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## Morphoagronomic characteristics display high genetic diversity in Murupi chili pepper landraces

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### ABSTRACT

Murupi chili pepper is a hot Amazonian pepper that has been used as flavoring in the region, yet its diversity is unknown. This paper aims to assess the diversity in 20 landraces from Peruvian, Colombian, and Brazilian Amazon. The experiment was installed in Manaus-AM, using a randomized complete block design with three replicates and five plants per plot. Analyses of variance showed significant differences for all nine descriptors. Fruit yield showed, in average, to be 208.08 fruits per plant, tantamount to 381.44 g per plant. Given 50% of relative distances estimated by generalized Mahalanobis distances and, nearest neighbor clustering, they displayed six groups. Cluster I comprised 50% of the landraces. Clusters IV, V and VI were represented by '8' (Putumayo, Colombia), '27' (Yurimaguas, Peru) and '24' (Jutaí-AM, Brazil), respectively. The highest yielding landrace showed to be '17' (Manicoré-AM, Brazil), bearing 685.5 fruits and 1.92 kg per plant. Our findings suggest Murupi pepper may possibly be improved by selection and/or hybridization.

**Keywords:** *Capsicum chinense*, Amazon pepper, hot pepper, Amazon vegetable, pre-breeding.

### RESUMO

#### Caracteres morfoagronômicos revelam elevada diversidade genética das variedades crioulas de pimenta Murupi

A pimenta Murupi é muito utilizada na região Amazônica como condimento, mas ainda se desconhece sua diversidade. O objetivo deste trabalho foi avaliar 20 acessos procedentes da Amazônia peruana, colombiana e brasileira. Para tanto foi conduzido um experimento em Manaus-AM, utilizando o delineamento de blocos completos ao acaso com três repetições e cinco plantas por parcela. As análises da variância mostraram que existe diferença significativa para todos os nove descritores. A produtividade média de frutos foi 208,08 frutos por planta o que equivale a 381,44 g por planta. Considerando 50% das distâncias relativas, obtidas a partir das distâncias generalizadas de Mahalanobis e o método de agrupamento do vizinho mais próximo, foram encontrados seis grupos. O grupo I foi composto por 50% dos acessos e os grupos IV, V e VI foram compostos por '8' (Putumayo, Colombia), '27' (Yurimaguas, Peru) e '24' (Jutaí-AM, Brasil), respectivamente. A maior produção de frutos foi do acesso '17' (Manicoré-AM, Brasil) com 685,50 frutos por planta e 1,92 kg por planta. Estes resultados indicam que a pimenta Murupi pode ser melhorada via seleção ou fazendo cruzamentos.

**Palavras-chave:** *Capsicum chinense*, pimenta amazônica, pimenta, hortaliça amazônica, pre-melhoramento.

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Murupi chili pepper (*Capsicum chinense*) (Photo 1) is greatly appreciated in the Amazonian region, because it exhibits a special, low pungency and sweetness combination. It grows up to 1.50 m high, containing a high content of terpenes, esters, and sesquiterpenes in volatile compounds (Bogusz Junior *et al.*, 2012, 2015). They also display a wide diversity of shape, size, and coloring in the fruits. It may be called the most Brazilian pepper when compared to other *Capsicum* genera (Silva Filho *et al.*, 2013), because it is well adapted to several Brazilian soils

and climates (Lannes *et al.*, 2007).

Family farmers are currently cultivating *Capsicum* landraces in the Amazon, yet Amazonian peppers have been undergoing genetic drift on account of being replaced by other crops or desertion of field activities.

Diversity studies can help to understand the genetic diversity level, identify useful genes and, at the same time assist on selecting genitors (Cruz & Regazzi, 2001). Multivariate analyses, such as distances and cluster analyses are frequently used to assess the diversity of quantitative and qualitative

characters. These approaches were useful to characterize various species, such as snap bean (Peixoto *et al.*, 2002); melon (Rizzo & Braz, 2000); wheat (Bertan *et al.*, 2006), pepper (Sudré *et al.*, 2005; Toquica *et al.*, 2003; Zewdie *et al.*, 2004); oat (Kurek *et al.*, 2002) and maize (Coimbra *et al.*, 2010).

In general, there are few studies addressing Amazon peppers. Antonious *et al.* (2009) have reported a wide diversity among 63 *C. chinense* accessions from different countries, some of them from Brazil. Other researchers have detected significant

diversity in 20 non-pungent Amazon landraces (*C. chinense*) (Batista *et al.*, 2013). Diversity was detected on some Murupi chili pepper landraces from the upper Rio Negro (Fonseca *et al.*, 2008). However, there are many landraces to be collected, and have their diversity assessed in the Amazon basin.

Our team has performed collections of various pepper types from the Amazon region for several years. The germplasm has been stored at the National Research Institute of Amazonia (INPA). This paper assesses the genetic diversity of 20 Murupi chili pepper landraces, most from the Amazonian region in Brazil, and some from Peru and Colombia.

## MATERIAL AND METHODS

Twenty Murupi chili pepper landraces from the INPA's germplasm bank (Manaus, Brazil), were assessed to determinate their diversity level. They originated from 20 different sites in the Amazon region (Figure 1).

The experiment was conducted at INPA's 'Alejo von der Pahlen' experimental station (2°59'48''S, 60°1'22''W, altitude 60 m). The Manaus climate is characterized as 'Af' type, according to Köppen climate ranking. In 2013, rainfall was equivalent to 2,732 mm, with 63 mm and 408 mm in the months with the lightest (October) and the heaviest rainfall (March), respectively. The low rainfall season extended from August (93 mm) to October (63 mm). The mean annual temperature was 26.8°C, ranging from 20.0 to 38.2°C with mean daily relative humidity of 88.7% (Scarazati, 2016).

The seedlings were started in a greenhouse using plastic cups filled with autoclaved organic compost (120°C during 2 h). Three seeds were sown in every cup and, when the plants had developed two true leaves they were rouged so as to attain the strongest seedling per cup. Next, 15 cm high seedlings were transplanted in the field at 1.5 m between rows and 1.0 m apart within rows, following a randomized complete block design with three replicates and five plants per plot. The

soil analysis results showed pH in water = 5.4; organic material = 20.8 g kg<sup>-1</sup>; C= 12.91 g kg<sup>-1</sup>; N= 0,64 g kg<sup>-1</sup>; P= 100.91 mg kg<sup>-1</sup>; K= 10.01 mg kg<sup>-1</sup>; Ca= 1.36 cmolc kg<sup>-1</sup>; Mg= 35.1 mg kg<sup>-1</sup>; Fe= 7.4 mg kg<sup>-1</sup>; Mn= 6.3 mg kg<sup>-1</sup>; Zn= 5.2 mg kg<sup>-1</sup>.

Each hole was 20 cm wide and deep. Each hole was fertilized with 2 kg of organic compost, 50 g of triple superphosphate, 40 g of potassium chlorate and 10 g of urea, as well. After transplanting to the open field, complementary fertilization with 10 g of urea per plant and weedings were performed every fortnight, until the start of fructification stage. In this experiment, no pesticide was used. The harvesting point was determined when the fruits displayed yellowish coloring.

The assessments were performed following the descriptors recommended by the International Plant Genetic Resources Institute (IPGRI, 1995). Nine quantitative descriptors, such as stem diameter (SD) 1.0 cm above ground, plant height (PH) after three months of transplanting, canopy diameter (CD), fruit length (FL), fruit diameter (FD), fruit pericarp thickness (FPT), fruit number per plant (FNP), mean fruit mass (MFM) and fruit mass per plant (FMP), were measured. Fruit data, such as FL, FD, FPT were obtained from the 10 first fruits per plant.

The analyses of variance and Skott-Knott tests were performed at 5% of probability. nearest neighbor method and generalized distances of Mahalanobis (D<sup>2</sup>) were used to calculate clustering and distances between landraces, respectively. All analyses were performed in GENES Software (Cruz, 2008).

## RESULTS AND DISCUSSION

Analyses of variance detected significant differences for all descriptors showing wide genetic diversity within Murupi chili pepper landraces (Table 1). Mean fruit mass (MFM) exhibited the highest coefficient of variation (18%), indicating a high experimental quality. Therefore, quality data and

interpretations are reliable.

The descriptors are associated with vegetative development indicators such as plant height (PH), stalk diameter (SD) and canopy diameter (CD), ranged from 48.06 to 100.12 cm; 1.80 to 3.36 cm and 57.8 to 111.65 cm, respectively. Plant height ranged from 55.08 to 100.12 cm, which is desirable for manual harvesting or landscaping (Neitzke *et al.*, 2010). High SD and CD may lead to increased fruit yield, like landrace '17', which showed the highest fruit yield. However, this is not always true because landrace '6' had high stem height and diameter, but poor fruit mass per plant (116 g).

On the other hand, fruit descriptors such as fruit length (FL), diameter (FD) and pericarp thickness (FPT) had the following range: 1.40 to 6.23 cm, 5.46 to 14.38 mm and 0.57 to 1.66 mm, respectively. In another type of non-pungent pepper (*C. chinense*) called 'Pimenta de cheiro' Domenico *et al.* (2012) found ranges for FL (2.1 to 7.7 cm) and FD (11 to 25 mm) showing that Murupi chili pepper tends to be smaller-sized than 'Pimenta de cheiro'. In another research with 330 accessions of *C. chinense*, Jarret & Berke (2008) found high amplitude for FL (0.8 to 11.0 cm) and FD (6 to 40 mm), since these accessions were likely to have included many pepper types. In non-pungent Amazonian peppers (*Capsicum* spp.), Batista *et al.* (2013) found amplitude from 2.04 to 6.02 cm, 7.7 to 38.9 mm and 0.77 to 2.30 mm for FL, FD and FPT respectively. Therefore, in general, our findings showed low amplitude for FL, FD and FPT. It suggests that probably Murupi pepper is one of the smallest peppers of *Capsicum chinense* species.

On the other hand, the descriptors related to fruit yield, such as fruit number per plant (FNP), fruit mean mass (FMM) and fruit mass per plant (FMP) exhibited amplitudes from 53,0 to 685.5; 0.41 to 2.96 g and 67.96 to 1919.4 g, respectively. It is interesting to note landrace '17' displayed the highest mean values for these three descriptors. This landrace was from the municipality of Manicoré (5°48'S, 61°17'W), which has a climate similar to that of Manaus (2°59'48''S, 60°1'22.4''W). In general,

**Table 1.** Descriptors means of Murupi pepper landraces (*Capsicum chinense*) collected from the Amazon basin. Manaus, INPA, 2013.

Landraces	Plant height (cm)	Stalk diameter (cm)	Canopy diameter (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit pericarp thickness (mm)	Fruit mean mass (g)	Fruit number per plant	Fruit mass per plant (g)
3	57.54k	2.44e	68.92l	4.13e	9.63l	1.38g	1.64c	318.25c	525.84e
4	68.22g	2.82c	73.06k	3.00f	9.69l	1.40f	1.48c	136.00m	194.74j
5	89.32c	3.16b	63.12p	1.40h	5.46p	0.57n	0.41d	87.50q	67.96n
6	100.12a	3.36a	95.31d	2.38g	10.88h	1.23k	1.33c	87.50q	116.21m
7	67.85g	3.21b	58.53s	4.08e	12.46f	1.48c	2.40a	124.00n	297.60h
8	77.49f	2.61d	76.31h	3.22f	9.58l	1.32i	1.44c	118.75o	167.15k
9	87.41d	3.14b	97.32c	3.27f	9.2m	1.29j	1.24c	112.51p	139.41l
10	85.42e	3.13b	62.18q	3.14f	10.07k	1.20l	1.14c	117.75o	134.24l
11	87.14d	2.87c	97.78b	3.33f	14.38a	1.66a	2.96a	53.00r	156.98l
12	76.88f	2.94c	68.1n	2.57g	7.35o	1.17m	0.76d	182.00j	148.0l
15	58.72k	3.35a	57.80t	3.09f	10.49i	1.47d	1.47c	157.00l	236.28i
16	48.81m	2.71d	78.38g	4.23e	8.31n	1.39g	1.99b	165.33k	296.36h
17	98.21b	3.11b	111.65a	5.42c	10.21j	1.57b	2.80a	685.50a	1919.40a
18	62.43i	2.11g	75.21i	6.12a	11.21g	1.42e	1.44c	215.00h	308.60h
20	65.22h	1.91i	82.34e	5.87b	10.30j	1.37h	1.32c	262.24f	346.12g
22	58.03k	2.28f	68.48m	4.91d	13.20d	1.28j	1.83b	191.99i	363.57g
24	66.04h	2.16g	81.20f	6.23a	13.50c	1.40f	1.75b	243.44g	426.02f
25	60.02j	1.80i	73.82j	6.11a	12.89e	1.38g	2.10b	278.41e	584.66c
26	55.08l	2.01h	59.34k	5.82b	13.86b	1.37h	1.97b	324.42b	638.95b
27	48.06m	2.02h	66.42o	5.77b	14.32a	1.38g	1.86b	301.46d	560.71d
Mean	70.90	2.65	75.76	4.20	10.85	1.33	1.66	208.08	381.44
CV (%)	0.81	2.66	0.30	4.80	0.73	0.47	18.25	1.26	3.07

Means with the same letter in the column indicate non-significant differences by Scott-Knott test ( $P < 0.05$ ).



**Photo 1.** Murupi chili pepper pod. Manaus 2013 (own source). Manaus, INPA, 2013.

the other landraces had a poor fruit yield in Manaus weather conditions; hence this landrace could be selected for further improvement.

The genetic dissimilarity values were estimated by generalized distances of Mahalanobis ( $D^2$ ) (Table 2). These distances are recommended when the assessed characters present different scales. Comparing the distances, we found maximum distance to have been between landraces 17 (Manicoré-AM, Brazil) and 5 (São Sebastião do Uatumã-AM, Brazil). Presumably, they are from different sites or domestication centers. It may be accounted for by the strong geographical isolation brought about by the Madeira, Amazon and Uatumã Rivers. The Madeira and Uatumã Rivers are major tributaries to the middle Amazon River. The Uatumã River is located 200 km downriver from the Amazon and

Madeira Rivers confluence. These geographical characteristics may lead to isolation of landraces, similar to allopatric evolution. Conversely, the minimum distance was found between landraces 4 (São Gabriel da Cachoeira-AM, Brazil) and 8 (Putumayo, Colombia). Geographically, these sites are not far from each other because São Gabriel da Cachoeira is on the border with Colombia. This suggests that there may be seed interchange between the upper Rio Negro region of Brazil and the southern areas of Colombia.

Six groups were found through nearest neighbor cluster method based on Mahalanobis distances (Figure 2). The clusters were formed with a 50% relative distance. We chose this relative distance to increase the probability of significant differences between groups.

The clustering results were plotted according to their geographical

**Table 2.** Some generalized Mahalanobis distances (D2) among Murupi chili pepper landraces (*Capsicum chinense*) from Amazonian basin. Manaus, INPA, 2013.

Landraces comparison	> Distance	Landraces comparison	< Distance
3 vs 17	72291.92	3 v 25	2794.83
4 vs 17	94036.35	4 vs 8	870.52
5 vs 17	146136.02	5 vs 12	12206.32
6 vs 17	82891.33	6 vs 9	1492.94
7 vs 17	120325.08	7 vs 15	1226.95
8 vs 17	92279.98	10 vs 12	2844.54
9 vs 17	74379.56	11 vs 6	7593.40
10 vs 17	118289.39	16 vs 4	2526.37
11 vs 17	84924.12	17 vs 20	60365.82
12 vs 17	99791.25	18 vs 20	1622.91
15 vs 17	119888.51	22 vs 18	1863.22
16 vs 17	83704.22	24 vs 20	1789.85
18 vs 17	76423.65	25 vs 18	1194.01
20 vs 17	60365.82		
22 vs 17	91097.08		
24 vs 17	62848.30		
25 vs 17	66492.41		
26 vs 17	88562.46		
27 vs 17	80297.14		
Distances mean	22928.73		

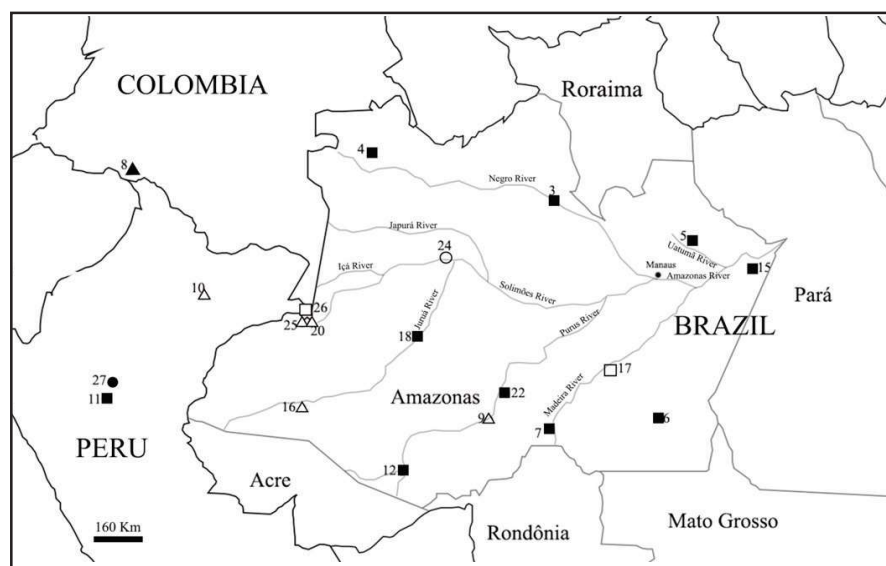
collection site (Figure 1), with each group being differently colored. Cluster I (Figure 1, bold square) included about 50% of the total landraces ('3', '4', '5', '6', '7', '11', '12', '15', '18' and '22'). In this cluster, the landraces' collection locals were extremely diverse, from Amazonas State (Brazil) to Tarapoto (Peru). These findings support the theory of interchanging seeds being frequent throughout the Amazon region, especially between Peru and Brazil.

Cluster II (Figure 1, unbold triangle) was equivalent to 25% of the studied landraces ('9', '10', '16', '20', and '25'). They are distributed from Iquitos (Peru) to the south of Amazonas State, Brazil. Similar behavior was observed on eggplant (Aramendiz-Tatis *et al.*, 2011), suggesting there to be an interchange of some seeds, which might include other species, in this region.

Cluster III (Figure 1, unbold square) included landraces '17' and '26' collecting from Manicoré-AM (Brazil) and Leticia (Colombia), respectively. Despite the large geographic distance between them (>980 km), they may be interchanging.

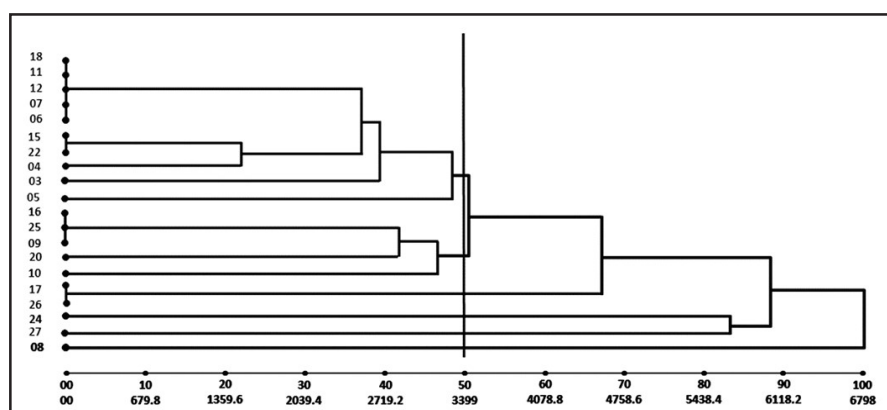
The last three clusters (IV, V and VI) showed to be exclusive to each country, '24' Jutai-AM (Brasil); '27' Yurimaguas (Peru) and '8' Putumayo (Colombia) respectively. The higher genetic divergence among some Murupi landraces suggests there being a possibility for a significant heterotic effect in their crossings, enhancing the agronomical characters such as sweet pepper (*C. annuum*) (Gomide *et al.*, 2003).

In conclusion, our findings point out that the 20 Amazonian Murupi chili pepper landraces may be clustered with at least six clusters; showing there to be genetic diversity in this region. Therefore, further efforts should be directed toward their conservation and breeding. Hybrid production may be the best and fastest Murupi chili pepper breeding method, because on the one hand in one generation can be grouped the better characteristics from two promising parents and on the other hand the heterotic effect can be used.



**Figure 1.** Geographical collection sites of Murupi chili pepper landraces (*Capsicum chinense*). Brazil: 3. Barcelos-AM; 4. São Gabriel da Cachoeira-AM; 5. São Sebastião do Uatumã-AM; 6. Apuí-AM; 7. Humaitá-AM; 9. Lábrea-AM; 12. Boca do Acre-AM; 15. Barreirinha-AM; 16. Eirunepé-AM; 17. Manicoré-AM; 18. Carauari-AM; 20. Benjamin Constant-AM; 22. Canutama-AM; 24. Jutai-AM; 25. Atalaia do Norte-AM. Colombia: 8. Putumayo and 26. Leticia. Peru: 10. Iquitos; 11. Tarapoto and 27. Yurimaguas. Same icons indicate that these landraces are included in a cluster, which was determined based on 50% of relative distances obtained from generalized Mahalanobis distances. Chart from César Ticona-Benavente based on Google Maps, 2015.





**Figure 2.** Dendrogram for 20 Murupi chili pepper landraces, from the Amazon basin, obtained by a nearest neighbor clustering method based on generalized Mahalanobis distances. Cluster I: 18, 11, 12, 07, 06, 15, 22, 04, 03, 05; cluster II: 16, 25, 09, 20, 10; cluster III: 17 and 26; cluster IV: 24; cluster V: 27; cluster VI: 08. Manaus, INPA, 2013.

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