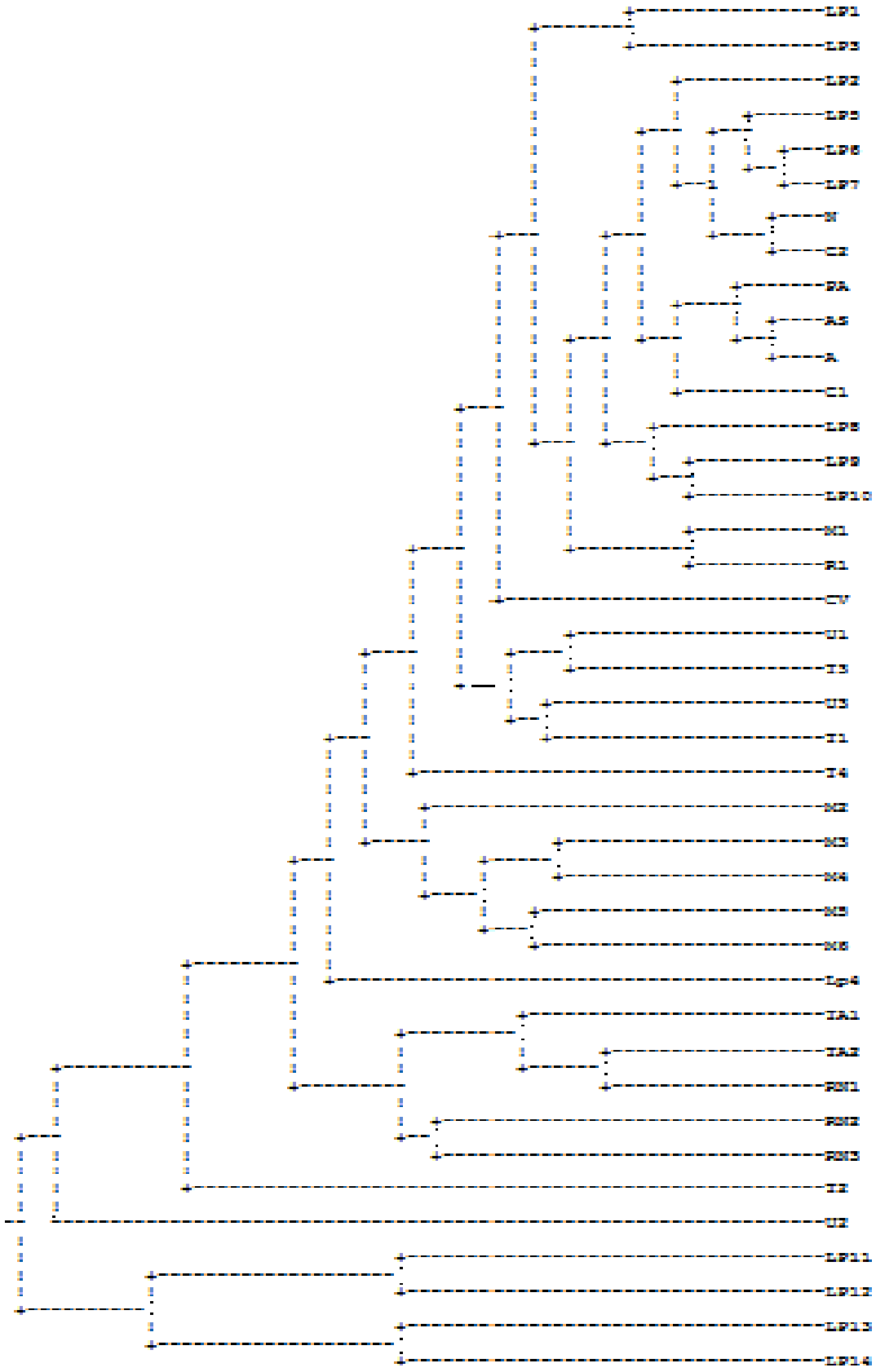

Figure 9: Dendrogram of Populations Based Nei's (1978) Genetic distance: Method = UPGMA, modified from NEIGHBOR procedure of PHYLIP Version 3.5

Genetic distance among regions: from one region to another, they were different. The most genetic distance was between Huanuco and Junin regions which was 0.0275, whereas the most similar was between Ucayali and San Martin regions that accounted for 0.0067. The differences between San Matin and Junin, Ucayali and Junin, Huanuco and San Martin, and Huanuco and Ucayali were 0.0266, 0.0205, 0.0103, and 0.0068, respectively. The detail of genetic distance among regions was exposed in **(Table 9).**

Table 9: The genetic distance among regions

Regions	***Huanuco***	***Ucayali***	***San Martin***	***Junin***
Huanuco	0.0000			
Ucayali	0.0068	0.0000		
San Martin	0.0103	0.0067	0.0000	
Junin	0.0275	0.0205	0.0266	0.0000

5.4. Analysis of Molecular Variance

There was high variation of gene within populations 72% in comparison to variation among populations, which was only 21%. The percentage of variation among the four regions was very low comprising of 7%. It meant that within population, it mixed the gene from other populations. That is the reason why the variation among populations was very low (**Figure 10**).

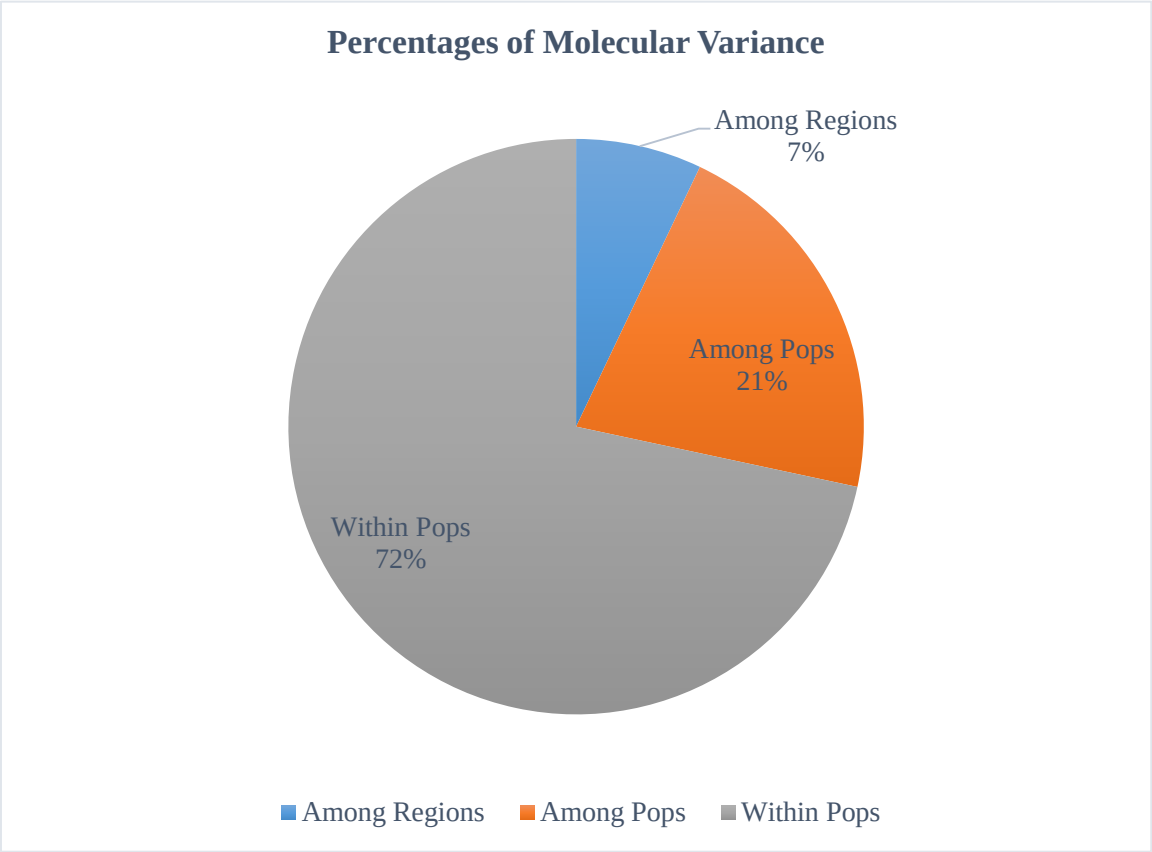

Figure 10: Pie chart of molecular variance

Table 10: Nei's distance among populations

POP	LP1	LP2	LTP3	LP4	LP5	LP6	LP7	LP8	LP9	LP10	LP11	LP12	LP13	LP14	PA	AS	A	C1	N	C2	CV
LP1	0.000																				
LP2	0.043	0.000																			
LP3	0.024	0.035	0.000																		
LP4	0.063	0.077	0.041	0.000																	
LP5	0.050	0.037	0.035	0.050	0.000																
LP6	0.045	0.016	0.029	0.052	0.017	0.000															
LP7	0.051	0.035	0.027	0.052	0.017	0.010	0.000														
LP8	0.064	0.057	0.048	0.078	0.042	0.028	0.026	0.000													
LP9	0.055	0.045	0.047	0.063	0.045	0.023	0.025	0.025	0.000												
LP10	0.046	0.042	0.034	0.059	0.037	0.021	0.026	0.027	0.022	0.000											
LP11	0.115	0.127	0.123	0.094	0.131	0.113	0.136	0.146	0.096	0.113	0.000										
LP12	0.120	0.100	0.112	0.094	0.118	0.081	0.104	0.105	0.079	0.096	0.051	0.000									
LP13	0.210	0.182	0.187	0.191	0.189	0.157	0.172	0.142	0.131	0.145	0.152	0.082	0.000								
LP14	0.132	0.124	0.118	0.124	0.121	0.095	0.103	0.072	0.085	0.081	0.121	0.065	0.050	0.000							
PA	0.047	0.034	0.032	0.082	0.033	0.020	0.021	0.027	0.029	0.017	0.136	0.107	0.161	0.094	0.000						
AS	0.043	0.034	0.036	0.101	0.035	0.030	0.037	0.032	0.044	0.033	0.153	0.116	0.174	0.094	0.013	0.000					
A	0.059	0.043	0.049	0.102	0.044	0.038	0.046	0.050	0.054	0.040	0.148	0.121	0.170	0.099	0.018	0.013	0.000				
C1	0.076	0.055	0.065	0.088	0.037	0.040	0.054	0.029	0.051	0.040	0.142	0.121	0.168	0.096	0.030	0.034	0.019	0.000			
N	0.038	0.023	0.025	0.063	0.023	0.017	0.024	0.033	0.043	0.025	0.134	0.103	0.158	0.082	0.019	0.017	0.020	0.023	0.000		
C2	0.050	0.029	0.028	0.038	0.027	0.020	0.030	0.047	0.034	0.024	0.086	0.064	0.123	0.062	0.030	0.033	0.042	0.039	0.010	0.000	
CV	0.076	0.065	0.069	0.090	0.043	0.046	0.053	0.049	0.047	0.044	0.125	0.100	0.148	0.070	0.045	0.031	0.063	0.053	0.042	0.028	0.000
U1	0.065	0.047	0.044	0.070	0.036	0.039	0.037	0.048	0.040	0.038	0.139	0.111	0.156	0.094	0.041	0.044	0.055	0.059	0.030	0.025	0.046
U2	0.136	0.109	0.132	0.141	0.133	0.115	0.136	0.164	0.113	0.106	0.166	0.159	0.216	0.204	0.120	0.150	0.141	0.150	0.135	0.116	0.168
U3	0.080	0.059	0.053	0.068	0.058	0.050	0.053	0.064	0.038	0.035	0.117	0.118	0.160	0.119	0.051	0.071	0.066	0.060	0.053	0.041	0.073
T1	0.087	0.073	0.059	0.073	0.061	0.056	0.051	0.058	0.057	0.041	0.158	0.130	0.145	0.096	0.054	0.076	0.052	0.050	0.042	0.047	0.088
T2	0.142	0.112	0.111	0.114	0.104	0.096	0.102	0.096	0.088	0.089	0.152	0.114	0.123	0.120	0.093	0.119	0.091	0.083	0.090	0.082	0.137
T3	0.067	0.055	0.047	0.071	0.037	0.049	0.048	0.057	0.057	0.039	0.141	0.126	0.162	0.115	0.047	0.052	0.061	0.054	0.046	0.040	0.050
T4	0.082	0.054	0.064	0.109	0.072	0.055	0.068	0.088	0.050	0.063	0.148	0.141	0.185	0.115	0.060	0.050	0.071	0.085	0.054	0.053	0.054
M1	0.052	0.030	0.056	0.083	0.034	0.031	0.047	0.055	0.040	0.040	0.114	0.110	0.175	0.106	0.037	0.037	0.042	0.034	0.031	0.034	0.034
R1	0.057	0.028	0.049	0.067	0.038	0.029	0.042	0.043	0.033	0.031	0.114	0.111	0.180	0.111	0.039	0.045	0.049	0.029	0.036	0.034	0.049
M2	0.098	0.066	0.079	0.094	0.063	0.050	0.048	0.086	0.057	0.057	0.156	0.151	0.226	0.159	0.062	0.087	0.096	0.084	0.069	0.067	0.083
M3	0.074	0.059	0.069	0.107	0.084	0.064	0.086	0.097	0.073	0.070	0.146	0.135	0.206	0.153	0.077	0.089	0.096	0.081	0.073	0.075	0.097
M4	0.084	0.059	0.063	0.081	0.069	0.057	0.064	0.091	0.068	0.056	0.137	0.121	0.205	0.150	0.062	0.093	0.076	0.068	0.061	0.063	0.104
M5	0.065	0.054	0.059	0.087	0.063	0.060	0.070	0.080	0.068	0.059	0.161	0.153	0.235	0.148	0.058	0.062	0.055	0.057	0.049	0.067	0.079
M6	0.086	0.077	0.079	0.105	0.082	0.069	0.082	0.087	0.060	0.070	0.139	0.156	0.223	0.150	0.064	0.090	0.079	0.065	0.073	0.078	0.087
TA1	0.125	0.088	0.100	0.108	0.101	0.071	0.082	0.068	0.063	0.065	0.158	0.109	0.128	0.081	0.073	0.100	0.092	0.075	0.087	0.073	0.101
TA2	0.097	0.073	0.083	0.105	0.073	0.050	0.063	0.035	0.052	0.045	0.146	0.095	0.119	0.069	0.043	0.062	0.062	0.050	0.062	0.059	0.075
RN1	0.053	0.054	0.042	0.065	0.042	0.031	0.032	0.018	0.036	0.021	0.141	0.101	0.143	0.071	0.017	0.032	0.032	0.024	0.028	0.035	0.053
RN2	0.102	0.079	0.086	0.126	0.077	0.057	0.071	0.051	0.063	0.047	0.206	0.162	0.185	0.114	0.037	0.064	0.059	0.049	0.061	0.069	0.083
RN3	0.108	0.115	0.094	0.145	0.114	0.093	0.103	0.075	0.107	0.067	0.229	0.183	0.196	0.115	0.070	0.081	0.066	0.075	0.082	0.104	0.115

Table 10: Nei's genetic distance among populations (continue)

POP ID	U1	U2	U3	T1	T2	T3	T4	M1	R1	M2	M3	M4	M5	M6	TA1	TA2	RN1	RN2	RN3
U1	0.000																		
U2	0.097	0.000																	
U3	0.037	0.066	0.000																
T1	0.041	0.108	0.032	0.000															
T2	0.079	0.120	0.061	0.041	0.000														
T3	0.026	0.116	0.039	0.044	0.064	0.000													
T4	0.047	0.146	0.059	0.075	0.115	0.043	0.000												
M1	0.036	0.115	0.047	0.061	0.106	0.044	0.037	0.000											
R1	0.039	0.110	0.040	0.051	0.098	0.040	0.054	0.012	0.000										
M2	0.080	0.152	0.072	0.081	0.146	0.077	0.077	0.051	0.033	0.000									
M3	0.077	0.150	0.081	0.084	0.127	0.061	0.064	0.048	0.034	0.070	0.000								
M4	0.071	0.127	0.060	0.049	0.103	0.067	0.098	0.056	0.034	0.048	0.029	0.000							
M5	0.077	0.156	0.081	0.070	0.137	0.070	0.079	0.045	0.031	0.057	0.051	0.033	0.000						
M6	0.084	0.171	0.079	0.075	0.119	0.071	0.066	0.046	0.041	0.057	0.045	0.046	0.034	0.000					
TA1	0.082	0.126	0.078	0.078	0.080	0.069	0.110	0.100	0.081	0.128	0.110	0.099	0.122	0.125	0.000				
TA2	0.071	0.114	0.074	0.074	0.094	0.075	0.118	0.076	0.070	0.111	0.128	0.098	0.109	0.115	0.027	0.000			
RN1	0.046	0.114	0.052	0.037	0.084	0.052	0.09	0.051	0.043	0.086	0.089	0.061	0.068	0.083	0.037	0.014	0.000		
RN2	0.071	0.145	0.083	0.078	0.138	0.095	0.106	0.070	0.069	0.114	0.123	0.104	0.104	0.114	0.075	0.046	0.029	0.000	
RN3	0.116	0.202	0.120	0.077	0.154	0.111	0.136	0.113	0.106	0.151	0.141	0.122	0.114	0.148	0.092	0.068	0.036	0.049	0.000

5.5 Cluster analysis based on the ISSR genotyping profile

The dendrogram was constructed with an aim to understand the similarity and dissimilarity of all the samples which had or did not have genetic relationship. The dendrogram revealed the division of all samples into 8 prominent clusters. Most of the cluster components were mixed samples from both different populations and regions, except *Cluster III* and *Cluster* VIII which had all the populations from one region; moreover, not all samples in one population were grouped together in one cluster except populations U2, LP11, LP12, LP13, TA1, T1, and T2 (Figure 11).

Cluster I: this cluster contained 26 individual samples from 3 regions. The dominant one was from San Martin, 3 samples from Huanuco, and 4 samples form Ucayali. It grouped population T3, T4, and M1 which belonged to San Martin region, LP5 (Huanuco), and CV (Ucayali) together.

Cluster II: it was formed by the populations from Ucayali region with a few samples in Junin and Huanuco, which was, of course, stated that population N, PA, AS, and RN1 were similar, especially, there were evident about the types of cultivation in these populations. The total number of samples in this group was 32.

Cluster III: it was absolutely included the populations from San Martin region. They were R1, M5, M3, M2, M6, and one only sample from M4. These populations were very close to each other. Exactly, these 5 populations had similar fruit shape (Round and small fruit) excluding population M4 whose fruit shape was round to oblong, particularly some populations have gold thick hair on fruits (M3 and M5 populations). This cluster had only 21 samples in total.

Cluster IV: The formation of this cluster was 3 populations from Ucayali and 2 populations from Huanuco. Most of the individuals belonging to LP2 population were involved in this group; it contained only 2 individuals from LP3. Half of this group was from few samples of the populations PA, AS, and A. This cluster comprised of 16 samples, which was the smallest cluster.

Cluster V: It was the second biggest cluster among the 8 clusters composed of all the 4 regions, which contained 35 samples. It included all samples from the populations LP11, LP12, LP13, and TA1, and almost samples from populations LP14 and TA2, a part of samples from population PA, RN2, and RN3. Based on geographical position, this cluster gathered the population located in high altitude from 600 up to 800 m a.s.l.

Cluster VI: The components of this cluster were populations from Ucayali, Huanuco, and San Martin regions. Half of the samples from LP10 population presented in the group, 2 samples from T3 and U2, 3 samples from U3, and 4 samples from U1.

Cluster VII: This group was constituted of populations from San Martin and Ucayali. All samples in T1 and T2 population were included in the cluster while U3, C2, CV, C1, and M4 contributed only a small number of samples. Most of the samples in this cluster had oblong fruit shape without hair, bar C2 population that had round shape with gold hair on fruit.

Cluster VIII: It was completely formed by the populations in Huanuco region such as LP1, LP3, LP4, LP5, LP6, LP7, LP9, and LP10. LP1 and LP3 populations were very similar in genetic relationship, for a particular reason, they were cultivated species and have the same fruit shape (oblong) with hair on fruit. However, other populations had similarity in fruit shape from round to nearly oblong or flat. It was the biggest cluster that carries 69 samples.

The factorial analysis of *S. sessiliflorum* Dunal by application of ISSR marker did not clearly separate 40 populations from each other, for the samples of each population bestrew throughout the surface, which did not distinguish the population according to the locations; however, it revealed that the population LP1 and LP3 scattered in the same plot together. All these evidence supported the construction of the dendrogram. More comments to this graph, it had grouped more samples together (Figure 12).

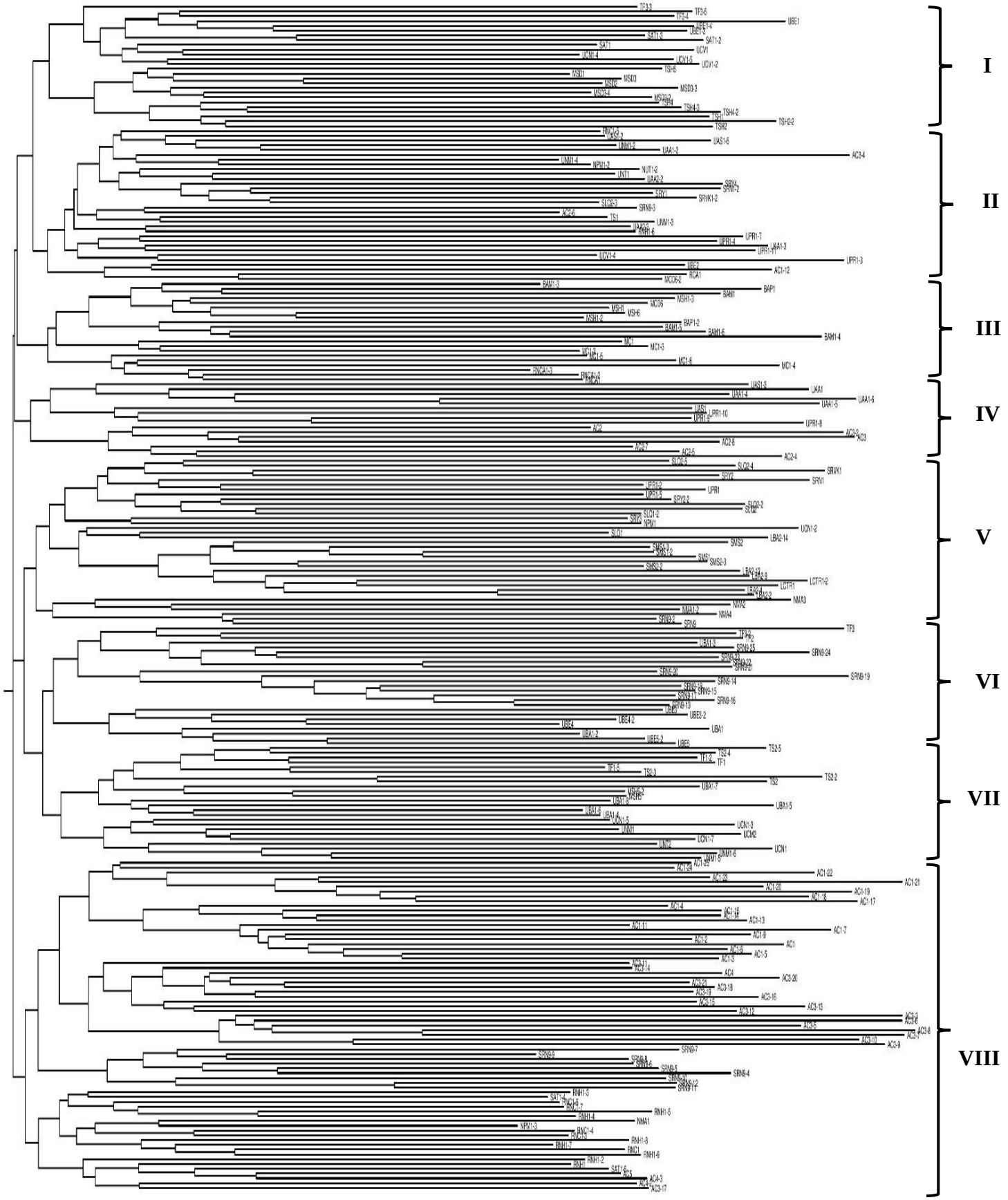

Figure 11: Dendrogram-Neighbour Joining of *Solanum sessiliflorum*

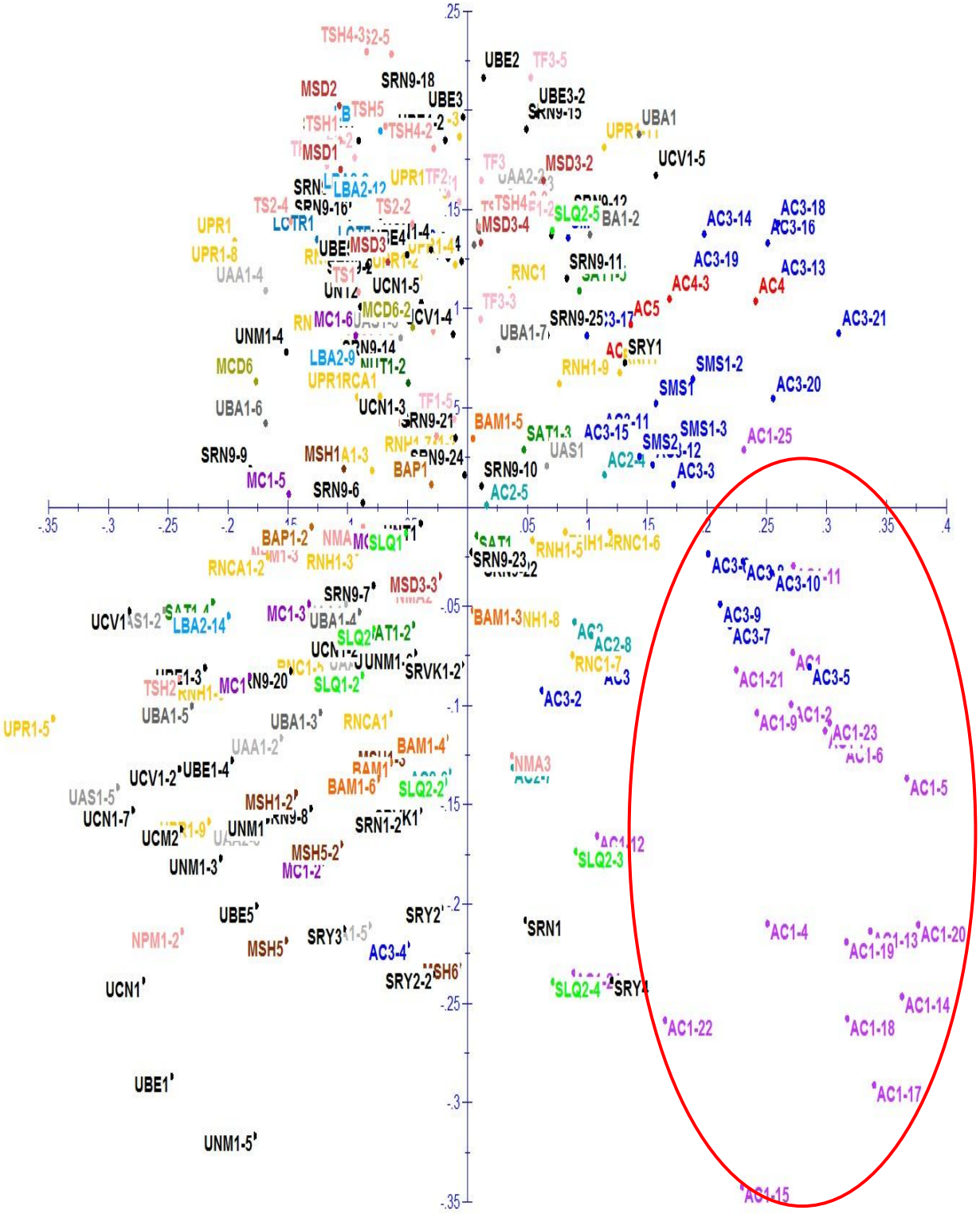

Figure 12: Factorial Analysis of 248 samples of *Solanum sessiliflorum* by ISSR marker

6. Discussion

6.1 Genetic diversity of *Solanum spp.* and ISSR marker

Solanum sessiliflorum is an exotic tropical fruit which contains many nutritive values necessary in contribution to human diet, yet the research on this special species is practically not link to genetic diversity even though there were some research having mentioned the high variation in fruit shape and size. A little research was done on genetic diversity of related species of *Solanum* (Lasiocarpa) including *Solanum sessiliflorum* in Columbia by using AFLP. It is reported to be similar in genetics of *Solanum quitoense* in the section Lasiocarpa (Sánchez, 2010). Another research was identified the phylogenetic relationships of *Solanum* section Lasiocarpa by using chloroplast DNA on 24 accessions from section Lasiocarpa and 14 accesssions of other *Solanum* species as outgroups. *S. sessiliflorum* was supposed in the section Lasiocarpa in the same group with *S. stramonifolium* and *S. pectinatum* ranged from 93–97% (Bohs, 2004).

ISSR had been used in genetic diversity study and also characterization of *Solanum* species and was reported to be polymorphic while it produced many polymorphic bands, which actually had the possibility to reveal the genetic diversity, for instance, in the case study of *Solanum melongena* L. (Tiwari et al., 2009). With good result and the same species, *S. melongena* used to be studied on genetic diversity among its varieties by ISSR marker with eleven primers (Channe et al., 2013). ISSR application on *S. lycopersicum* obtained acceptable result in comparison to RAPD and IRAP markers even though they had individual results during analyzing ten tomato cultivars (Mansour et al., 2010). One recent research focusing on relationship analyzed cultivated, wild, and hybrid of potato proved by ISSR (Giglou et al., 2015). Other research on different genus indicated the high genetic diversity among populations and a number of scored bands by using ISSR done on *Magnolia wufengensis* on the ground that it showed the considerable morphological variation (Chen et al., 2014); ISSR marker was used in the same genus of *Magnolia* spp to identified the genetic variation within and among populations of *Magnolia officinalis* (Yu et al., 2010). These studies totally supported the result of *Solanum sessiliflorum* as many scored bands had been produced and generated the total number of 6143 bands with 6 primers.

6.2 Morphological Diversity

Solanum sessiliflorum varied in fruit shape andaccording to the samples had been collected in different places in Peru. Some of populations had been collected in wild and the others were cultivated varieties. The difficulty of isolation was occurred between wild or weed population because they almost looked the same; however, it was able to distinguish wild or weed population from cultivated one. This population contained spiny stem with small fruit and located along the river or steam bank or disturbed habitat and some were at safe habitat from herbivore, but cultivated populations were spineless. The shapes of fruit were divided into oblong, round, and flat (Salick, 1992), while other authors divided the fruit shape into cylindrical, heart, round and flat fruits (Pereira and Filho, 2010). In response to this statement, populations gathered from wild such as LP2, LP5, LP6, LP7, LP8, and LP9, which belonged to Huanuco had small fruits in comparison to cultivated populations whose size of fruits were, of course, bigger than those of from wild. They were LP1 and LP3, especially their shapes were in the same form, which were noticeably trangular.

6.3 Genetic diversity within and among populations

Shannon index was not so high within population from 0.0318 to 0.2677 with a mean of 0.1398. It meant that in one population, it mixed few samples from other population, only 13.98% in average. There was one research about breeding between naranjilla/lulo (*Solanum quitoense*), a shrubby perennial plant with cocona (*Solanum sessiliflorum*) in Colombia (Pizzinato et al., 2008). It showed the hybrid variety, so it might mix the gene in cocona in these samples having been collected because cocona has high gene flow. Nei's gene diversity ranged from 0.0218 to 0.1650 with an average of 0.0905. The genetic variation within population might mix the gene from near locations, naturally crossed in nature between wild and cultivated populations for the reason that wild or weed populations had been normally observed as a weed in cultivated populations (Salick, 1992).

The overall Shannon index of all the samples was 0.2783; the Nei's genetic distance was 0.1672 and gene flow presented high among the populations which was 0.5871 and Gst was 0.7277. Gst was very high for all the samples and was clearly indicated that the variation was 72.77%, so only 27.27% were similar following with gene flow which was quite high 58.71%. It seemed that there was no hard obstacle for cocona in spreading the pollination. Based on crop domestication and evolution of cocona studied by (Salick, 1992) in Iscozacin, Palcazu valley at 350 m in altitude where the variations in cocona types were

formed including wild, weed, and domesticated forms. He inspected that cocona had high gene flow for several reasons, one of which was because of physical property of cocona itself while it continuously blossom and it accepted the pollen almost all the times. Furthermore, the pollen could be spread by wind, and the most important one was a very active pollinator that was always bee. The most common one was due to seed dispersal by animal and intentionally by human for cocona domestication. Cocona seeds were remarkably emerged in cow dung and transported to the field of cocona cultivation. Therefore, the weedy form was situated among the cultivated one. In spite of the fact that farmers saw this weedy species, they ignored owing to spine of weedy cocona. As a consequence, cultivated populations normally had variation in morphology. Some of people just go to the forest and collect the fruits of cocona from wild or half domesticated ecotypes for food (Melgarejo et al, 2003). This could be the crucial source of gene flow.

Related to analyze of molecular variant, it totally agreed with these reasonable proofs. The molecular variation within population was extremely high 72%, 21% among populations and 7% among regions. In almost every population, it contained the gene from other populations to make it less different even though the physical appearance of fruits was similar in every population.

7. Conclusion

This research will become the basic data for genetic study of *S. sessiliflorum* Dunal while there have not been any studies about it before. This study indicated that *Solanum seesiliflorum* Dunal had higher genetic diversity within populations rather than among populations along with high gene flow that was the reason why the variations of molecule among populations and regions were small.

Based on phenotype of cocona, it demonstrated high differences in fruit shapes and sizes, which was divided into five shapes including triangular, heart, oblong, round, and flat shape, particularly, the most dominant shapes were triangular and heart. Three populations had hair surrounding the fruit: TA2, M3, and M5 in San Martin region; wild populations had smaller fruit size if compared to cultivated populations.

Even though the samples collected from the study sites contained less information about the origin of cultivation, basic information focused on morphological data, genetic study by application of ISSR marker was able to reveal the high genetic diversity of *S. sessiliflorum* Dunal with six ISSR primers. However, it generated many scored bands and polymorphic loci in order to evaluate the whole genetic structure. Thus, ISSR is an exceptional choice for genetic study.

Cocona is a valuable plant and high in phenotypic and genetic variation; in addition, the breeding between cocona and naranjilla on disease and insect resistance received good result, for the disease was not the problem for hybrid generation; therefore, more research should focus on breeding program of cocona as it takes long time for cocona to produce fruits, more research on nutritive values of it, especially the genetic study of cocona for the purpose of gene conservation. If not, it will be lost and decrease the gene pool in nature. Inasmuch as the cross pollination cannot be controlled in nature as well as the factors of pollination, genetic study is absolutely important.

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Appendix

Fruit shape of samples collected from Peru