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*Article*

# Variation in nutritional components in roots from ahipa (*Pachyrhizus ahipa* (Wedd.) Parodi) accessions and an interspecific hybrid (*P. ahipa* × *P. tuberosus* (Lam.) Spreng.).

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**Abstract:** Among the many neglected underutilized species, tuberous Andean root crops like the ahipas (*Pachyrhizus ahipa*) constitute a promising alternative for increasing diversity in nutrient sources and food security at a regional level. In this study, we present the content of some functional compounds in tuberous roots from several ahipa accessions and the progenies of the interspecific hybrid X207 (*P. ahipa* × *P. tuberosus*). A significant objective was to determine protein and free amino acids in the roots to evaluate their food quality as protein supply. The interspecific hybrids have been found to possess the root quality to provide the crop with a higher dry matter content. The high dry matter content of the *P. tuberosus* Chuin materials is retained in the root quality of the hybrids. Food functional components like carbohydrates, organic acids, and proteins were determined in several ahipa accessions and a stable (non-segregating) progeny of the interspecific hybrid, X207. The X207 roots showed a significantly higher dry matter content and a lower content in soluble sugars, but no significant differences were found in starch content or organic acids compared to the ahipa accessions. About the root mineral contents, Fe and Mn concentrations in X207 were significantly raised compared to the average of ahipa accessions. Among the ahipa and the hybrid, no prominent differences in protein content or protein amino acids were found, being both partially defective in providing sufficient daily intake of some essential amino acids. Root weight, a central component of root yield, was significantly higher in X207, but thorough field studies are required to substantiate the hybrid's superior yield performance..

**Keywords:** leguminous root crop, high quality protein, dry matter yield.

## 1. Introduction

In the Andean region, several crops have been improved by local farmers for centuries. However, they may yet be considered neglected and underutilized species (NUS). At present, they are subjected to a gradual loss of genetic variability or even verging extinction because of their reduced demand and the competition of readily marketable crops [1]. Potentially, they may contribute to regional food security while providing a wide range of functional elements for healthy diets [2,3]. For a long time, these crops have been almost ignored, but more recently, new interest appeared for being highly nutritious and sources of functional compounds [4,5,6]. Furthermore, they constitute essential components of farm agrobiodiversity, playing a significant role by increasing food security and yield stability either by reducing pests impact [7] or providing nutrients to soils, e.g. N2-fixing legumes [8].

Among these NUS, the ahipa (*Pachyrhizus ahipa* [Wedd.] Parodi), is a legume with a tuberous root, that has been used from the time of the Incas and still cultivated locally in small areas in Southern Bolivia [9]. The species provides valuable starch for food or industrial applications [10–14]. The available ahipa landraces have shown competitive yield figures for Mediterranean irrigation agriculture compared to traditional starch sources, e.g. potato (*Solanum tuberosum* L.), and sugar-beet (*Beta vulgaris* L. subsp. *vulgaris* Cultivar group Altissima Group) [15]. The ahipa roots might also provide essential amino acids, vitamins, sugars, minerals, and antioxidants with a low content of anti-nutrients (phytic acid, oxalate, tannins) [15,16]. In a previous study, Forsyth and Shewry [10] did not find storage proteins in ahipa roots but proteins related to tuber metabolism and growth. Recently, root proteins have been characterized, and their possible food applications have been suggested [17].

Attempts to increase the crop dry matter yield have led to interspecific hybridization experiments involving the lowland South American species *P. tuberosus* (Lam.) Spreng., a related species complex where holding higher dry matter contents in their roots [18]. It should be stressed that the Chuin materials are the only cultivar group within the *P. tuberosus* complex known to possess this trait [19]. Breeding could improve crop competitiveness even further by delivering cultivars with desirable agronomic traits, e.g. reduced flowering, shorter cycles, and mono-tuberous roots. At present, ahipa may be an attractive food to complement other traditional sources, which might be defective in some functional elements. In this report, we have looked at the content of energy sources (starch, sugars, and organic acids) and have extended the analysis of structural compounds like protein amino acids.

## 2. Materials and Methods

*Plant material*

Ahipa genotypes evaluated in this work were six different accessions of *P. ahipa* (AC216, AC229, AC521, AC524, AC525, AC526) and an F7 progeny from a hybrid between *P. ahipa* AC524 and *P. tuberosus* TC361 Chuin genotype designated as X207. Hybrid seeds (F1), supplied by M. Sørensen, were multiplied annually for seven years and continuously selected for root size and dry matter content; i.e. during the seven years of cultivation, the progenies were segregated according to leaf and root morphology, shoot/root ratio, and root dry matter content. In this study, only F6 seeds of one line of the X207 hybrid, which remained stable regarding the desired traits (root size, high dry matter content), were used. The seeds were planted in 25-liter pots filled with potting mix and irrigated with drip-lines from April to October 2019. A peat-based rhizobial inoculant (PAC51) was used for coating seeds before planting [20]. Three replicates for each accession and hybrid progeny were grown outdoors at the Jardin Arvense (University of Seville). A slow-release fertilizer (5 g per pot, of 16-7-15 (2MgO), Floramid Permanent, Compo) was provided during the growth cycle. Traditional flower pruning recommended for increasing root yield [21 was not performed. Root harvest took place 210 days after sowing. Roots and shoots were separated and weighed; then after washing and peeling, the roots were diced for dry matter determination by drying in an oven at 65$^0$C for 48 hours. Root samples of equal size were frozen and lyophilized for later determination of minerals and organic compounds.

*Minerals*

The concentration of N in the samples was determined after Kjeldahl digestion in a Technicon Autoanalyzer. The remaining macro and micronutrients content was analysed after acid digestion with HNO3 by ICP (Varian ICP 720-ES).

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*Starch analyses*

Starch was measured following sample dilution and hydrolysis recommended in the R-Biopharm Starch kit (Boehringer Mannheim). Ground samples were dissolved with DMSO and 8 M HCl by incubation at 60ºC for one h, cooled quickly, and adjusted to pH 4–5. Starch hydrolysis was performed with amyloglucosidase to D-glucose. D-glucose was then determined by NADPH formation after incubation with hexokinase and glucose-6-phosphate-dehydrogenase. Starch concentration was then corrected by subtracting the initial content of soluble sugars in samples as recommended by the kit supplier, which substantially reduced the final starch concentration. The apparent content of amylose in starch from different accessions was determined according to Washington et al. [22].

*Sugars*

Sugars were measured in samples after extraction with hot water (90°C, one h). Enzymatic kits from R-Biopharm (Boehringer Mannheim) for sucrose, D-glucose, and D-fructose were used.

*Organic acids*

Malate and citrate were extracted from lyophilized samples in water and determined using enzymatic kits (L-malic and citric acid, R-Biopharm). Ascorbate content was analyzed in fresh or frozen samples. Ascorbate was assayed with a similar kit (L-ascorbic acid, R-Biopharm), but concentration was significantly reduced after freezing and thawing in comparison with fresh samples, and therefore recorded values may only be considered indicative.

*Protein hydrolysis and amino acid analysis*

For a complete analysis of root protein amino acids and free amino acids, only one accession of ahipa (AC521) and the X207 hybrid were used. Peeled roots were stored deep-frozen (-70ºC) before drying in a vacuum freeze dryer. Freeze-dried samples were dissolved in 6.0 M HCl with D, L-$\alpha$ aminobutyric acid as internal standard. The samples in HCl acid were gassed with nitrogen and sealed in hydrolysis tubes under nitrogen, then incubated in an oven at 110ºC for 24 h. Derivatisation and chromatography of amino acids were performed as in Alaiz et al. [23]. Dried samples of protein hydrolysates were dissolved in 1 M sodium borate buffer (pH 9) and derivatized with diethyl etoxymethylenemalonate. Separation was performed in a reversed-phase column using sodium acetate and acetonitrile as eluents [23,24]. Tryptophan was measured separately by HPLC after alkaline hydrolysis of samples [25].

*Protein determinations*

Protein contents in the samples were estimated as the concentration of amino acids after the protein hydrolysis (in g amino acids 100 g dried sample-1) minus the concentration of free amino acids. In addition, estimation of root protein contents from Kjeldahl N concentration was also calculated using the conversion factor 5.1 reported by Dini et al. [17].

*Statistical analysis*

From each determination, data were analyzed using a statistical package (Statistica) to perform ANOVAs in a completely randomized model and further post-hoc comparisons between genotypes. Data means and their corresponding standard deviation are presented in Tables 1−5, 7 and 8. When F values were not statistically significant among ahipa accessions, they were pooled as ahipa replicates to compare with the hybrid.

## 3. Results

*Variation in root morphology, dry matter content, and starch yield*

Tuberous roots showed a significant variation in morphology among accessions (Fig. 1), from a single tuber (mono-tuberous root) to a divided root system (multi-tuberous root) with few thickened secondary roots. The X207 hybrid plants showed larger-sized tuberous roots from all ahipa accessions, except for ahipa AC229 (Fig. 1, Table 1). Root dry matter content in ahipa accessions ranged from 16.7 to 20.1 % (Table 1), while it reached up to 25.9 % in the hybrid progeny. Meanwhile, root starch content varied from 28.3 to 35.5%, with no significant differences between genotypes. The amylose content in root starches ranged from 9.6 to 13.5 (Table 1), but significant differences were found between ahipas (mean 10.8%) and the X207 hybrid (13.5%).

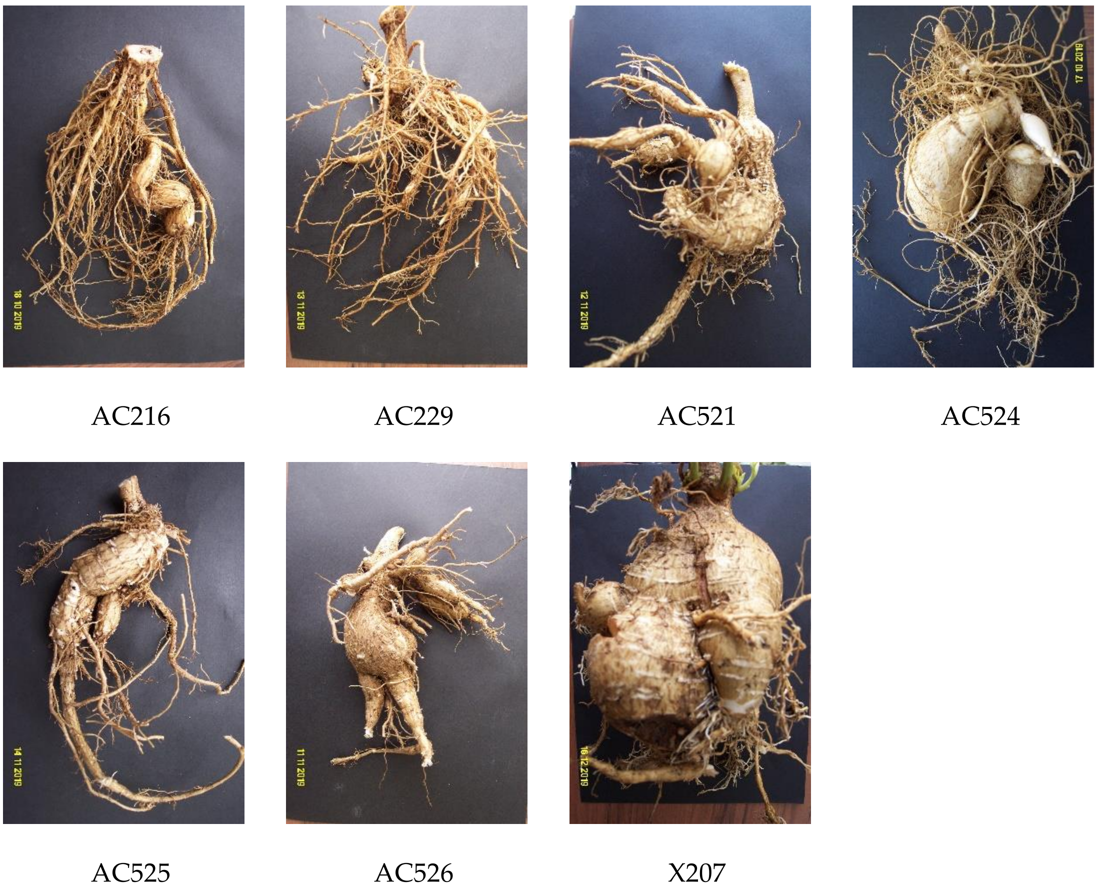


**Figure 1.** Root morphology in representative samples of the ahipa accessions and the interspecific hybrid X207.

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**Table 1.** Root weight (g plant$^{-1}$) and root dry matter, starch and amylose content (in % dry weight) in six different ahipa accessions and the X207 *P. ahipa* × *P. tuberosus* hybrid. Mean ± standard deviation. Below, significance of Snedecor's F for the genotypic source of variation.

| Genotype | Root weight | Dry matter | Starch | Amylose |
|---|---|---|---|---|
| AC216 | 121.7 ± 67.4 | 20.1 ± 2.9 | 30.8 ± 11.4 | 10.7 ± 1.1 |
| AC229 | 416.6 ± 88.8 | 18.4 ± 1.8 | 29.6 ± 2.2 | 11.4 ± 1.1 |
| AC521 | 287.9 ± 23.2 | 17.7 ± 0.8 | 32.0 ± 1.9 | 10.5 ± 0.9 |
| AC524 | 189.2 ± 38.8 | 19.1 ± 1.4 | 35.5 ± 6.1 | 10.8 ± 1.4 |
| AC525 | 327.6 ± 107.1 | 16.7 ± 2.1 | 32.6 ± 6.9 | 9.6 ± 1.3 |
| AC526 | 85.1 ± 40.2 | 19.9 ± 2.3 | 30.5 ±1.2 | 11.8 ± 1.8 |
| X207 | 429.4 ± 184.5 | 25.9 ± 1.8 | 28.3 ± 4.7 | 13.5 ± 2.2 |
| ANOVA | **Root weight** | **Dry matter** | **Starch** | **Amylose** |
| Genotype | P<0.05 | P<0.01 | ns | P<0.01 |

ns, not significant.

*Sugars*

The root concentration of soluble sugars (sucrose and glucose) showed significant differences between the ahipas and the hybrid (Table 2), with the latter presenting a lower sugar concentration. No fructose was detected in the samples.

**Table 2.** Sucrose and glucose contents in roots of six different accessions and the *P. ahipa* × *P. tuberosus* hybrid X207 (in % dry weight). Mean ± standard deviation. Significance of Snedecor's F for the genotypic source of variation.

| Accession | Sucrose | Glucose |
|---|---|---|
| AC216-145 | 7.08 ± 0.27 | 2.33 ± 0.13 |
| AC229-150 | 9.34 ± 1.30 | 3.50 ± 0.47 |
| AC521 | 10.72 ± 0.84 | 4.27 ± 0.33 |
| AC524 | 8.54 ± 0.89 | 3.46 ± 0.37 |
| AC525-171 | 8.82 ± 0.49 | 2.11 ± 0.13 |
| AC526 | 10.21 ± 0.60 | 3.94 ± 0.25 |
| X207 | 6.22 ± 0.52 | 1.55 ± 0.12 |
| ANOVA | **Sucrose** | **Glucose** |
| Genotype | P<0.001 | P<0.01 |

*Organic acids*

No significant differences in malate, citrate and ascorbate contents were found between ahipa accessions and X207 (Table 3).

**Table 3.** Malate and citrate contents in roots of six different accessions and the X207 hybrid (*P. ahipa* × *P. tuberosus*). Means in % dry weight ± standard deviation. Significance of Snedecor's F for the genotypic source of variation.

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| Accession | Malate | Citrate |
|---|---|---|
| AC216-145 | 0.17 ± 0.03 | 0.16 ± 0.03 |
| AC229-150 | 0.31 ± 0.07 | 0.14 ± 0.04 |
| AC521 | 0.20 ± 0.04 | 0.15 ± 0.01 |
| AC524 | 0.26 ± 0.07 | 0.39 ± 0.04 |
| AC525-171 | 0.37 ± 0.06 | 0.14 ± 0.03 |
| AC526 | 0.19 ± 0.03 | 0.08 ± 0.02 |
| X207 | 0.29 ± 0.05 | 0.22 ± 0.04 |
| ANOVA | **Malate** | **Citrate** |
| Genotype | ns | ns |

ns, not significant.

*Protein and amino acids*

Total root protein concentration of the ahipa and the hybrid was somewhat similar when converting Keldahl N into protein content using Dini's factor or calculated from the sum of total amino acids after acidic protein digestion (Tables 4 and 5). A similar conclusion may be drawn from the assessment of roots protein quality (Table 6), where both AC521 (the only ahipa accession analyzed) and the interspecific hybrid X207 were defective in sulfur amino acids (cysteine and methionine) and tryptophan. However, they provided more than half of the nutritional requirements of several essential amino acids like leucine, lysine, threonine, and aromatic amino acids (phenylalanine and tyrosine). Furthermore, the analysis of free amino acids in the root flesh showed asparagine as the predominant amino compound in both the ahipa and the hybrid (Table 7).

**Table 4.** Root protein content in accession AC521 and the hybrid X207 (*P. ahipa* × *P. tuberosus*). Lipids and fibers determined only in ahipa AC521. Mean ± standard deviation. Values in g · 100 g dry matter$^{-1}$.

| Genotype | Proteins[1] | Proteins[2] | Lipids | Fibers |
|---|---|---|---|---|
| AC521 | 4.15 ± 0.04 | 4.87 ± 1.07 | 0.54 ± 0.02 | 7.91 ± 0.24 |
| X207 | 4.36 ± 0.09 | 6.24 ± 1.77 | nd | nd |

[1], protein concentration calculated as the total amount of amino acids determined after protein digestion minus the free amino acids determined in similar samples. [2], protein concentration calculated using the N to protein conversion factor of 5.1 [17]. nd, not determined.

**Table 5.** Protein amino acids in roots of ahipa AC521 and the interspecific hybrid X207. Means in g · 100 g dry weight$^{-1}$ ± standard deviation.

| Amino Acid | AC521 | X207 |
|---|---|---|
| Asp + Asn | 2.23 ± 0.017 | 3.29 ± 0.007 |
| Glu + Gln | 0.37 ± 0.002 | 0.30 ± 0.016 |

| | | |
|---|---|---|
| Ser | 0.19 ± 0.011 | 0.15 ± 0.000 |
| His | 0.12 ± 0.002 | 0.14 ± 0.002 |
| Gly | 0.12 ± 0.003 | 0.10 ± 0.001 |
| Thr | 0.12 ± 0.003 | 0.12 ± 0.004 |
| Arg | 0.23 ± 0.005 | 0.44 ± 0.006 |
| Ala | 0.16 ± 0.003 | 0.12 ± 0.004 |
| Pro | 0.56 ± 0.036 | 0.39 ± 0.033 |
| Tyr | 0.08 ± 0.003 | 0.09 ± 0.002 |
| Val | 0.20 ± 0.009 | 0.24 ± 0.058 |
| Met | 0.01 ± 0.002 | 0.00 ± 0.000 |
| Cys | 0.02 ± 0.001 | 0.02 ± 0.003 |
| Ile | 0.15 ± 0.003 | 0.13 ± 0.001 |
| Trp | 0.01 ± 0.002 | 0.01 ± 0.001 |
| Leu | 0.19 ± 0.002 | 0.14 ± 0.003 |
| Phe | 0.13 ± 0.001 | 0.10 ± 0.001 |
| Lys | 0.19 ± 0.005 | 0.18 ± 0.003 |

**Table 6.** Quality assessment of root proteins based on their essential amino acid scoring pattern from ahipa AC521 and interspecific hybrid *P. ahipa* × *P. tuberosus* X207.

| Amino acid | pattern[1] | AC521 | X207 |
|---|---|---|---|
| His | 27 | 110 | 122 |
| Ile | 35 | 102 | 88 |
| Leu | 75 | 62 | 44 |
| Lys | 73 | 62 | 57 |
| SAA[2] | 35 | 23 | 17 |
| AAA[3] | 73 | 70 | 64 |
| Thr | 42 | 66 | 67 |
| Trp | 12 | 19 | 16 |
| Val | 49 | 101 | 114 |

[1]Tissue amino acid pattern based on amino acid composition of whole-body protein (in mg · g protein$^{-1}$). Source: Milward [26].

[2]SAA, sulphur amino acids (met+cys); [3]AAA, aromatic amino acids (Phe+Tyr)

**Table 7.** Free amino acids in roots of ahipa AC521 and the interspecific *P. ahipa* × *P. tuberosus* hybrid X207. Means in g · 100 g dry weight$^{-1}$ ± standard deviation.

| Amino acid | AC521 | X207 |
|---|---|---|
| Asp | 0.099 ± 0.004 | 0.100 ± 0.001 |
| Glu | 0.066 ± 0.002 | 0.065 ± 0.000 |
| Asn | 0.616 ± 0.018 | 1.097 ± 0.011 |
| Ser | 0.035 ± 0.000 | 0.030 ± 0.001 |
| Gln | 0.005 ± 0.000 | 0.000 ± 0.000 |
| His | 0.017 ± 0.001 | 0.023 ± 0.001 |

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| | | |
|---|---|---|
| Gly | 0.010 ± 0.000 | 0.009 ± 0.000 |
| Thr | 0.010 ± 0.001 | 0.031 ± 0.000 |
| Arg | 0.040 ± 0.001 | 0.134 ± 0.001 |
| Ala | 0.016 ± 0.000 | 0.006 ± 0.000 |
| Pro | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Tyr | 0.007 ± 0.002 | 0.015 ± 0.000 |
| Val | 0.031 ± 0.001 | 0.044 ± 0.005 |
| Met | 0.001 ± 0.002 | 0.000 ± 0.000 |
| Cys | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Ile | 0.019 ± 0.001 | 0.024 ± 0.000 |
| Trp | 0.008 ± 0.001 | 0.000 ± 0.000 |
| Leu | 0.007 ± 0.000 | 0.016 ± 0.000 |
| Phe | 0.013 ± 0.001 | 0.010 ± 0.000 |
| Lys | 0.006 ± 0.000 | 0.018 ± 0.000 |

*Minerals*

From all the mineral elements determined in roots (Table 8), statistically, significant differences only were found in Fe and Mn concentration between the ahipa accessions and the X207 hybrid, which showed a higher concentration in both nutrients.

**Table 8.** Comparison of the concentration of minerals in roots from six ahipa accessions and the interspecific hybrid X207. Mean ± standard error.

| Genotype | K | Ca | Mg | P | S |
|---|---|---|---|---|---|
| | | | g · 100 g dry matter$^{-1}$ | | |
| Ahipas | 0.82 ± 0.06 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.16 ± 0.01 | 0.12 ± 0.02 |
| X207 | 0.79 ± 0.11 | 0.13 ± 0.03 | 0.08 ± 0.01 | 0.19 ± 0.02 | 0.15 ± 0.03 |

| Genotype | Fe | Mn | Cu | Zn | B |
|---|---|---|---|---|---|
| | | | mg · kg dry matter$^{-1}$ | | |
| Ahipas | 24.9 ± 2.1 | 2.3 ± 0.9 | 1.1 ± 0.3 | 8.4 ± 1.2 | 4.8 ± 0.5 |
| X207 | 35.7 ± 3.6 | 6.7 ± 1.5 | 0.9 ± 0.6 | 10.3 ± 2.0 | 6.0 ± 0.8 |
| | **Co** | **Mo** | **Ni** | **V** | **Na** |
| Ahipas | 0.43 ± 0.1 | 1.8 ± 0.4 | 1.1 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.0 |
| X207 | 0.47 ± 0.1 | 2.5 ± 0.8 | 1.1 ± 0.2 | 0.2 ± 0.1 | 0.2 ± 0.1 |

## 4. Discussion

The interspecific hybrid showed higher root weight and a superior dry matter content compared to the different ahipa accessions. Interestingly, its sugar content was lower than in the ahipa accessions, but starch accumulation did not show significant differences between genotypes. Protein contents were relatively similar between ahipa and X207, as it was the composition of protein amino acids, defective in both cases in essential sulfur amino acids. However, roots from X207 showed a significantly higher accumulation of Fe and Mn, whereas the content of other minerals was similar.

The hybridization of ahipa with a Chuin genotype of *P. tuberosus* proved to be a successful way of increasing root dry matter content in the ahipa species, as previously reported [18]. Although root dry matter content in the hybrid was significantly higher than in the evaluated ahipa accessions, other reports found significantly greater dry matter values in progenies of interspecific crossings [27]. In X207, root dry matter content was somewhat similar to the dry matter content of Chuin genotypes reported by Grüneberg et al. [28]. Interestingly, some ahipa accessions and the hybrid produced a significant high root yield despite not performing flower pruning. Starch contents in *P. ahipa* and X207 were higher than reported values of 9.1% for the cultivated *Pachyrhizus* relative, *P. erosus* (L.) Urb., or Mexican yam bean [29], species widely distributed and cultivated mainly in Central America and Southeast Asia [9]. The amylose content in X207 was higher than the amylose content in ahipas, but still, it was significantly lower than in jicama (*P. erosus*) (approx. 24%) [30] or other root crops like cassava (*Manihot esculenta* Crantz) [31]. Thus, amylopectin, the main component of the stored starch in ahipas and X207, may provide interesting applications from the food to plastic industry [13,32].

Root sugars, which provide the characteristic sweet flavor of ahipa, were at a lower concentration in X207 (Table 2). The most popular use of ahipa roots is either as a fruit [33] or as fresh juice in urban markets consumed as a folk medicine [34]. Roots may supply from 8.8 ± 2.6 (X207) to 10.6 ± 2.1 (mean among ahipa accessions) mg of ascorbate in 100 g fresh weight, a concentration in the range provided by yambean or potato [29,35]. Malate and citrate contents (Table 3) reached values similar to other root or tuber crops [3,36].

Protein contents in the *Pachyrhizus* roots (Table 4) were significantly higher than in other roots crops used for human consumption as dietary energy sources [37]. The protein content in roots was not remarkably high. However, it was among values found in other root and tuber crops cultivated in the Andean region like potato, racacha (*Arracacia xanthorrhiza* Bancr), yacón (*Smallanthus sonchifolius* (Poepp.) H.Rob.), cassava, or achira (*Canna indica* L.) [2,4,10,36]. For a human diet, the supply and composition of essential amino acids are deficient in sulphuric amino acids and tryptophan (Tables 4 and 5). Hence, the necessary essential amino acids may be acquired from other plant or animal sources [38]. In both ahipa and the X207 hybrid, the primary amino acid found in proteins was aspartate (Table 4), as it was also reported in proteins isolated from market-purchased ahipa roots [17]. The relatively high concentration of free amino acids provided by fresh roots (Table 6), where the amide asparagine was predominant, followed by the amino acids glutamate and arginine (Table 7), should not be despised for their nutritional, functional values. The role of non-essential amino acids in humans is a matter of interest for improving health like arginine as immuno-stimulant [39] or asparagine and its role in avoiding apoptosis when cellular glutamine-deficiency is induced by human tumors [40].

Mineral contents in roots are good sources for macro and micronutrients (Table 8) comparable to potato or other Andean root and tuber crops [3,36].

From an agronomic perspective, the interspecific hybrid X207 and a few of the ahipa accessions assessed in this study may indeed provide economic root yields if cultivated extensively without requiring the labour intensive field operation of flower pruning [41]. In addition, the dry matter yield obtained from X207 roots is similar to that of potatos, and it may approach the DM content of other root and tuber crops, cassava and sweet potato (*Ipomoea batatas* (L.) Lam.) [42] after selection and appropriate management [15].

The tuberous roots of this genus provide a valuable food source to compensate for nutritional imbalances in the diet in different regions of the world [43,44]. They are also an alternative source of fresh products for the development of new food products, e.g. gluten-free bread, cookies and food additives [12,14] or even industrial uses like, e.g. biodegradable films [13,32].

**Supplementary Materials:** N/A

**Author Contributions:** Conceptualization, E.O.L-M.; design of the study and supervision. E.O.L.-M. and S.R.-O.; a collection of materials and maintenance of the field experiment Y.E.-L. and E.O.L.-M.; data acquisition Y.E.-L. and E.O.L.-M.; data curation and statistical analysis E.O.L-M. and S.R.-O.; interpretation of results and drafting the first manuscript E.O.L-M., S.R.-O. and M.S.; Writing, review and final editing E.O.L-M. and M.S.; Funding acquisition E.O.L-M. and M.S. All authors have read and agreed to the published version of the manuscript.

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