

GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF TRADITIONAL RICE AND BEAN VARIETIES FROM THE PANTANAL OF MATO GROSSO, BRAZIL

CARACTERIZAÇÃO GENÉTICA E MORFOLÓGICA DE ARROZ E FEIJÃO DO PANTANAL MATO-GROSSENSE, BRASIL

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ABSTRACT: Farmers from the district of São Pedro de Joselândia, belonging to the municipality of Barão de Melgaço, Pantanal of Mato Grosso, have been cultivating for some time two rice plant materials and three types of beans that, according to them, are not commercial. For this reason, the objective of this work was to test the originality of the materials by means of genetic and morphological characterization in order to identify whether the nature of the subsamples would be effectively traditional or whether they are varieties already known in the market. Morphological analyzes of the cultivated plants (phenotypic characteristics, cycle and flowering) and seed of the rice subsamples, hereinafter called Agulhinha Vermelho and Branquinho and of the Amarelinho, Rosinha and Roxinho subsamples were performed. Also, the evaluation of distribution of comparative genetic variability with other materials of the same species, already cataloged, was determined. The subsamples were characterized as traditional varieties different from those already cataloged and they are not found in the Nuclear Collection of Rice and Beans of the Brazilian Agricultural Research Corporation (Embrapa). They have distinct morphological characteristics, being potential species for use in the market of new technologies. This should guarantee to the residents of that community that they are breeders of specific materials of cultural heritage adapted to the local conditions.

KEYWORDS: Genetic heritage. Genetic resources. *Oryza sativa*. *Phaseolus vulgaris*.

INTRODUCTION

Several studies have been carried out showing the potential of using traditional materials in studies on the genetic improvement of cultivated species in the area of genetics of populations with species (CARGNIN et al., 2010).

The genetic basis of agronomic crop breeding programs is relatively narrow, so that new materials adapted to specific environmental conditions, which may be carriers of genes different from those already found, are extremely valuable because they allow the inclusion of new genes of interest in new and more adapted cultivars.

In Brazil, the cultivation of native materials is conducted predominantly by subsistence and has as main characteristic a periodic acquisition of seeds. Farmers use their grains as seeds for several years. If, on the one hand, this system of using the genetic material itself contributes to a low productivity, on the other hand it is an excellent source of genetic diversity (COSTA; OLIVEIRA; ABREU, 2013). Thus, traditional varieties are a

source of favorable genes to be used by breeding programs, subsidizing a sequence of research and evaluations (COSTA et al., 2011).

The traditional and scientific knowledge of native species, which have added value, be it nutritional or economic, among other roles subsidizes not only the understanding of the species, but also integrates the communities in concern with their development and biodiversity seeking a balance between nature and the use of its richness (BERTAZZONI; DAMASCENO-JUNIOR, 2011).

The Pantanal of Mato Grosso presents landscape heterogeneity, with a physiognomy that distinguishes it from several wetlands, with its own ecologically and floristically differentiable characteristics (GUARIM NETO, 2008).

Between two of the largest formative rivers of the Pantanal North of Mato Grosso (Cuiabá and São Lourenço) and the surroundings of the Private Reserve of Natural Patrimony of the Social Service of Commerce (RPPN Sesc) in Pantanal, there is the district of São Pedro de Joselândia, belonging to the

municipality of Barão de Melgaço (PIGNATTI; CASTRO, 2010).

Given the existence of rice and bean seeds characterized as non-commercial by the inhabitants of São Pedro de Joselândia, Barão de Melgaço district, the objective of this work was to characterize the morphology and the genetic nature of two rice subsamples and three subsamples of beans from São Pedro de Joselândia, Barão de Melgaço district, in the Pantanal of Mato Grosso, providing new potential genetic material for breeding.

CONTENTS

Two subsamples of locally named "Branquinho" and "Agulhinha Vermelho" rice and three subsamples of beans locally called "Amarelinho", "Rosinha" and "Roxinho" were collected, and a propagation experiment was carried out in a greenhouse at the Federal University of Mato Grosso, Cuiabá campus.

The soil used in the experiment was collected from the arable layer in previously cultivated areas of rice and beans in the district of São Pedro de Joselândia, municipality of Barão de Melgaço. After air-drying, the soil samples were sieved and transferred to pots of 10 dm³, containing 9.0 dm³ of soil.

For sowing, 40 pots were used for cultivating rice and 36 pots for cultivating beans. Ten seeds were sown per pot and then thinned, leaving two seedlings per pot. For rice, flooding in the tillering period - four leaves - began with water addition in order to maintain a blade of 5.0 to 10 cm throughout the experiment, simulating the traditional form of cultivation. For the cultivation of beans, a daily irrigation was performed.

Molecular analysis was carried out at the Biotechnology Laboratory of Embrapa Arroz e Feijão, in Santo Antônio de Goiás. Samples of nine plant leaf tissues were collected per subsample for the extraction and quantification of genomic DNA by the alkaline lysis method and for the characterization of genetic diversity. For the molecular analysis, a panel composed of 24 SSR markers was used (BORBA et al., 2009).

Polymerase chain reactions (PCR) followed the following amplification pattern to a final volume of 5 µl: 2.5 µL of Master Mix, 0.5 µL of Q solution, 0.1 µL of RNase free water (Commercial Kit Qiagen® Multiplex PCR), 0.8-1.0 µL of SSR primers (10 µM), and 1.0 µL of DNA (3 ng/µL). The thermocycler used to conduct the PCR reactions was the GeneAmp PCR System 9700 (Applied

Biosystems, Foster City, CA, USA). The amplified products were diluted to obtain uniform and defined signals on an ABI 3100 automatic fragment analyzer (Applied Biosystems). Alleles were identified by the GeneMapper 3.5 software (Applied Biosystems).

Factorial matching analysis (FMA) was conducted using the Genetix software, version 4.03 (BELKHIR et al., 2001).

The analyses were performed by comparing the nuclear collections of rice belonging to the Nuclear Collection of Embrapa Rice and Beans using the sub-samples of the research.

The analysis was performed using the Structure 2.3.4 software (PRITCHARD; STEPHENS; DONNELLY, 2000) (Burn-in 25,000/MCMC 75,000, with 5 replicates for each subgroup) for a possible identification of the origin center of the subsamples.

Morphoagronomic characterization of the varieties of Rice: The evaluation of the agronomic traits and grain quality was performed on materials collected in the field (plant descriptors) or in laboratory (seed descriptors).

For rice, after describing the seeds, we analyzed the distribution of aristae in the panicle, Pubescence of glumellae, Coloring of the apiculus in maturation, Coloring of the lemma and the palea, Coloring of the apiculus, Weight of a thousand seeds, Length of the grain without hulls (caryopsis), Grain form (caryopsis), Color of the pericarp, Whole grains in 100 g and Grains broken in 100 g, according to Bioversity international, Irri and Warda (2007), using the methodological procedures adopted according to recommendations of Fonseca and Castro (2003).

The descriptors that contemplate the seedling and the different parts of the plant of rice, i.e., stem, leaf, flower, fruit and seed, as well as its morphological and agronomic characteristics at different stages of development, were Color of leaf, Leaf blade position, Leaf pubescence, Auricula coloring, Ligule color, Plant height, Last leaf length, Last leaf width, Last leaf angle, Panicle grain detachment, Panicle length, Resistance of stem to bedding, Senescence of leaves, Distribution of branches in panicle, Number of days for flowering, and Number of days for maturation.

The use of minimum rice descriptors is specific for the *Oryza sativa* species, and its application methodology is that recommended by the National Service for the Protection of Cultivars (SNPC) in accordance with the Law no. 9,456/97 of the Ministry Agriculture, Livestock and Supply (BRASIL, 2007).

Morphoagronomic characterization of the varieties of Bean: For beans, the subsamples were characterized according to the descriptors used by Silva (2005).

The following was evaluated in seeds: Color of the seed, Primary color of the integument, Secondary color of the integument, Secondary shape of color of the integument, Color of the seed halo, Seed brightness, Cultivar (commercial group to which it belongs), Seed shape, Level of flattening of seed, and Weight of a thousand seeds (BRASIL, 2009).

The minimum descriptors that contemplate the characteristics of the evaluated bean plant were Cycle (days from emergence to flowering), Cotyledon pigmentation, Growth habit, Color of the flower on the 4th stem node, Color of the standard, Color of the wings, Primary color of the mature pod (in the case of pods with only one predominant color), Secondary color of the mature pod (in the case of pods with two colors = bicolor) and Pod profile.

To determine the total protein, the method used followed the Kjeldahl method (DETMANN; QUEIROZ; CABRAL, 2012), which is based on the nitrogen content in the sample.

For the physical and biochemical analysis of seeds, the design was completely randomized, using four replicates for each rice variety (Branquinho and Agulhinha Vermelho). Analysis of variance (ANOVA) and *F* test at 5% probability were performed (FERREIRA, 2011).

For seed descriptors, ten replicates of 50 seeds were tested for each variety in a completely randomized design.

For plant descriptors, the experimental design was completely randomized with ten replicates for each variety, where each replicate consisted of two plants. Data were analyzed by analysis of variance with a probability of 0.05%.

Figure 1 shows the factorial analysis of rice subsamples and their spatial distribution pattern of the genetic variability of the cultivars and samples of the materials from the Embrapa Rice Nuclear Collection (CNAE).

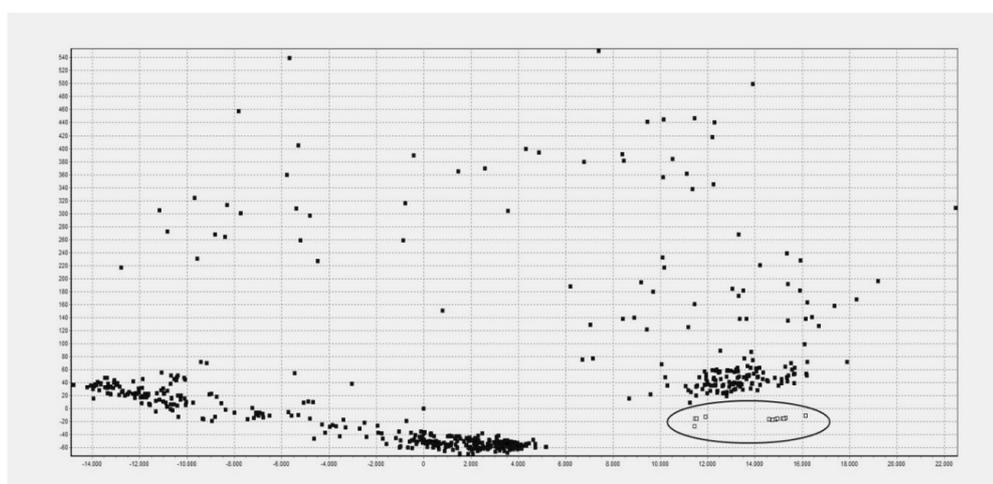


Figure 1. Factorial Matching Analysis (FMA) and its spatial distribution pattern of genetic variability among the subsamples cataloged at the Embrapa Rice Nuclear Collection (black) and subsamples studied: Branquinho and Agulhinha Vermelho (white)

The materials of the Nuclear Rice Collection are shown in black and the evaluated subsamples are shown in white (identified with a circle). This indicates that the rice samples from São Pedro de Joselândia, a district of the municipality of Barão de Melgaço, were materials actually specific to the studied region, and are not found at the Embrapa Nuclear Rice Collection.

This information is relevant since the knowledge of the existence of materials other than those commercially known means potential materials for use in genetic improvement, since they can express important characteristics of adaptation,

also guaranteeing gains for the residents as breeders of a specific material of the community adapted to local conditions.

By the factorial analysis of correspondence, we observed a division of distinct groups, which may be related to the region of origin of the subsamples, since they originate from rice breeding programs from abroad and from Brazil. The presence of expressive genetic variability provides indications that the subsamples evaluated can be used by rice improvement programs in Brazil as a source of variability.

Among the studied groups for rice materials, both subsamples evaluated were grouped together with accessions of a japonica background, or accessions of Várzea, characteristic of the cultivation of the materials in São Pedro de Joselândia.

According to Borba et al. (2009), floodplain adhesions of the Japanese group present a greater diversity and are genetically more divergent than upland accessions. This assumption was confirmed when the accessions were evaluated in order to determine the extent of the genetics in 242 strains and cultivars of the Embrapa Nuclear Rice Collection.

According to Gao (2005), it is reasonable to expect a greater source of genetic diversity of these floodplain adhesions when one considers that the

greatest number of them occupy wide and diverse environments around the world, leading to different adaptive processes compared to upland rice.

Table 1 shows the morphological, flowering and cycle descriptors of Branquinho and Agulhinha Vermelho rice varieties.

Branquinho and Agulhinha Vermelho rice genotypes do not differ in leaf color, leaf blade position, leaf pubescence, auriculum color, color of the collar, color of the ligule, and detachment of panicle grains. These characteristics are qualitative because they define the variety and are usually controlled by few genes. They have a high heritability and do not change, or are little influenced by the environment (FONSECA; RODRIGUES; GUSMÃO, 2008).

Table 1. Morphological, flowering and cultural cycle descriptors of rice varieties of the Pantanal of Mato-Grosso

Plant descriptors	Branquinho	Agulhinha Vermelho
Leaf Color	Medium green	Medium green
Position of the leaf blade	Erect	Erect
Leaf pubescence	Glabrous	Glabrous
Auriculum color	Whitish	Whitish
Collar coloring	Green	Green
Ligule color	Colorless to green	Colorless to green
Plant height (cm)*	97 B	125 A
Length of last leaf (cm) ^{ns}	41 A	47 A
Last Leaf Width (cm) ^{ns}	2.89 A	2.71 A
Last Leaf Angle (°)*	23 A	18 B
Last Leaf Position	Semi-erect	Erect
Detachment of panicle grains	Difficult	Difficult
Panicle Length (cm) ^{ns}	20 A	22 A
Resistance of the Stem to Lodging	Very strong stem	Strong stem
Leaf Senescence	Precocious	Precocious
Distribution of Branches in the Panicle	Semi-erect	Semi-erect
Cycle until flowering (days)*	89 B	107 A
Crop cycle (days)*	124 B	137 B

* Significant at a 5% probability level by Tukey test.

^{ns} Not significant at 5% probability by Tukey test.

As for the angle of the last leaf, position of the last leaf and cycle until flowering, there was a variation between both materials (Table 1).

The genotype Agulhinha Vermelho obtained a lower insertion angle of the last leaf: 18°. Among the various characteristics of the leaf, the insertion angle seems to be the most important. The photosynthetic rate of horizontal leaves tends to be lower than that of upright leaves (KEULEN, 1976).

In rice, leaf length is more variable than width, and is associated with the leaf angle. A better distribution of leaves increases the interception of solar radiation. Leaf thickness is mentioned as an

important morphological characteristic. However, according to Yoshida (1972), the experimental results are controversial and corroborate with the one found in this work, where rice plants with thin leaves are generally more productive.

Although different, the angle of the last leaf was classified as erect in both cultivars studied. According to Fonseca et al. (2002), the angles of last leaf and sprouts are inherent characteristics of the cultivar and are rarely modified by the environment.

The shortest cycle (124 days) was observed for the Branquinho genotype, whose material was

characterized as having an average cycle (Table 1). Under the conditions evaluated, the Agulhinha Vermelho genotype presented a semi-late cycle (137 days).

The identification of cultivars is carried out, most times, by using morphological descriptors. The interest in the characterization of cultivars in the last

decades is related to the growing need to protect commercial cultivars in increasingly competitive economic markets (BONOW et al., 2007).

The morphological characteristics of seeds described in Table 2 show the identification of materials, which allow distinguishing the materials visually.

Table 2. Morphological description of seeds of rice varieties of the Pantanal of Mato-Grosso

Grain Descriptors	Branquinho	Agulhinha Vermelho
Aristae	White	Absence of arista
Pubescence of Glumellae	Glabrous	Glabrous
Coloring of Lemma and Palea	Golden	Palea
Color of the apiculus	Reddish brown	Straw
Weight of Thousand Seeds (g)*	22.70 A	19.25 B
Length/Width Ratio*	2.18 A	3.69 B
Grain shape	Medium elongated	Very elongated
Pericarp color	White	White
Whole grains (g)*	54.52 B	66.84 A
Broken grains (g)*	8.42 A	2.97 B

* Significant at a 5% probability level by Tukey test.

^{ns} Not significant at 5% probability by Tukey test.

For the descriptors arista, color of the lemma and the palea and color of the apiculus, there were differences between the evaluated materials.

Agulhinha Vermelho rice seeds have a lower weight of one thousand seeds and a greater length/width ratio, which allows them to be identified as a very elongated grain material. Moreover, there was a greater resistance to breaking in processing when characterized into whole grains and broken grains after processing. Grains can thus be characterized as potential for the market of new technologies.

This information provides the best differential characteristics between cultivars and acceptance by the market. The national preference, according to Utumi et al. (2016), is for the elongated and narrow, spotless, unbleached grain, technically termed long thin, allowing to infer that the Agulhinha Vermelho variety has a potential for acceptance by the food market.

The determination of the reserve protein content is an excellent source of information for the

planning of the development of rice genotypes with a better nutritional quality of the grains. The protein content observed for the Branquinho and Agulhinha Vermelho varieties was 8.56% and 8.89%, respectively, which does not differ from the average protein content observed for commercial rice cultivars: 7%. However, studies indicate a great variation in the concentration of this nutrient, with values varying between 4.3 and 18.2% (WALTER; MARCHEZAN; AVILA, 2008), which characterizes the varieties evaluated as potential for the germplasm enrichment of the species.

For bean subsamples, the molecular analysis was performed comparing the nuclear bean collections with the evaluated subsamples.

In Figure 2, according to the factorial analysis by correspondence, the subsamples are shown in white (identified with a circle), while the subsamples of beans cataloged in the Nuclear Collection of Embrapa Rice and Beans are shown in black.

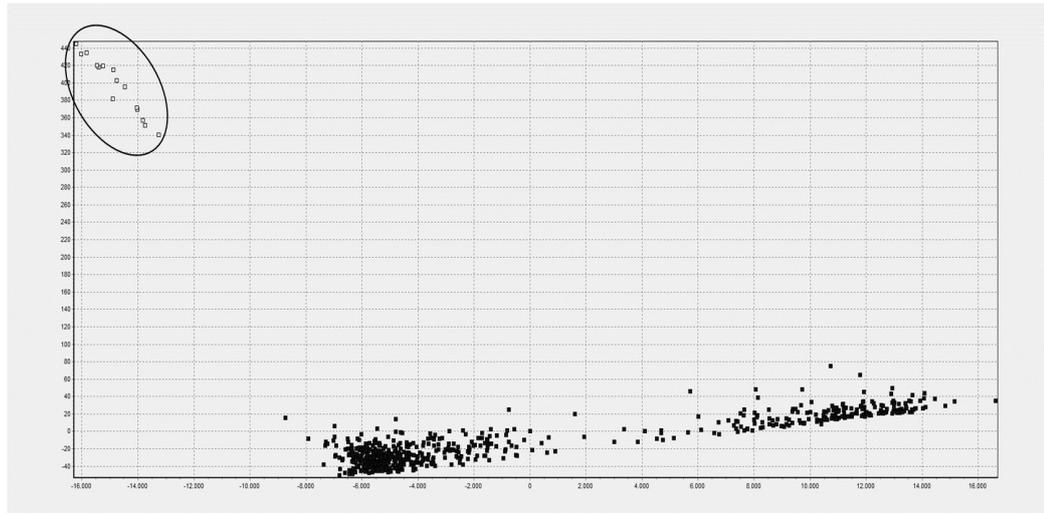


Figure 2. Factorial Matching Analysis (FMA) and its spatial distribution pattern of genetic variability among the subsamples cataloged at the Embrapa Bean Nuclear Collection (black) and subsamples studied (white)

The evaluated subsamples are different from those already cataloged. The use of SSR markers allowed determining the genetic relationship between subsamples, which form a separate group and allows new genetic parameters to be estimated, helping the selection of new accessions to be introduced in CNAFE (Embrapa Nuclear Bean Collection).

The analyzed bean materials form a group apart from the accessions that were classified as Andean and Mesoamerican. The Amarelinho,

Rosinha and Roxinho subsamples are included in the group of traditional varieties, strains and cultivars and their accessions (corresponding to 80% of accessions).

For the morphological characteristics of bean plants cotyledon pigmentation, hypocotyl pigmentation and primary and secondary color of the pods, there were variations that proved highly heritable and which can be easily observed expressing in all environments (Table 3).

Table 3. Morphological description of plants of Amarelinho, Roxinho and Rosinha bean subsamples from São Pedro de Joselândia, Pantanal of Mato Grosso.

Plant descriptors	Amarelinho	Roxinho	Rosinha
Cycle (days) ^{ns}	30 A	33 A	30 A
Harvest (days) ^{ns}	93 A	94 A	94 A
Cotyledon pigmentation	Absent	present	absent
Hypocotyl pigmentation	Absent	absent	absent
Growth habit	determined	determined	determined
Flower color	uniform	uniform	uniform
Last leaf color	white	white	white
Wing Color	white	white	white
Primary color of mature pod	yellow	purplish	purplish
Secondary color of the pod	yellow	purplish	purplish
Profile	arched	arched	arched

* Significant at a 5% probability level by Tukey test.

^{ns} Not significant at 5% probability by Tukey test.

For the protein contents of the subsamples Amarelinho, Rosinha and Roxinho, contents of 23.98%, 23.85% and 23.04% were found. These values are among the average protein contents observed for commercial bean cultivars, reaching 28.7% and 16% (SOARES JUNIOR et al., 2015), and 35.2% for beans (SANTALLA et al., 2004).

This allows inferring that the evaluated accessions are potential for nutritional enrichment, since the economic and social importance of beans translates into important information for the development of genotypes with a better nutritional quality.

The variation in the cycle between the bean subsamples studied was small, characterizing them

as normal cycle materials, according to Embrapa Arroz e Feijão (2014), varying from 85 to 95 days.

Table 4 shows the morphological description of seeds of bean subsamples.

Table 4. Morphological description of seeds of Amarelinho, Rosinha and Roxinho bean subsamples from the Pantanal of Mato Grosso.

Grain Descriptors	Amarelinho	Roxinho	Rosinha
Uniformity of color	uniform	uniform	Uniform
Primary color of tegument	beige	brown	Beige
Primary color of tegument	beige	brown	Beige
Secondary color of tegument	not applicable	not applicable	not applicable
Halo color	yellow	brown	Pink
Brightness	opaque	bright	Opaque
Commercial group	beige/brownish	brown	pink/rosy
Seed shape	elliptic	oblong	Elliptic
Degree of flattening of seed	full	full	semi-flattened
Weight of thousand Seeds ^{ns}	250 A	280 A	260 A

* Significant at a 5% probability level by Tukey test.

^{ns} Not significant at 5% probability by Tukey test.

The materials differ in the hue of the integument, color of the halo, brightness and seed shape. The morphological description of the seeds allows the subsamples to be classified into different groups and facilitates the characterization.

Samples of rice and beans from São Pedro de Joselândia, a district of the municipality of Barão de Melgaço, are characterized by materials specifically coming from the studied region, which are not found in the Nuclear Rice Collection of Embrapa.

They present different morphological descriptors both for plants and for seeds that allow

differentiating them. Such characteristics make the varieties a cultural heritage of the Pantanal of Mato Grosso, and a potential for use in the market of new technologies.

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RESUMO: Agricultores do distrito de São Pedro de Joselândia, pertencente ao município de Barão de Melgaço – Pantanal Norte do estado de Mato Grosso, vêm cultivando há tempos dois materiais vegetais de arroz e três materiais de feijão que, segundo os mesmos, não são comerciais. Por essa razão, com este trabalho o objetivo foi realizar a caracterização genética e morfológica desses materiais a fim de identificar se a natureza das subamostras seria efetivamente tradicional, ou se trataria de variedades conhecidas no mercado e descrever suas características. Foram realizadas análises morfológicas das plantas cultivadas (características fenotípicas, ciclo e florescimento) e de sementes das subamostras de arroz, doravante denominadas Agulhinha vermelho e Branquinho e das subamostras de feijão Amarelinho, Rosinha e Roxinho. Ainda, determinou-se a avaliação de distribuição de variabilidade genética comparativa com demais materiais das mesmas espécies, já catalogados. Observou-se que as subamostras foram caracterizadas como variedades tradicionais, diferentes daquelas já catalogadas e que não se encontram na Coleção Nuclear de Arroz e Feijão da Embrapa. As mesmas possuem características morfológicas distintas, podendo ser materiais com potencial para o uso no mercado de novas tecnologias, o que deve garantir aos moradores da comunidade, serem obtentores de materiais específicos e adaptado às condições locais.

PALAVRAS-CHAVE: *Oryza sativa*. Patrimônio genético. *Phaseolus vulgaris*. Recursos genéticos.

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