Collecting and evaluation of germplasm of spiked pepper from Brazilian Amazon

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ABSTRACT

Spiked pepper (Piper aduncum L.) is an aromatic plant species with high essential oil production. It is a species that occurs abundantly in the Brazilian Amazon. Its essential oil has exploitable biological properties in the human health and agriculture. Aiming to study its germplasm toward future use in genetic breeding programs, collecting was carried out (inflorescences, cuttings, leaves and thin branches) in ten provenances from the Brazilian Amazon. Twelve morphoagronomic traits were determined to take the data: number of leaves by branch, length of leaf, width of the leaf, circumference of the older branch, height of the plant, number of orthotropic branches, number of plagiotropic branches, length of the internodes, number of spikes per branch, yielding of oil, content and production of dillapiole, as well as data on the environment and populations of spiked pepper. The inflorescences and cuttings were encoded and sent for the Federal Rural University from Pará State (UFRA), Brazil, for propagation. The leaves and thin branches were sent for the Emílio Goeldi Museum, from Pará State (MPEG) for extraction of essential oil (hydrodistillation). Estimators of amplitude of variation, mean, standard deviation and coefficient of variation were utilized to study the phenotypical variability. The morphoagronomic traits of largest variability were number of orthotropic branches, number of spikes per branch, circumference of the older branch and the content and production of dillapiole. This species has adapted to many different environments of vegetation, soil, climate, relief and drainage becoming easy the domestication and cropping. There is morphoagronomic variability pleasing the selection and genetic breeding.

Keywords: Piper aduncum L., genetic resources, conservation of germplasm, morphoagronomic traits, genetic breeding.

RESUMO

Pimenta-de-macaco: coleta e avaliação de germoplasma na Amazônia Brasileira

Piper aduncum L. é uma planta que ocorre na Amazônia Brasileira com elevado teor de óleo essencial e que apresenta propriedades biológicas utilizáveis na agricultura e saúde humana. Com o objetivo de avaliar germoplasma visando ao melhoramento genético e cultivo econômico, realizaram-se coletas (inflorescências, estacas, folhas e ramos finos) em dez municípios da Amazônia Brasileira (Manaus, Marabá, Goianésia, Moju, Belém, Santa Izabel, Americano, Bonito, Santarém Novo e Aveiro). Tomaram-se dados do ambiente, populações e de doze caracteres morfoagronômicos (número de folhas por ramo, comprimento da folha, largura da folha, circunferência do ramo mais velho, altura da planta, número de ramos ortotrópicos, número de ramos plagiotrópicos, comprimento do entrenó, número de espigas por ramo, rendimento de óleo, teor e produção de dillapiole). As inflorescências e estacas foram identificadas e encaminhadas para a UFRA em Belém-PA e, as folhas e ramos finos, para o Museu Paraense Emílio Goeldi-MPEG, para extração do óleo essencial (hidrodestilação). Utilizaram-se estimadores de média, desvio padrão, coeficiente de variação e amplitude total para estudo da variabilidade fenotípica. As matrizes prevaleram em ambientes antropizados, solos argilosos, condições de drenagem variáveis, terrenos planos e clima Ami, como também predominaram populações definíveis pela agregação dos indivíduos, em terra alta e a pleno sol, serrapilheira, tamanho das populações e presença de plântulas no chão muito variáveis. Os caracteres de maior variabilidade foram número de ramos ortotrópicos, número de espigas por ramo, circunferência do ramo mais velho (morfológicos), teor e produção de dillapiole (agronômicos). Concluiu-se que a espécie apresenta adaptação a diferentes ambientes com relação à vegetação, solo, clima, relevo e drenagem, facilitando o cultivo e domesticação. Há variabilidade morfoagronômica favorecendo a seleção e fitomelhoramento.

Palavras-chave: Piper aduncum L., recursos genéticos, conservação de germoplasma, caracteres morfoagronômicos, melhoramento genético.

Spike pepper is a piperaceous species found in the Brazilian Amazon, presenting a great potential for economic use due to the exploitable biological properties in agriculture and human health, attributed to the essential oil, rich in dillapiole, whose yielding can vary from 1.2% to 3.4% (Maia et al., 2000). Such percentage is high, compared to other aromatic species, demonstrating the advantages of its exploration.

In agriculture, this species demonstrated phytosanitary properties against insects and banana phytopathogens (Bastos & Albuquerque, 2004), bean plants (Fazolin et al., 2005), stoked corn (Estrela et al., 2006), flour, bran and rations (Fazolin et al., 2007) and in the tomato plant (Silva et al., 2007). Concerning human health, isolated elements contained in this oil showed properties which are able to combat the leishmaniosiss protozoa (Torres-Santos et al., 1999).

This species grows fast in open places in the wood as well as in degraded soils,
reproducing by self-pollination, presents substantial level of self compatibility and seed dispersion through zoohory. Its cuttings' propagation does not require the use of phyto hormones (Leme et al., 1998; Figueiredo & Sazima, 2000; Francis, 2003).

In long-pepper (Piper hispidinervium C. DC.), studies on the oil yielding variation depending on place of collecting, plant age, botanic and phytochemical characterization of germplasm, period and frequency of harvest and their effects on the oil yielding, are contributing for the domestication and cropping of this species (Pimentel et al., 1998; Silva & Oliveira, 2000; Bergo et al., 2005).

The aromatic plants originated in Brazil require a minimum level of selection and domestication to make its cropping economically viable. Therefore, studies involving collecting practices, characterization, evaluation, besides techniques related to conservation and utilization of genetic resources are also required. The germplasm collecting is recommended for species which represent alternatives for the research on their potential use, as well as for that ones under risk of extinction due to its excessive exploration or the antropic pressure over the biomes. The collecting must generate basic information on the local of collection and the visible variation, considering that the preferable locals are those which concentrate variability and diversity of species (Querol, 1993; Valois et al., 2001).

The evaluation must be done in two levels. In the first one it is necessary to find and discriminate the variability of the collecting and then analyze the characteristics and variations among them. In the preliminary evaluation, the used traits must be easily measurable and defined according to the users' consensus. The analyses of the obtained information may be given in descriptive graphics and evaluation of central tendency as well as through multivariate statistics (Querol, 1993).

The objective of this study was to collect and evaluate spiked pepper germplasm (Piper aduncum L.) in terms of phenotypic variability, in order to select genotypes for economic cropping, as well as to subsidize future programs for its genetic breeding improvement.

**MATERIAL AND METHODS**

The collecting of spiked pepper germplasm was carried out in ten cities of five meso regions in the Brazilian Amazon (the Northeast of Pará State, South of Pará State, Mid Amazon area in Amazon State, Mid Amazon area in Pará State and in the Lower Area of Tocantins State), from January to April, 2001. The collecting was carried out along the roads, also taking informations on the populations and the collecting sites. The geographic position of the cities was determined by their geodesic coordinates, such as latitude and longitude.

The information about the environment characteristics of the local of collection, such as the vegetation type, soil, drainage and relief, as well as the population characteristics, such as population limits defined by the type of individual aggregations in the population, local topography, luminosity conditions, presence of litter in the soil, presence of plantlets on the ground and the estimated area of the population were evaluated by established classification through visual considerations, and the climates, according to the classification of Koeppen for tropical climates. The botanic identification of the collected materials was done by The Botanic Department of Emílio Goeldi Museum in Pará State (MPEG).

The collected material (inflorescences, cuttings, leaves and thin branches) was tagged with an alpha numeric registration code and the initials of the scientific names as well as the collecting sequence (PA-000). The cuttings and inflorescences were sent to the Federal Rural University of Amazon (UFRA) and the leaves and thin branches to MPEG, for the analysis of the essential oil, whose extraction was done by hydro distillation, using Clevenger extractors during three hours. The analysis of the chemical composition of the essential oil was done in a gas chromatograph, model Hewlett Packard 5890, using capillary column WCOT, of fused silica, 25 m long, internal diameter of 0.25 mm, film thickness of 0.25 μm and temperature program of 60°C/240°C (3°C per minute).

During the collection, data were taken from nine morphologic characters, from which five measurements of each one were sampled as follows: 1) average number of leaves per branch (NFR), taken from five branches in the mid part of the plant; 2) leaf length (CF, in cm), measured on the fifth alter leaf, counting from the intersection point of the plant branch and the branch, counting from the beginning of the mid part of the plant, which was determined visually; 3) leaf width (LF, in cm), taken from the fifth leaf of the fifth branch from the mid part of the plant, using the same counting procedure as used in the previous trait; 4) circumference of the oldest branch (CRV, in cm), considered the most developed in thickness and size, from the stem, around 20 cm from the ground; 5) plant height (AP, in m), measured from the ground to the highest part of the plant; 6) total number of orthotropic branches (NRO, obtained by simple counting); 7) total number of plageotropic branches (NRP, obtained by simple counting); 8) length of internodes CEN, in cm), obtained at 50 cm from the ground; 9) number of spikes per branches (NER, corresponding to the means of five branches taken randomly). Three agronomic traits were also evaluated: 1) essential oil yielding (RO, in %), extracted from 100 g of dry leaves in shaded environment, 2) dillapiole level (TD, in %), taken from the essential oil extracted from 100 g of dry weight in shaded environment and 3) dillapiole production (PD, in g), dillapiole weight corresponding to the level (dillapiole) in 100 g of essential oil, considering the oil density equal to one.

The quantification of phenotypic variability was estimated by statistic parameters (variation amplitude, means, standard deviation, coefficient of variation) and test of normality Lilliefors, preceded by data standardizing. The agronomic traits were also analyzed by the standard deviation numbers, above and below the means, by subtracting from the highest value (when above)
or from the lowest (when below), the mean value and then dividing by the respective deviation standard. The data were obtained by the computer program GENES (Cruz, 2006).

RESULTS AND DISCUSSION

Table 1 contains the description of the matrixes sampled as well as the environment characterization of the local of collection considering the vegetation (VG), soil (SL), drainage (DR), climate (CL) and relief (RL) of each city and the characterization of the populations regarding the population limits (LP), local topography (TL), luminosity conditions (CL), presence of litter in the soil (PSS), presence of plantlets on the ground (PPC) and estimated area of the population (AEP) in each meso region.

Regarding the collected materials, thirteen matrixes occurred in Amazon State, in the city of Manaus, while the others were sampled out in nine cities of Pará State: nine in Marabá, five in Belém, four in Goianésia; four in Moju; two in Santa Izabel, two in Bonito, one in Americano, one in Santarém Novo and one more in Aveiro (Table 1). These cities are distributed in five meso regions: Mid Amazon in Amazon State, the South of Pará State, the Lower region of Tocantins River, the Northeast of Pará State and Mid Amazon in Pará State. In the Mid Amazon in Amazon State, the material was collected from thirteen matrixes (Manaus); in the South of Pará State, equally, from thirteen matrixes (Marabá and Goianésia); in the Lower region of Tocantins River, from four (Moju); in the Northeast of Pará State, from eleven (Belém, Santa Izabel, Americano, Bonito and Santarém Novo) and in the Mid Amazon in Pará State, from one (Aveiro).

In general, the habitats were characterized by environments with some kind of anthropization, such as secondary forests, pastures, road margins, etc, with a predominance of secondary forest (Table 1). Some matrixes were observed in more specific environments, like areas with great tendency of flooding (PA-002), areas of shifting agriculture (PA-021) and of permanent agriculture (PA-029).

The matrixes occurred in different kinds of soil: clay, stoned, sanded and alluvial, predominating in well drained soil, although also occurring in badly drained and flooded. Francis (2003) reported that, spiked pepper is able to

<table>
<thead>
<tr>
<th>NMT/ NMA</th>
<th>Municipio/ MR</th>
<th>( \varphi; \lambda )</th>
<th>Matrizes</th>
<th>Caracterização local</th>
<th>Caracterização das populações</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Manaus/ MAA</td>
<td>3(^\circ) 06’ 07”S; 60º 01’ 30”O</td>
<td>PA-001 a</td>
<td>Capoeira; argila amarela; bem drenado, clima Ami; planície</td>
<td>Definível; terra alta; variada; fina; ausentes; 101 a 500</td>
</tr>
<tr>
<td>9/22</td>
<td>Marabá/ SUP</td>
<td>5(^\circ) 22’ 07”S; 49º 07’ 04”O</td>
<td>PA-012 a</td>
<td>Capoeira; argiloso; drenagem variada; clima Awi; ondulada</td>
<td>Dispersa; variada; pleno sol; fina; ausentes; 10</td>
</tr>
<tr>
<td>4/26</td>
<td>Goianésia/ SUP</td>
<td>3(^\circ) 50’ 33”S; 49º 05’ 49”O</td>
<td>PA-022 a</td>
<td>Capoeira; argiloso; drenagem variada; clima Ami; ondulada</td>
<td>Dispersa; variada; pleno sol; fina; ausentes; 10</td>
</tr>
<tr>
<td>4/30</td>
<td>Moju/BAT</td>
<td>1(^\circ) 53’ 02”S; 48º 46’ 08”O</td>
<td>PA-026 a</td>
<td>Capoeira; argiloso; bem drenado; clima Ami; Placien</td>
<td>Definível; terra alta; pleno sol; variada; variada; 10</td>
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<tr>
<td>5/35</td>
<td>Belém/NEP</td>
<td>1(^\circ) 27’ 21”S; 48º 30’ 16”O</td>
<td>PA-030 a</td>
<td>Outros; argila arenosa; bem drenado; clima Afi; planície</td>
<td>Definível; terra alta; pleno sol; variada; variada; variada</td>
</tr>
<tr>
<td>2/37</td>
<td>Sta Izabel/ NEP</td>
<td>1(^\circ) 17’ 55”S; 48º 09’ 38”O</td>
<td>PA-035 e</td>
<td>Outros; argiloso; bem drenado; clima Afi; planície</td>
<td>Definível; terra alta; pleno sol; variada; variada; variada</td>
</tr>
<tr>
<td>1/38</td>
<td>Americano/ NEP</td>
<td>1(^\circ) 18’ 21”S; 48º 03’ 05”O</td>
<td>PA-037</td>
<td>Outros; argila arenosa; mal drenado; clima Afi; planície</td>
<td>Definível; terra alta; pleno sol; variada; variada; variada</td>
</tr>
<tr>
<td>2/40</td>
<td>Bonito/NEP</td>
<td>1(^\circ) 21’ 45”S; 47º 18’ 21”O</td>
<td>PA-038 e</td>
<td>Capoeira; argiloso; variada; clima Ami; planície</td>
<td>Definível; terra alta; pleno sol; variada; variada; variada</td>
</tr>
<tr>
<td>1/41</td>
<td>Santarém Novo/ NEP</td>
<td>0º 55’ 44”S; 47º 23’ 49”O</td>
<td>PA-040</td>
<td>Capoeira; outros; alagado; clima Ami; planície</td>
<td>Definível; terra alta; pleno sol; variada; variada; variada</td>
</tr>
<tr>
<td>1/42</td>
<td>Aveiro/ MAP</td>
<td>3º 36’ 20”S; 55º 19’ 54”O</td>
<td>PA-041</td>
<td>---; ---; ---; Ami; ---</td>
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NMT: número de matrizes (number of matrixes); NMA: número de matrizes acumulado (accumulated number of matrixes); MR: mesorregião (meso region); LP: limites populacionais (populational limits); TL: topografia local (local topography); COL: condições de luz (light conditions); PSS: presença de serrapilheira no solo (litter observed in the soil); PPC: presença de plântulas no chão (plantlets observed on floor); Coordenadas geodésicas: (latitude; longitude); *PA-003 e PA-009, possuem duas formas (a e b) e PA-017 é inexistente, totalizando 42 matrixes. (PA-003 e PA-009 have two forms (a and b) and PA-017 is not existing, summing up 42 matrixes); †Considerado por mesorregião (considered in a meso region).
live in an excessively silty soil as well as in dry soil, but not in excessively drained nor saline soil, as observed in collecting expeditions.

The predominant relief was flat or undulating terrain, in which 22 matrixes occurred in flat terrain and ten in undulating terrain. Regarding the climate, 25 occurred in locals with climate type Ami (annual average precipitation of 1,500 to above 3,000 mm), nine occurred in locals with climate AwI (1,000 to 2,500 mm annually) and eight in locals with climate AfI (2,000 to above 3,000mm).

Concerning the characterization of the populations, the population limits, based on the type of individuals aggregation, was definable in the cities of Manaus (Mid Amazon in Amazon State), Santa Izabel, Americano and Bonito (Northeast of Pará State), whereas the populations of Marabá (South of Pará State) presented dispersed individuals. In the cities of Belém (Northeast of Pará State), Goianésia (South of Pará State) and Moju (Lower region of Tocantins River) the populations presented aggregated individuals, defining limits as well as dispersed individuals.

Regarding the local topography, the matrixes were observed in lowlands, highlands and hillsides, in which 22 occurred in highlands, ten in hillsides and seven in lowlands, thus indicating a preference for highlands.

Concerning the luminosity conditions, 32 matrixes (78%) occurred in the sunlight. The matrixes from Manaus presented the highest variation in terms of luminosity conditions, being also present in shaded environment (3) and partially shaded (4), whereas the ones from Pará State were found in sunlight condition. As observed in collecting expeditions, Francis (2003) also described spiked pepper as a species, moderately intolerant to shade.

The litter predominated in the thin form, although variations were observed from medium to thick in all the meso regions, in which eighteen matrixes occurred in soil with thin litter, eleven in soil with medium litter and four with thick litter.

Concerning the presence of plantlets in the ground, in 26 populations it was absent, in ten abundant and in three scarce. In the South of Pará State and the Low region of Tocantins River, only populations with absence of plantlets were observed, whereas in the other meso regions, there were detected populations with absence, scarceness and abundance of plantlets. The predominance of matrixes with populations presenting absence of plantlets in the soil could be related with the mechanism of seeds dispersion, mainly occasioned by the frugivorous bats from the species Carollia perspicillata (Leme et al., 1998).

The estimated area of the populations demonstrated the predominance of modules of 10 ha, particularly in the South of Pará State and Low Region of Tocantins River. In the Northeast of Pará State the estimated areas varied from 10 to above 1,000 ha and in the Mid Amazon in the Amazon State there was a higher number of areas with modules from 101 to 500 ha.

Evaluating the phenotypic variability of matrixes (Table 2), we found that the number of leaves per branch (NFR) presented a variation amplitude (AV) of eleven, with the highest value registered in matrix PA-009/02 (Manaus) and the lowest in matrix PA-012 (Marabá), with means (m) of 9.6, standard deviation (S) of 2.6 and variation coefficient (CV) of 27.1%. For leaf length (LF) we encountered AV = 9.3, presenting, in the matrix PA-012, the highest value and, in the matrix PA-011 (Manaus), the lowest.
with m = 18.4, S = 2.4 and CV = 13.0%. The width of the leaf (LF) presented AV = 4.9, with the highest value in the matrix PA-012 and the lowest in the matrix PA-030 (Belém), with m = 6.8, S = 1.1 and CV = 16.2%.

In the plant height (AP), there were registered AV = 4.9, with the highest value in the matrix PA-029 (Moju) and the lowest in the matrix PA-033/02 (Manaus), with m = 3.9, S = 1.1 and CV = 28.2%. The circumference of the oldest branch (CRV) had AV = 42.5, with the highest value in matrix PA-018 (Marabá) and lowest in the matrix PA-010 (Manaus), m = 16.2, S = 8.6 and CV = 53.1%. For the number of orthotropic branches (NRO), we observed AV = 33.0, with the highest value in the matrix PA-033 (Belém) and the lowest in nine matrixes, among which the PA-002 (Manaus), m = 6.1, S = 6.1 and CV = 100.0%.

For the number of plagiotropic branches (NRP), there were registered AV = 16.0, with the highest value in the matrix PA-037 (Americano) and the lowest, also in nine matrixes, among which the PA-002, m = 20.3, S = 4.4 and CV = 21.7%. The length of the internodes (CEN) had AV = 21.0, with the highest value in the matrix PA-024 (Goianésia) and lowest in the matrix PA-005 (Manaus), m = 15.9, S = 5.3 and CV = 33.3%. The number of spikes per branch (NER), presented AV = 10.1, with the highest value in the matrix PA-038 (Bonito) and the lowest in the matrix PA-019 (Marabá), m = 3.7, S = 2.8 and CV = 75.7%.

The existence of phenotypic variability was verified in all the morphologic traits and in the number of orthotropic branches. The number of spikes per branch and the circumference of the oldest branch achieved CVs above 50%. The others obtained CVs below 50% with the lowest variation in length and width of leaf. Studying the variability of watermelon accesses, (Citrus lannatus), Queiroz et al. (2001) observed a great variation amplitude in the traits interesting to improve the yielding, such as the fruit length and diameter, skin thickness and brix, which stayed close to the commercial varieties.

The test of normality in the morphogenetic traits was significant in eight of the twelve traits, indicating absence of normality, which denoted heterogeneity in the data, probably as a reflex of the heterogeneity in the different environments and meso regions where the collecting was carried out. The level of dillapiole was the most heterogeneous agronomic trait of highest interest and probably the one with highest variability.

Regarding the essential oil yielding (RO), the lowest value was detected in matrix PA-033 (Belém) and the highest in matrix PA-010 (Manaus), with AV = 2.9, m = 2.3, S = 0.6 and CV = 26.1%. Nineteen matrixes, which represented near 50% of the examined matrixes, stayed below the means and 19 within the means (1) or above the means (18). Therefore, the referred dispersion standard around the means followed the symmetry model, typical of the normal distribution, in accordance with the test of normality (non-significant). In this case, the class of values superior to the means constitutes the selectable part for cropping and genetic improvement.

The dillapiole level (TD) varied from 51.1, in matrix PA-033 (Belém), to 86.6, in matrix PA-014 (Marabá), with AV = 31.1, m = 76.7, S = 7.7 and CV = 10.0%. Sixteen matrixes, which represented approximately 42% of them, stayed below the means and 22, representing approximately 58% of the matrixes, stayed above the means, thus constituting a dispersion standard around the asymmetric means, which denotes existence of variability, in accordance with the test of normality (significant), favoring the selection of material for cropping and improvement.

The dillapiole production (PD) varied from 0.4, in matrix PA-033, (Belém) to 2.9, in matrix PA-010 (Manaus), with AV = 2.5, m = 1.8, S = 0.5 and CV = 27.8%. Over half of the matrixes (20) presented a value which was equal (3) or above the means (17), representing around 53% of the total examined and 18 stayed below the means, representing around 47%. Therefore, the dispersion around the means, practically presented a symmetry, in accordance with the test of normality (non significant). In this case, the matrixes with values above the means are selectable.

The analysis of the standard deviation indicated that the dispersion of data around the means presented similar intervals in three of the evaluated traits, in which the dillapiole level presented a higher number of matrixes above the means. However, the means of this trait demonstrated elevated magnitude, determinant to reduce the number of standard deviations and lower the CV (10.0%), which was much inferior when compared to other traits’ yielding of essential oil (26.1%) and dillapiole production (27.8%).

The number of standard deviations of oil yielding, level and dillapiole production was respectively around 2S (two standard deviations), 1S and 2S above the means. Similarly, the 3S was below the means in these three traits. Thus, the analyses indicated that there exist higher variability in the dillapiole level and bigger normality in the essential oil yielding and dillapiole production, despite the fact that these traits presented superior CVs, compared to the dillapiole level and a higher possibility of selection due to a bigger number of matrixes above the means.

Silva et al. (2006), evaluating 69 banana accesses (Musa spp) of two types and four originated from SP, MG, BA and SC States, through twelve traits, contrarily to what was observed in matrixes of spike pepper, did not observe significant variability among the accesses. However, two samples were possible to be selected for cropping.

The morphologic traits presenting higher variation were the number of orthotropic branches, the number of spikes per branch and the circumference of the oldest branch. The dillapiole level was the agronomic trait presenting the highest variation.

The easy adaptation of the species to a diversity of edaphoclimatic and ecological conditions can facilitate its fast domestication and economic cropping. The analysis of morphoagronomic traits indicated, in general, that there exist variability for selection which aims at the improvement of the species.

ACKNOWLEDGEMENTS
The author thanks PROBEM and CNPq for the resources provided in the initial phase of the collecting procedures and for the installation of the germplasm collection, as well as the researching team and trainees from Emílio Goeldi Museum in Pará State (MPEG) for the contribution in the analyses of the essential oil and botanic identification of the collected material; particularly CAPES for conceding the scholarship to the first author.

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