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Variation in Growth and Yield of Sacha Inchi (*Plukenetia Volubilis* L.) under Different Ecological Regions in Vietnam

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ABSTRACT

The sacha inchi seeds (Plukenetia Volubilis L.), collected from different ecological regions of Vietnam (the Northern Mountainous region, the North Central region, and Central Highlands Area), were sown in the spring crop (March 2020) in Gia Lam district, Hanoi city, Vietnam. The variety S18 was copyrighted by Ministry of Agriculture and Rural Developmentof Vietnam in 2019 as a special medicinal plant variety. During the period of 26 months after planting, the agronomical parameters, growth and development characteristics (leaf form, number of inflorescences per branches, number of female flowers per inflorescences, etc.), pests and diseases, yields and yield components (number of seeds per fruit, seed weight, seed grain, etc.), ratio of kernel per seed and ratio of oil per seed were evaluated. The evaluation results show that the collected sacha inchi lines have a time from sowing to flowering and fruiting of 108-125 days, a fruiting to ripening of 123-125 days, and a time from sowing to harvesting the first batch of 244-250 days. The annual harvest is carried out at two main times: May-June and November–January. The actual yield of the first two periods is high (2.66–3.07 tons per hectare), and the highest vield is collected in the Northern Mountainous area is 3.07 tons per hectare (in Tuyen Ouang province), while and 3.01 tons per hectare are collected in the North Central region (in Thanh Hoa province). In the nursery, root rot caused 2.1–5.3% deaths and green wilt caused 3.1–4.8% deaths of seedlings. In the planting garden, fruit borers, stem borers, cavity worms and red spiders were discovered, causing more serious damage than other types (from 5 to over 50%). The results of evaluation of growth, development, yield components and yield showed that the sacha inchi lines collected in the North Central region (in Thanh Hoa province) and the Northern Mountainous region (in Tuyen Quang province) is the best.

Keywords: ecological region, growth, sacha inchi, selection, yields.

INTRODUCTION

Sacha inchi (*Plukenetia Volubilis* L.), belongs to the *Euphorbiaceae* family, and originates from the Amazon rainforest, where the altitude is below 200 meters above sea level. To date, scientists have collected, preserved, and studied 20 species of *Plukenetia*, which range from Bolivia to Mexico, being most common in Peru (12 species); the rest are in Ecuador, Colombia, and other places (Hamaker et al. 1992). According to Hufstader and Chris (2009), sacha inchi is a multipurpose climbing plant, planted once to harvest many times a year and lasting 15–20 years. The first year's seed yield is 0.7–1.0 tons per hectare, the second year's yield is 2.0–3.0 tons per hectare, and the third year's yield is stable at 3.0–4.0 tons per hectare. The garden has reasonable nutrition and irrigation, the grain seed can reach 4.0–5.0 tons per hectare for 10–15 years in a row. Guillén et al. 2003 showed that sacha inchi seeds have high oil content (35–60%); the oil composition includes unsaturated fatty acids, fiber, iodine, vitamins A, E, B1, B6, and antioxidants. Natural chemicals are good for health. The previous studies confirmed that value of pure sacha inchi oil with high levels of omega-3 (48%), omega-6 (33.5%), omega-9 (9%), and protein (27–33%). Old leaves are used as herbal teas,peeled seeds are roasted to be eaten directly or used as candy, e.g. chocolate-coated seeds, cold-pressed fresh seeds are used for salad dressings, mix baby food or process 3-6-9 capsules are used as as functional foods, skin creams, hair conditioners, and dry oils to make protein powders (Bondioli et al. 2006, Hamaker et al. 1992, Follegatti-Romero et al. 2009, Gutiérrez et al. 2011, Maurer et al. 2012).

In 2012, the first sacha inchi seeds were imported and planted at Vietnam National University of Agriculture (VNUA). After studying and evaluating the adaptability, agro-biological characteristics, and use value, the scientists here have selected the S18 line, which is recognized by the Ministry of Agriculture and Rural Development as a medicinal plant of Vietnam (Nguyen et al., 2018). Thus, sacha inchi is a completely new plant with very few starting seeds as well as an unknown name and seed origin. Pure line S18 was cloned from one of the best individuals, high harvested grain, and multiplied many times under many different conditions. In some provinces, people themselves buy seeds available on the market, which can create sacha inchi gardens that are different in terms of growth, adaptability, tolerance, yield, and quality. To make sacha inchi medicinal plants have a reasonable position in the cultivation system, help the plant bring high economic efficiency to famers as well as bring medicinal value and good nutritional value to human, it is necessary to study it on a large scale, collect more species and varieties, as well as collect natural and artificial variations to form a starting material garden to help researchers in pharmacology and nutrition. Nutritionists and breeders have an extensive gene bank to conduct industry-targeted research. In February 2020, the authors went to a number of sacha-inchi gardens in the Northern Mountainous region, the North Central region, and the Central Highlands area to investigate and assess the ecological adaptability and collect natural variations that contribute to the starting material of this imported cultivar.

MATERIALS AND METHODS

Sacha inchi genotypes were collected from some gardens in three ecological regions of Vietnam (the Northern Mountains region, the North Central region, and the Central Highlands area), and S18 (checking variety). To understand the morphological, mutant and yield characteristics of sacha inchi, field surveys, interviews with gardeners, data collection, and fruit collection from mutant individuals sacha inchi gardens were conducted (in the Hoa Binh and Tuyen Quang provinces belonging to the Northern Mountains region, in the Thanh Hoa province belonging to the North Central region, and in the Dak Lak province belonging to the Central Highlands area) (Fig. 1).

This study was conducted at experimental area of Plant Research and Development Institute (CRDI) of VNUA. The experimental design was randomized complete block (RCBD) with three replicates, a plot area of 30 m^2 , ten plants in each plot, a distance of $2.0 \times 1.5 \text{ m}$, and a density of 3,333 plants per hectare.

The soil was plowed, cleared of grass, raised in beds, holes were dug in the middle of the beds, as well as lined with 1.0 kg of microorganic fertilizer and 0.5 kg of NPK (3:10:2), mixed with loose soil in each hole. Soaked seeds germinate, are sown in potting soil, arranged in a nursery when seedlings are 35-40 days old andthen they can be planted. After 40-50 days of planting, stakes are buried, making a trellis for climbing plants. When the tree begins to flower, the first fertilizer is applied; then, weeding, and rooting are carried out. After collecting the first fruit, fertilizer is applied the second time, pruning is performed, and leaves are remove to improve ventilation. The amount of fertilizer applied the second time is: 5.0 kg of organic fertilizer + 0.2 kg of superphosphate for one plant.

Agro-biological characteristics, including the time from sowing to branching, male and female flowering, fruiting, and harvesting, the number of female flowers per inflorescence, the number of inflorescences per branch, and the number of seed zones per fruit, were measured in this study.

Disease and pest types were recorded and evaluated: the time of occurrence of the disease in the nursery (root rot disease, green wilt, yellow wilt), and in the planting garden (yellowing of leaves, browning of pods, fruit drop). Assessment of pests in plantations (stem borers, fruit borers, cavity worms, and red spiders) was conducted according to the National Regulation on methods of investigation and detection of plant pests (MARD of Vietnam-QCVN 01-38: 2010/BNNPTNT, 2010); The yield components including the number of fruits per plant; the number of seeds, seed weight per plant (kilogram), seed weight per 100 dry seeds (gram), actual harvested grain (ton per hectare)



Many female flowers/inflorescences



Lots of fruit/bunch



Two year old garden



Many bunches of flowers/trees

Fig. 1. Photos of flowers

were determined and oil quality traits including ratio of kernel per seed (%) and ratio of oil per seed (%) were measured in this study. Data analysis was done by using biological statistical software IRRI-STAT 5.0 and Microsoft Excel program.

RESULTS AND DISCUSSION

The morphological, mutant and yield characteristics of sacha inchi

In February 2020, a survey of a number of sacha inchi gardens were conducted in the provinces belonging to three different ecological regions: the Northern Mountains region (in the Hoa Binh province, and the Tuyen Quang province), the North Central region (in the Thanh Hoa province), and the Central Highlands area (in the Dak Lak province). The survey results show that most sacha inchi gardens are designed on low hillside land, with raised beds along contour lines. Some gardens are covered with black plastic, others are covered with garbage straws, grass, etc. Seeds are freely bought, unnamed, and sourced from Thailand and China. The age of trees in different gardens (ranging from 2 to 3 years), harvested 2 to 5 batches of fruit. The plant grows and develops well. The stem has a diameter of 3–7 cm, the height of the branches is 30–50 cm, and the leaves are heart-shaped, large, shallowly lobed, light green to dark green. The time from sowing to flowering varies from 105–115 days; from sowing to fruiting in the first batch, 245–260 days. The number of ripe fruits per plant in the first age ranges from 230–245 fruits, the number of seeds per fruit is 4–6 seeds. The percentage of fruits with four seeds was high (> 98%) in all plantations. The number of seeds per fruit was the highest in the Thanh Hoa province and the lowest in the Dak Lak province.

The yield of the first harvest varied from 0.90 (in the Dak lak province) to 1.33 tons per hectare (in Tuyen Quang province) (Table 1). There were no major differences in plant morphology between gardens. In each garden, some individuals were observed with variations in fruit bunches (the garden in the Tuyen Quang province had 4–5 fruits per cluster, the gardens in the Thanh Hoa province and the Dak Lak province had plants with 3–4 fruits per cluster), the number of seeds per fruit (in the Tuyen Quang, Hoa Binh and Thanh Hoa provinces there are 4–6 seeds per fruit, and the weight variation of 100 seeds is large (in the Tuyen Quang

province, a quantity of 100 seeds is 118 grams), variation in the number of inflorescences per stems is high (in the Dak Lak province). However, the morphological characteristics of these mutants were not significantly different from those of other individuals. From that, it can be initially assessed that the sacha inchi being grown in Vietnam may be different batches of seeds derived from the same variety grown in some Southeast Asian countries (Thailand, Laos, and Vietnam) and China.

Evaluation and selection of sachi inchi genotypes

Time of growth stages of the sacha inchi lines sown in Gia Lam district, Hanoi City, Vietnam, is shown in the Table 2. The seeds collected from gardens in localities were planted in the spring crop at the laboratory of the CRDI, sown in October to March 2020; then, assessment of morphological characteristics, growth and development, natural pests and diseases in nurseries and plantations was conducted, whereas yield and yield

Table 1. Growth and yield characteristics of sacha inchi gardens in some surveyed localities

No	Indiaatar		Lo	cal name	
INO.	Indicator	Hoa Binh	Tuyen Quang	Thanh Hoa	Đak lak
1	Origin of the Seed	Purchase in Thailand	S18 in Vietnam (selection and recognition of varieties)	Purchase in Thailand	Purchase in China
2	Planting period	June 2017	January 2018	February 2017	February 2018
3	Tree age (at the time of survey) (months)	31	24	35	23
4	Days from sowing to flowering	105	110	115	115
5	From sowing to the first nine litters (days)	245	245	249	260
6	No. of ripe fruits per tree in the first year	230	235	240	245
7	No. of seeds or fruits collected in the first batch	4-5	4-6	4-6	4-5
8	Weight 100 seeds (grams)	88	118	105	83
9	Individual productivity in the first year (kilogram per plant)	0.93	1.33	1.23	0.90
10	Comments on "growth characteristics and pests"	Healthy growth, many branches, many flowers, large fruits, very few pests.	Strong growth, many inflorescences, many female flowers/ inflorescences and 4-6 seeds/fruits, few pests.	Healthy growth, many inflorescences, many female flowers per inflorescence, and 4-6 seeds per fruit, with very few pests.	Healthy growth, many inflorescences, almost no pests and diseases.
11	Distinct trees appear (*)	The fruit has 4-6 seeds.	Mass of 100 large seeds (138grams), 4-5 female flowers per inflorescence, 4-6 seed segments	There are clusters of 3-4 fruits. Each fruit has 4-6 seeds.	There are many inflorescences and branches, and 3–4 fruits per inflorescence.

Note: Survey data in February 2020, from the records of the farmer households owning the garden; (*) Findings of the investigator

Ne	Collection lesstion		From sow to branch (days)		Sow to Male flowers (days)		Sow to Female flowers (days)		Fruiting until	From sowing to
INO.	Collection loc	ation	Level 1 of the branch	Level 2 of the branch	Flower Buds	Bloom	Flower Buds	Fruit set	fully ripe (days)	ripening (days)
1	The Northern	Tuyen Quang	60	85	110	121	116	122	123	245
2	Mountains region	Hoa Binh	62	85	110	119	115	121	124	245
3	The North Central Area	Thanh Hoa	58	80	108	118	113	120	124	244
4	The Central Highlands region	Dak Lak	63	90	115	125	118	125	125	250
5	S18 (Control variety)	Hanoi	66	95	118	128	123	130	125	255

Table 2. Growth stages of the sacha inchi lines collected and reviewed in Gia Lam district, Hanoi City, Vietnam (in 2020)

components were evaluated. The time from sowing to branching level 1, level 2 is 58–63 and 80– 90 days, respectively. The time from sowing to the appearance of male and female buds was 108–115 days and 113–118 days, respectively. From sowing to fruiting, the time is 120–125 days, from fruiting to ripening, it is 123–125 days, shorter than that reported by Niu et al. 2014, who stated that from fruit set to harvest is 145 days in Xishuangbanna. The time from sowing to ripening to harvesting the first fruit is 244–250 days (Table 2).

Situation of pests and diseases of sacha inchi

Disease occurrence in nurseries and plantations

In nurseries at different sources of collected materials, the occurrence of root-neck disease causing seedling death, a variation of 2.1–5.3% and green wilt disease, a variation of 3.1–4.8% was observed. In the planting garden, there were no records of any diseases that kill the plants. At the end of the cold spell of January–February 2021 and after the storm of July–August 2021, the phenomenon of yellowing and falling leaves was observed in all collected varieties (no trace of disease) at a rate of 1.5–3.1%. However, the disease

samples collected from the Thanh Hoa province and Tuyen Quang province are less severe. Some brown marks appear on the pods, which may be caused by harmful fungi that drop young fruit at a low rate of 0.2 (fruit melasma disease in the North Central region) to 1.6% (fruit drop disease in the Central Highlands region) (Table 3).

Pests monitoring results in planted gardens

At the nursery, there were no insects. At the plantation from March to June 2021, four species of harmful insects were recorded, including: fruit borer, stem borer, cavity worms, and red spiders. It was found that the frequency of occurrence of fruit borers was the highest, followed by cavity worms, red spiders, and stem borers (Table 4).

Fruit borers appear during the growth of sacha inchi, the most in May (over 50%) and the least in March (5–25%). The young caterpillars roll up the leaves to make a nest, then crawl out to gnaw on the leaves, reducing the photosynthetic area, affecting the growth and development of the plant. Then, they bore fruit into the young seeds, causing fruit rot, which reduces yield. Cavity worms (*Spodoptera litura*) appear less in March (5–25%) and gradually increase in April, May,

Table 3. Occurrence of diseases in nurseries and plantations in Gia Lam district, Hanoi city, Vietnam (2020–2021)

			*				
		Nursery (% of plants)		From planting to 18 months of age (%)			
No.	Locatio	n	Root-neck disease	Green Wilt Disease	Leaf Yellow Disease	Fruit Melasma Disease	Fruit Drop Disease
1	The Northern	Tuyen Quang	3.0	3.1	1.2	0.2	0.3
2	Mountains region	Hoa Binh	5.3	4.8	3.1	0.3	0.6
3	The North Central Area	Thanh Hoa	2.1	3.5	1.5	0.2	0.2
4	The Central Highlands region	Dak Lak	4.3	4.2	2.8	0.3	1.6
5	S18 (Control variety)	Hanoi	4.3	3.8	3.6	0.3	0.6

No	Post type	Domogod parts	Popularity by months				
110.	r esi type		3 4 5		5	6	
1	Fruit borer (Conogethes punctiferalis)	Fruit, leaves	+	++	+++	++	
2	Stem borer (Nadezhdiella cantori Hope)	Roots, stems and branches	-	+	-	-	
3	Cavity worms (Spodoptera litura)	Leaves	+	++	++	++	
4	Red spider (Tetranychus sp.)	Leaves (back side)	+	+	-	-	
Note:		Symbol - very + very ++ +++ very	Popularity very rarely appear very little popular popular very popular		Occurrence rate < 5% 5-25% 25-50% > 50%		

 Table 4. Composition of harmful worms and spiders and their prevalence by months in 2020–2021, in Gia Lam

 district, Hanoi City, Vietnam

and June (25–50%). A baby worm mainly damages young leaves, gnawing on leaf defects, reducing the photosynthetic area.

The stem borer appears very rarely (mainly in April); the young stem borer (at the end stem and branches) is located there and gnaws on the vessels, leading to the death of the tree. Although rare, this species is very dangerous because it causes tree death, so it is necessary to observe it regularly and cut off the damaged branches to kill pests. Red spiders appear in March–April and do not appear in May and June. Young and adult spiders sting the leaf tissue, creating tiny stings that initially appear pale white, then yellowish, and many spots aggregate. The leaves curl and die from being sucked out of the sap.

Evaluation results of the sacha inchi seed yield through different harvests

Sacha inchi flowered and produced results in two periods: late spring and early summer (March-May) and late summer-early autumn (July-September). The results of evaluation of yield and yield components of sacha inchi in the first fruit collection (September-October 2020) and the second stage of fruit collection (April-May 2021) are as follows: in three different ecological region, the number of fruits per plant in two seasons fluctuated from 303 to 335 fruits (in the Tuyen Quang, Hoa Binh, and Thanh Hoa provinces). The number of fruits per tree after the first two harvests was significantly higher than that of S18-control variety ($p \ge 99.5\%$). The number of seeds per fruit fluctuated from 4.1 to 4.6 seeds. The weight of 100 seeds in different harvested season and ecological regions fluctuates from 85-125 grams, reaching its highest in the

Tuyen Quang province – the Northern Mountainous region (in the second harvested batch), is 125 grams. The yield of the two harvests fluctuated from 2.66 to 3.07 tons per hectare, of which the highest was in the Tuyen Quang province (3.07 tons per hectare) and the Thanh Hoa province (3.01 tons per hectare) at the probability level statistically significant ($p \ge 99.5\%$) (Table 5).

Evaluation of some characteristics of the sacha inchi seeds and oil collected

The results in Table 6 show that the sacha inchi seeds collected in different ecological regions have a variable ratio of kernel per seed from 59.0 to 63.0%, and the ratio of oil per seed fluctuates between 47.0 and 48.0%, equivalent to the control variety (S18) for these two criteria, 61.8% and 47.6%, respectively.

CONCLUSIONS

The sacha inchi variety in the collected gardens has a time from sowing to flowering of 105– 115 days; in turn, time to the first harvested fruit is 245–260 days. Each year, the fruit ripens in two main periods: May–June and December–January.

In the nursery root rot caused 2.1–5.3% of deaths, and green wilt caused 3.1–4.8% of deaths. Yellowing leaves diseases, fruit melasma diseases, and drops fruit diseases also appeared, but causes very rarely damage. In the nursery there were almost no pests. In the garden, there were four main pests: fruit borer, stem borer, cavity worms and red spider. The damages caused by fruit borers and cavity worms are common (5 to over 50%) and more severe more than other types.

1	Location	Harvest	tedbatch	Number of fruits per plant	Number of seeds per fruit	mber of eds per fruit (gram) (gram) Individual productivity (kilogram per plant)		Theoretical yield (ton per hectare)	Actual productivity (ton per hectare))
1		Tuyen	1	110.2	4.5	115.0	0.57	1.91	1.12
	The	Quang	2	225.0	4.6	125.0	1.29	4.31	1.95
	Northern	То	otal	335.2	-	-	1.86	6.22	3.07
	Mountains	Ноа	1	98.8	4.5	90.0	0.40	1.33	1.01
2	region	Binh	2	208.5	4.4	88.0	0.81	2.69	1.87
		Total		307.3	-	-	1.21	4.02	2.88
	The North Central	he North Thanh entral Hoa	1	100.0	4.5	106.0	0.48	1.59	1.11
3			2	210.2	4.5	110.0	1.04	3.47	1.90
	Area	Total		310.2	-	-	1.52	5.06	3.01
	The Central	Central Data lat	1	98.5	4.1	86.0	0.41	1.37	0.69
4	Highlands region	Dak lak	2	205.0	4.2	85.0	0.73	2.44	1.97
		region		otal	303.5	-	-	1.14	3.81
		Llamai	1	90.5	4.2	86.0	0.33	1.09	0.71
5	S18 (Control)	18 Hanoi	2	207.5	4.2	84.0	0.73	2.44	1.92
		То	otal	298.0	-	-	1.06	3.53	2.63
	CV%			12.4					0.9
	LSD _{0.05}			7.9					0.28

 Table 5. Components of yield and yields of the sacha inchi seeds through two harvested batches (November 2020 and June 2021) in Gia Lam district, Hanoi City, Vietnam

Table 6. Effect of collected materials on the percentage of kernel and oil of sacha inchi seeds

STT	Location		Ratio of kernels per seeds (%)	Ratio of oil per seed (%)
1 The Nerthern Mountaine read		Tuyen Quang	62.5	48.0
2	The Northern Mountains region	Hoa Binh	60.2	47.3
3	The North Central Area	Thanh Hoa	63.0	47.8
4	The Central Highlands region	Đak lak	59.0	47.0
5	S18 (Control variety)	Hanoi	61.8	47.6

The actual yield of the first two harvested batches of sacha inchi materials collected from three ecological regions was high (2.66–3.07 tons per hectare), reached the highest grain of seeds collected in the Northern Mountainous region was 3.07 tons per hectare (in the Tuyen Quang province) and 3.01 tons per hectare in the North Central region (in the Thanh Hoa province).

The ratio of kernel per seed and the ratio of oil per seed had little variation between ecological regions and were similar to the control variety (S18). Selection of two sources of saha inchi materials from the North Central region: 2-year-old trees (in the Thanh Hoa province) and the Northern Mountainous area, and nearly 3-year-old trees (in the Tuyen Quang province) capable of growing healthy growth, less infection with pests and diseases, and high yield.

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