

Uncaria tomentosa Willd. ex Roem. & Schult.: genetic and chemical diversity of accessions cultivated in vitro and their introduction into the native environment

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Abstract

Uncaria tomentosa Willd. ex Roem. & Schult., known as cat's claw, is a medicinal plant that is native to the Amazon and used to treat inflammatory diseases. Currently plants are harvested in a predatory manner leading potentially to extinction of the species. The aims of this study were to develop an efficient protocol for the micropropagation of the species, to evaluate the genetic and chemical diversity of genotypes cultivated *in vitro*, and to determine the survival rate of plants introduced into the native environment. The optimised micropropagation protocol employed medium-sized flasks containing 20 mL of woody plant medium (WPM) supplemented with 5.0 μ M 6-benzylaminopurine (BAP), with four explants per flask maintained under a 16 h photoperiod provided by light emitting diodes. Under these conditions, explants produced an average of 2.13 shoots bearing a total of 8.13 buds per explant. Twenty accessions of *U. tomentosa*, each originating from seeds of individual plants collected in three states of the Brazilian Amazon, were maintained *in vitro* and analyzed by start codon targeted (ScoT) molecular markers and chemically by liquid chromatography. These genotypes showed genetic diversity *in vitro* that was comparable with natural populations, and the majority of accessions accumulated mitraphylline and isomitraphylline. Plantlets cultivated *in vitro* were acclimatized and introduced into the native environment with a survival rate of $46 \pm 18\%$ after eighteen months. It is concluded that *in vitro* propagation of *U. tomentosa* is efficient for the production of vigorous plants capable of surviving in the natural conditions of the Amazon rainforest.

Key message

An micropropagation protocol for enabled successful reintroduction of this medicinal species into the Amazon, supporting both its conservation and the development of sustainable production systems.

Introduction

Uncaria tomentosa Willd. ex Roem. & Schult., commonly known as cat's claw, is a medicinal plant from the Amazon region and used mainly in the treatment of inflammatory diseases (Arado et al. 2024). Based on its utilization in the traditional medicine of the indigenous peoples of the Amazon and its scientifically proven therapeutic efficacy, the species is included in monographs of selected medicinal plants by the World Health Organization (Blumenthal 1997), and in the National List of Essential Medicines of the Brazilian government (Ministério da Saúde 2013) and the United States Pharmacopeia and National Formulary (United States Pharmacopeial Convention 2022).

Despite the popular use of *U. tomentosa* and the well-documented body of evidence regarding its pharmaceutical properties, exploitation of this valuable resource depends exclusively on predatory collection since efficient propagation of the species is limited by the scarcity of agronomic studies. As a consequence, natural populations of *U. tomentosa* are being compromised with increasing risk of genetic erosion (Honório et al. 2016). In fact, the vulnerability of *U. tomentosa* to extinction was first documented a decade earlier by Zavala and Zevallos (1996) and confirmed in a more recent study by

Honório et al. (2017) involving the use of sequence-related amplified polymorphism (SRAP) markers. These authors reported the occurrence of low gene flow and an island-type genetic structure among the natural populations of *U. tomentosa* analyzed, and emphasized the importance of conserving accessions in *in vitro* germplasm banks.

Moreover, field studies in the Amazon performed by members of our group have revealed further compounding factors that serve to increase the difficulty of collection from natural populations, including the minute size of the seeds (< 1 mm) and the height of the lianas (> 30 m). For these reasons it is likely that the *in vitro* culture of *U. tomentosa* will prove to be a sustainable solution for the large-scale production of phytomedicine.

Although several researchers have reported the *in vitro* cultivation of *U. tomentosa* (Pereira et al. 2008; Venutolo 2010; Raposo and Teixeira 2011; Luna-Palencia et al. 2013), a protocol for the efficient micropropagation of the medicinal plant has yet to be developed. Moreover, the multiplication of *U. tomentosa* under *in vitro* conditions should be preceded by genetic analysis of introduced accessions in order to preserve genetic variation in the field and to conserve species diversity.

Genetic stability and diversity in tissue culture-derived plants is typically assessed using molecular markers as, for example, the robust and cost-effective start codon targeted (SCoT) polymorphism technique (Sirikonda et al. 2023; Vrundha and Thomas 2023; Rai 2023). Thus, the aims of the present study were to establish protocols for the multiplication of *U. tomentosa*, to employ SCoT markers to verify genetic diversity among micropropagated plantlets maintained *in vitro*, to evaluate the chemical diversity of these accessions by high-performance liquid chromatography (HPLC), and to introduce the micropropagated plants into their natural environment in the Brazilian Amazon.

Materials and methods

Plant material

Plants of *U. tomentosa* were identified by Dr. Piero Giuseppe Delprete (Herbier de Guyane, Institut de Recherche pour le Développement, Cayenne, French Guiana) and a specimen was deposited in the Medicinal Plant Herbarium of the Universidade de Ribeirão Preto (UNAERP) under accession number HJBOP 2723. All accessions used in the experiments were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number AB94A2F.

The accessions of *U. tomentosa* maintained *in vitro* at UNAERP were established from seeds of individual plants collected from the municipalities of Bannach (BN, State of Pará), Tarauacá (TA, State of Acre) and Boca do Acre (BC, State of Amazonas), all of which are located in the Brazilian Amazon rainforest as presented in Fig. 1.

Figure 1 here

Seed sterilization and *in vitro* germination

Seeds were sterilized by immersion in 1% w/v Cercobin® for 24 h under constant shaking, followed by treatment with 1% w/v sodium hypochlorite solution for 20 min under similar conditions. After surface asepsis, the seeds were washed with sterile distilled water and transferred to plates containing sucrose-free basal Murashige and Skoog (MS) medium (Murashige and Skoog 1962) solidified with 3 g·L⁻¹ of Gelzan™ CM (Sigma-Aldrich, St. Louis, MO, USA; # G1910) and adjusted to pH 6.0. Incubation was carried out in a growth chamber at 22 ± 2°C under a 16 h photoperiod provided by white fluorescent tubes (25 μmol·m⁻²·s⁻¹).

In order to test the influence of light on germination (photoblastism), batches of 50 seeds were incubated in the growth chamber under a 16 h photoperiod or dark conditions and the experiment repeated four times. The longevity of seeds was assessed by storing seeds at room temperature for one prior to germination.

A total of 114 seedlings were established *in vitro* and analyzed by HPLC to determine the proportions of the alkaloids considered to be the chemical markers of the species, namely mitraphylline and isomitraphylline. Twenty seedlings derived from seeds originating from the three different areas of the Brazilian rainforest were selected for further investigation on the basis of vigor and alkaloid content.

Optimization of culture conditions for *in vitro* micropropagation

In order to determine the influence of culture conditions on the efficiency of *in vitro* micropropagation, explants comprising 1 cm nodal segments from the 20 selected seedlings were cultivated either individually or in groups on different culture media, with or without growth regulators, in diverse types of culture vessels and under various light sources.

The experiment was of a completely randomized design and involved the following treatments: (i) Culture medium: individual explants were cultured in glass test tubes containing 5 mL of MS medium, woody plant medium (WPM; Lloyd and McCown 1980) or Schenck and Hildebrandt (SH; Schenck and Hildebrandt 1972) medium supplemented with 3% sucrose and solidified with 0.30% Gelzan CM at pH 6.0; (ii) Addition of growth regulators: individual explants were cultured in large jars containing 20 mL of WPM supplemented with cytokinins 6-benzylaminopurine (BAP), kinetin or zeatin at concentrations of 0, 0.5, 1.0, 2.5, 5.0 and 10.0 μM, and/or auxins naphthalene acetic acid (NAA) or indolebutyric acid (IBA) at concentrations of 0, 0.1 and 0.5 μM, or a combination of BAP (5.0 μM) and NAA or IBA (0, 0.1 and 0.5 μM). (iii) Size of glassware: individual explants were cultured in large jars (12 cm height x 5.4 cm diameter; 20 mL WPM), medium jars (8.5 cm height x 5.9 cm diameter; 20 mL WPM) or small test tubes (10 cm height x 2 cm diameter; 5 mL WPM) (Fig. 2A); (iv) Light source: individual explants were cultured in small test tubes containing 5 mL WPM and incubated under a 16 h photoperiod provided by light emitting diode (LED) arrays or fluorescent tubes (25 μmol·m⁻²·s⁻¹); and (v) Co-cultivation interaction: individual explants or groups of two to four were cultured in large jars containing 20 mL of WPM (Fig. 2B).

Figure 2 here

Unless otherwise stated, all explants were cultured on WPM supplemented with 3% sucrose and solidified with 0.30% Gelzan CM at pH 6.0, and maintained in a growth chamber at 22 ± 2 °C under a 16 h photoperiod provided by LED arrays ($25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The variables of interest, namely height of plantlets, number of buds, number of shoots and number of roots), were measured 60 days after cultivation of explants. Each treatment was repeated 10 times and each repetition included 10 plantlets (replicates).

Acclimatization of plantlets to greenhouse conditions and field cultivation

Plantlets were transferred to 162-cell plastic trays (model 7100235; Nutriplan, Cascavel, PR, Brazil) containing commercial substrate (Carolina Soil, Santa Cruz, RS, Brazil) and maintained for 60 days in a climate-controlled greenhouse (Van der Hoeven, Delft, The Netherlands) with a relative humidity of 70% achieved by operating micro-sprinklers for 5 min, three times daily. Plants were subsequently relocated to 1 L plastic bags containing 900 g of a mixture of soil, manure and sand (3:2:1) and remained in the greenhouse for 10 months with micro-sprinkler irrigation twice a day.

Acclimatized plants (40 ± 5 cm height) were transferred to forested areas in the municipality of Santarém, PA, Brazil, during the rainy season. Planting pits (20 cm x 20 cm x 20 cm) were dug near to native trees to provide support for the plants. The survival of the transplanted individuals was evaluated eighteen months after planting.

Analysis of the genetic variability of the *U. tomentosa* accessions

The genetic diversity of the 20 selected individuals maintained *in vitro* was determined by SCoT polymorphism analysis. Genomic DNA was extracted following the method of Doyle and Doyle (1987) as modified by Honório et al. (2017), and analyzed using the SCoT molecular marker technique following the method proposed by Collard and Mackill (2009). The 36 SCoT 18-mer primer sequences described by these authors were screened and eight (5, 8, 13, 15, 19, 24, 29 and 30) presenting the highest polymorphism with sharply resolved bands were selected for the full analysis.

Polymerase chain reaction (PCR) amplifications were performed in a thermal cycler (Jingle Scientific, Shanghai, China) with a reaction mixture containing 1 μL of 10x reaction buffer, 1 μL of dNTP mix (2.5 mM), 1 μL of primer (8 μM), 0.1 μL of Taq DNA polymerase (5 U/ μL), 2.5 μL of DNA (10 ng/ μL^{-1}) and deionized water to final volume (10 μL). Amplifications were carried out under the following conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 5 min. The PCR products were separated by electrophoresis on 3% agarose gel prepared in 1x tris-borate-EDTA (TEB) buffer containing ethidium bromide (0.5 $\mu\text{g}\cdot\text{mL}^{-1}$) and visualized under a Gel Logic 112 digital imaging

system (Bruker BioSpin, Billerica, MA, USA) and analyzed using Carestream Molecular Imaging (Rochester, NY, USA) software. The bands produced were counted manually to generate a binary matrix (presence or absence of bands) for subsequent data analysis.

Chemical analysis of the *U. tomentosa* accessions

The alkaloid content of the *U. tomentosa* accessions was determined using a previously validated HPLC-diode array detector method (Honório et al. 2017) with external standards mitraphylline (LGC Standards CDx, Teddington, Middlesex, UK; # CDX-00013955–005) and isomitraphylline (Chromadex/Niagen Bioscience, Los Angeles, CA, USA; # ASB-00009417). The limits of detection (LOD) and quantification (LOQ) were: mitraphylline (0.22 and 0.75 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively) and isomitraphylline (0.12 and 0.24 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively). Validation of the analytical data (LOD, LOQ, linearity, precision and accuracy) was performed in accordance with the guidelines of the Agência Nacional de Vigilância Sanitária (2003). Calibration curves were constructed using triplicate injections of the standard alkaloids at concentrations of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.9 $\mu\text{g}\cdot\text{mL}^{-1}$. The ratio of peak areas of the target alkaloids were plotted as a function of the corresponding concentration using linear regression of the standard curves as described by Honório et al. (2017).

Data analysis

Data relating to the micropropagation experiments were submitted to analysis of variance (ANOVA) and, when significant, means were compared using the Scott-Knott test at $\alpha = 0.05$. Statistical analyses were performed with the aid of the program Sisvar (Ferreira 2011).

Binary data (presence or absence of bands) obtained from the ScoT marker analysis were employed to estimate the genetic similarity between the 20 accessions of *U. tomentosa* using the Jaccard coefficient and unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis. Phylogenetic trees were constructed and analysed with the aid of Free Tree software (Hampl et al. 2001). Genetic variability was investigated using principal coordinates analysis (PCoA) with the help of GenAlEx 6.5 software (Peakall and Smouse 2012).

Bayesian assignment analysis was conducted using STRUCTURE (Pritchard et al. 2000) to infer the genetic structure of the accessions, grouping individuals into K distinct clusters (K = 1 to 20) based on genetic similarities. Analyses were run under the correlated allele frequencies and no admixture model, which assumes that each individual originated from a single cluster, with a burn-in of 300,000 steps followed by 500,000 Markov chain Monte Carlo (MCMC) iterations. The most likely number of clusters (K) was determined using the ΔK method of Evanno et al. (2005).

Results and discussion

Germination and establishment of seedlings in vitro

Seeds of *U. tomentosa* showed positive photoblastism. Germination under a 16 h photoperiod occurred between 10 and 15 days after inoculation onto the medium, with a germination rate of $92 \pm 3.4\%$, while no seeds germinated during this time period under dark conditions. Seeds exhibited low longevity since the germination rate declined to $10 \pm 0.5\%$ after one year of storage under the experimental conditions, and no germination was observed following storage for two years.

The 114 seedlings established *in vitro* were assessed for vigor and alkaloid content and 20 were assigned for the micropropagation study. The selected accessions comprised eight originating from TA (UTTA1, UTTA2, UTTA5, UTTA9, UTTA10, UTTA19, UTTA20 and UTTA30), 11 from BN (UTBN207, UTBN233, UTBN332, UTBN354, UTBN358, UTBN499, UTBN750, UTBN767, UTBN847, UTBN902 and UTBN985) and one from BC (UTBC1).

Optimization of micropropagation conditions

The effects of culture conditions on the development of *U. tomentosa* explants are presented in Table 1. Regarding culture medium, WPM favored increased plantlet height and number of shoots compared with MS and SH media. This finding may be related to the lower concentration of mineral salts in WPM relative to MS and SH media, a factor frequently associated with superior responses in woody species and plants native to oligotrophic environments. Considering that *U. tomentosa* plants occur naturally in nutrient-poor Amazonian soils, it is reasonable to assume that the physiology of the species is better adapted to low nutrient concentrations as already discussed for tropical forest species (Lloyd and McCown 1980; Ferreira et al. 2006).

Table 1

Effects of diverse culture conditions on the development of *Uncaria tomentosa* explants *in vitro*

Treatments	Variables evaluated			
	Height of plantlets (cm)	No. of shoots [#]	No. of buds [#]	No. of roots
<i>Culture medium</i>				
MS	2.80b	1.28b	4.48a	-
SH	1.29b	1.05b	3.52b	-
WPM	3.58a	1.81a	4.28a	-
<i>Addition of growth regulators</i>				
BAP (μM)				
0	2.37a	1.33b	6.47b	3.93a
0.5	1.50b	1.33b	5.93b	2.00a
1.0	1.25b	0.93b	9.67a	1.87a
2.5	1.16b	1.80a	7.27b	0.40b
5.0	1.16b	2.13a	8.13a	1.60a
10.0	1.21b	2.06a	6.40b	0.80b
Kinetin (μM)				
0	2.07a	1.40a	5.93a	4.27a
0.5	1.33a	1.53a	3.80b	2.27b
1.0	1.98a	1.00a	3.00b	1.93b
2.5	1.75a	1.33a	2.73b	1.53b
5.0	1.96a	1.27a	2.96b	1.67b
10.0	1.57a	1.47a	2.57b	0.47b
Zeatin (μM)				
BAP - benzylaminopurine, IBA - indolebutyric acid, MS - Murashige and Skoog, NAA - naphthalene acetic acid, SH - Schenck and Hildebrandt, WPM - woody plant medium, LED - light emitting diode				
Within each treatment, mean values in the columns followed by dissimilar lowercase letters are significantly different according to the Scott-Knot test at 5% probability				
[#] Variables submitted to $\sqrt{x + 0.5}$ transformation				

Treatments	Variables evaluated			
	Height of plantlets (cm)	No. of shoots [#]	No. of buds [#]	No. of roots
0	1.96a	1.40a	5.93a	2.73a
0.5	1.86a	1.53a	4.27a	1.20a
1.0	2.34a	1.00a	3.33b	1.27a
2.5	1.45b	1.33a	2.47b	0.20b
5.0	1.43b	1.27a	2.43b	0.33b
10.0	1.20b	1.47a	2.23b	0.13b
NAA (μM)				
0	1.46b	1.37b	3.27b	1.20c
0.1	1.78a	1.40b	4.37a	2.73b
0.5	2.10a	1.76a	4.63a	3.97a
IBA (μM)				
0	1.46b	1.37b	3.27b	1.20c
0.1	1.04c	1.27b	2.57b	1.90b
0.5	2.12a	1.77a	5.90a	3.30a
Cytokinin (μM) + auxin (μM)				
0	2.50a	1.28b	3.85b	0.21a
BAP (5) + NAA (0.5)	2.52a	2.06a	5.13a	0.40a
BAP (5) + IBA (0.5)	2.08a	1.20b	3.92b	0.34a
<i>Size of glassware</i>				
Large jar	1.90a	1.86a	5.96b	2.46a
Medium jar	2.35a	1.86a	7.80a	3.03a
Small test tube	2.31a	1.53a	5.29b	2.90a
BAP - benzylaminopurine, IBA - indolebutyric acid, MS - Murashige and Skoog, NAA - naphthalene acetic acid, SH - Schenck and Hildebrandt, WPM - woody plant medium, LED - light emitting diode				
Within each treatment, mean values in the columns followed by dissimilar lowercase letters are significantly different according to the Scott-Knot test at 5% probability				
[#] Variables submitted to $\sqrt{x + 0.5}$ transformation				

Treatments	Variables evaluated			
	Height of plantlets (cm)	No. of shoots [#]	No. of buds [#]	No. of roots
<i>Light source</i>				
LED arrays	2.14a	1.32a	6.60a	3.66a
Fluorescent tubes	1.90a	1.50a	6.14a	2.30b
<i>Co-cultivation of explants</i>				
1 explant	2.38a	1.53a	7.33a	3.13a
2 explants	2.69a	1.23a	7.35a	3.13a
3 explants	1.77b	1.42a	4.93b	2.27b
4 explants	2.25a	1.57a	6.68a	2.95a
BAP - benzylaminopurine, IBA - indolebutyric acid, MS - Murashige and Skoog, NAA - naphthalene acetic acid, SH - Schenck and Hildebrandt, WPM - woody plant medium, LED - light emitting diode				
Within each treatment, mean values in the columns followed by dissimilar lowercase letters are significantly different according to the Scott-Knot test at 5% probability				
[#] Variables submitted to $\sqrt{x + 0.5}$ transformation				

Table 1 here

Supplementation of WPM with cytokinins BAP, kinetin or zeatin in the concentration range 0.5 to 10.0 μM revealed that 5 μM BAP was most efficient in breaking apical dominance and stimulating the production of buds and shoots in *U. tomentosa*. Similar results have been reported for this species and other woody medicinal plants (Luna-Palencia et al. 2013; Okao et al. 2025). Addition of auxins to tissue culture medium may also assist in the production of multiple shoots, and in the present study, a concentration of 0.5 μM of NAA or IBA induced significantly higher numbers of shoots. WPM supplemented with a combination of 5 μM BAP and 0.5 μM NAA produced a statistically greater number of buds (5.13 ± 0.43) and shoots (2.06 ± 0.36) compared with 5 μM BAP + 0.5 μM IBA. However, the combination treatment also gave rise to some undesirable effects such as swelling of the base and reduced vigor of plantlets when maintained for prolonged periods. These effects have been associated with cytokinin/auxin imbalance, which causes morphophysiological changes and reduction in shoot quality (Manchanda et al. 2025). Interestingly, no significant differences were observed regarding plantlet height or number of roots in any of the cytokinin-auxin combinations.

The *in vitro* cultivation of *U. tomentosa* in WPM, MS and quarter strength MS media supplemented with BAP at concentrations ranging from 2.2 to 44.4 μM has been described previously, with reported

multiplication rates ranging between 1.5 and 5 ± 2 shoots per plantlet (Pereira et al. 2008; Venutolo 2010; Raposo and Teixeira 2011; Luna-Palencia et al. 2013). Although these studies demonstrated the feasibility of the *in vitro* cultivation of *U. tomentosa*, none of them established an optimized protocol that integrates multiplication efficiency, maintenance of genetic diversity and post-acclimatization performance.

As shown in Table 1, medium sized glass jars afforded the best conditions for the multiplication of *U. tomentosa* since the number of buds was significantly higher than those produced in the larger or smaller vessels. In tissue culture laboratories, it is common practice to evaluate different types of vessels to determine the most suitable growing conditions. .

Whilst the application of different sources of light exerted no significant influence on the height of plantlets or the numbers of shoots or buds, exposure to LED arrays induced a higher number of roots compared with fluorescent tubes (3.66 and 2.30 per plantlet, respectively). The use of fluorescent tubes in growth chambers has always been very common, although the tendency is to replace them by LED arrays owing to their lower energy consumption.

The use of LEDs as an alternative to conventional lighting in plant tissue culture installations reportedly gives satisfactory results regarding rhizogenesis, stem elongation, axillary shoot formation and increased photosynthetic capacity, all of which can be regulated by adjusting the spectral properties of the source (Gupta and Jatothu 2013; Marino et al. 2025). The reported advantages of LED arrays, such as greater durability, lower operating cost and higher energy efficiency, will serve as a stimulus to upgrade the lighting systems in growth chambers.

There were no significant differences in the height of plantlets or the number of shoots, buds or roots of explants cultivated in isolation or in co-cultivation with two or four explants per vessel. However, the height of plantlets and the number of buds and roots were reduced significantly when three explants were cultivated in the same vessel. Thus, co-cultivation of four explants is recommended for *U. tomentosa* to maximize the use of space and economize culture medium without detriment to the growth of plantlets. Co-cultivation of explants can be a cost-effective technique for the large-scale micropropagation of species whose development is not impaired under such conditions. In the case of *U. tomentosa*, large-scale micropropagation with two or four explants per vessel proved to be a productive approach since it optimizes the use of physical space, culture medium and labor without impairing *in vitro* morphogenesis. The assessment of co-cultivation on solid medium is particularly relevant since liquid or temporary immersion bioreactors, such as the RITA® system developed by CIRAD, although efficient, can cause physiological disturbances, including hypoxia and hyperhydricity, especially in woody species (Bethge et al., 2023; Ersali, 2024).

The optimised micropropagation protocol developed in this study employed medium-sized flasks containing 20 mL of woody plant medium (WPM) supplemented with 5.0 μ M BAP, with four explants per flask maintained under a 16 h photoperiod provided by LED arrays. Under these conditions, explants produced an average of 2.13 shoots bearing a total of 8.13 buds per explant.

Acclimatization of plantlets and survival in forested areas

The acclimatization of micropropagated *U. tomentosa* plantlets was considered excellent considering that the species is restricted climatically to tropical humid environments. The survival rate of the plantlets after two months under *ex vitro* conditions was 100%, while that of plants transferred to their natural habitat in the Amazonian forest was $46 \pm 18\%$ after eighteen months of planting (Fig. 3).

Figure 3 here

Genetic diversity of *U. tomentosa* accessions maintained *in vitro*

Genomic DNA was extracted efficiently from the 20 selected *U. tomentosa* genotypes maintained *in vitro*. The eight primers employed in the ScoT marker analysis of genetic diversity amplified between 23 and 40 bands per primer, totaling 150 bands and exhibiting an average polymorphism of 88%. This high level of polymorphism was in accord with the value (92.22%) reported by Pinto et al. (2022) for accessions of *U. guianensis* maintained in an *in vitro* germplasm bank. Such notable degree of polymorphism within the genus *Uncaria* is significant and essential for conservation of the species.

The UPGMA dendrogram (Fig. 4) and the dispersion (variance) PCoA plot (Fig. 5) revealed genetic structuring and variability among the 20 individuals cultivated *in vitro*. Cluster analysis indicated grouping patterns among the 20 accessions that mirrored their geographic origin in which the genetic similarity among genotypes from TA was higher than among those from BN, whilst the accession from BC (UTBC1) formed an independent branch signifying comparatively greater genetic divergence.

Is it worth noting that while UTBC stands out as genetically dissimilar from the UTТА and UTBN accessions (Figs. 4 & 5), it is UTТА1 that is assigned entirely to one cluster whilst UTBC has mixed ancestry like all of the other accessions (Fig. 6).

Bayesian model-based clustering performed using the software STRUCTURE revealed the presence of two genetic groups, as represented by the red and green bands in Fig. 6. The bars of all accessions, with the single exception of UTТА1, showed both red and green segments indicating that the vast majority of individuals were of mixed ancestry with different proportions of membership in both clusters. In contrast, the uniform red bar presented by UTТА1 suggests that it is genetically assigned entirely to one cluster with no admixture.

The results of the present study are analogous to those reported by Honório et al. (2017) in which the existence of genetic structuring and low gene flow was demonstrated in eight natural populations of *U. tomentosa*. In particular, the conclusions of these authors regarding the ancestry of the natural populations align with the inference from STRUCTURE analysis of the presence of two gene groups within the 20 *in vitro* accessions. This result is significant because it implies that the *in vitro* germplasm bank of *U. tomentosa* is representative of the genetic groups found in natural populations. Maintaining this genetic diversity in accessions cultivated *in vitro* is especially relevant from a conservationist point

of view, since it reduces the risks of genetic erosion during clonal propagation and allows the selection of elite genotypes for the pharmaceutical industry without compromising natural populations.

Few actively managed germplasm collections have been assessed for the genetic diversity of their accessions. Knowledge of genetic variation is particularly important in the case of *in vitro* collections since they maintain accessions free from disease and in their specific allelic state, thereby assisting in the identification of useful traits that can be used in genetic improvement programs (Anglin et al., 2025).

Chemical characteristics of the selected individuals

HPLC analysis revealed significant quantitative and qualitative variability in alkaloid content among the 20 accessions of *U. tomentosa* (Table 2). Thus, UTBN233 presented the highest concentration of mitraphylline ($3.329 \text{ mg}\cdot\text{g}^{-1}$ fresh weight), while UTTA5 exhibited the highest concentration of isomitraphylline ($1.422 \text{ mg}\cdot\text{g}^{-1}$ fresh weight). Although the majority of accessions produced both alkaloids, two individuals from BN (UTBN358 and UTBN750) contained no mitraphylline and two (UTBN233 and UTBN332) contained no isomitraphylline, whereas UTBN207 accumulated neither of these alkaloids.

Table 2
Alkaloid content of the 20 selected *Uncaria tomentosa* individuals

Individuales	Mitraphylline (mg·g ⁻¹ fresh weight)	Isomitraphylline (mg·g ⁻¹ fresh weight)
UTTA 1	0.08 m	0.00 j
UTTA 2	2.52 c	0.53 f
UTTA 5	2.24 d	1.43 a
UTTA 9	1.63 g	1.14 b
UTTA 10	1.87 f	1.00 c
UTTA 19	2.80 b	1.17 b
UTTA 20	2.07 e	0.69 e
UTTA 30	2.27 d	0.94 c
UTBN 207	0.00 m	0.00 j
UTBN 233	3.32 a	0.00 j
UTBN 332	1.04 i	0.00 j
UTBN 354	0.90 j	0.25 h
UTBN 358	0.00 m	0.32 g
UTBN 499	0.88 j	0.20 h
UTBN 750	0.00 m	0.31 g
UTBN 767	1.04 i	0.12 i
UTBN 847	0.73 k	0.83 d
UTBN 902	1.01 i	0.30 g
UTBN 985	1.35 h	0.00 j
UTBC 1	0.21 l	0.04 j
Mean values in each column followed by dissimilar lowercase letters are significantly different according to the Scott-Knot test at 5% probability.		

This variation in alkaloid constituents appears to be a normal pattern in *U. tomentosa* since it has been described for natural populations of the species in previous studies (Peñaloza et al. 2015; Torrejón et al. 2010). The existence of chemical heterogeneity reinforces the importance of conserving multiple genotypes, especially considering the interest of the phytotherapeutic industry in plant materials with high levels of these bioactive compounds. In this context, the introduction of genetically diverse and

chemically distinct accessions into *in vitro* germplasm banks is a pertinent strategy for both the conservation and sustainable use of the species.

Tissue culture has been widely recognized as an important conservation strategy for threatened medicinal species that are subject to predatory exploitation (Moraes et al. 2021). Furthermore, micropropagation and *in vitro* germplasm maintenance allow not only the rapid multiplication of selected genotypes but also the preservation of genetic variability, especially for species with difficult sexual propagation or low seed viability (Singh and Chokheli 2025).

Conclusions

Genetic analysis by SCoT markers of 20 accessions of *U. tomentosa* cultivated *in vitro* indicated that they descended from two inferred ancestral genetic clusters, a situation similar to that established for natural populations. Fifteen of the 20 genotypes accumulated both mitraphylline and isomitraphylline, albeit in varying concentrations, which is typical of the species. In contrast, four of the genotypes accumulated only one of the alkaloids and one genotype contained neither. The survival rate of the plants introduced into the natural environment was highly satisfactory considering the environmental and abiotic constraints, such as nutrient and light limitation and high humidity and temperature, posed by the Amazon rainforest. The results obtained reinforce the potential of *in vitro* culture as a complementary approach to the *in situ* conservation of *U. tomentosa*, since both contribute to the protection of natural populations and the sustainable supply of plant material.

Declarations

Ethical approval

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest

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Author Contribution

Conceptualization: I.C.G.H., J.S.C and A.M.S.P.; Methodology: I.C.G.H., J.S.C., G.H.T.P., B.S.R., R.N.S.M., E.F.S and W.S.; Data curation: I.C.G.H., G.H.T.P. and B.W.B.; Formal analysis: I.C.G.H., J.S.C. and B.W.B.; Investigation: I.C.G.H., J.S.C. and B.W.B.; Visualization: I.C.G.H. and S.C.F.; Writing - Original draft: I.C.G.H., B.S.R., S.C.F and B.W.B.; Validation: A.M.S.P; Writing – review & editing: A.M.S.P; Supervision: A.M.S.P
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Data availability

Data is available upon reasonable request.

References

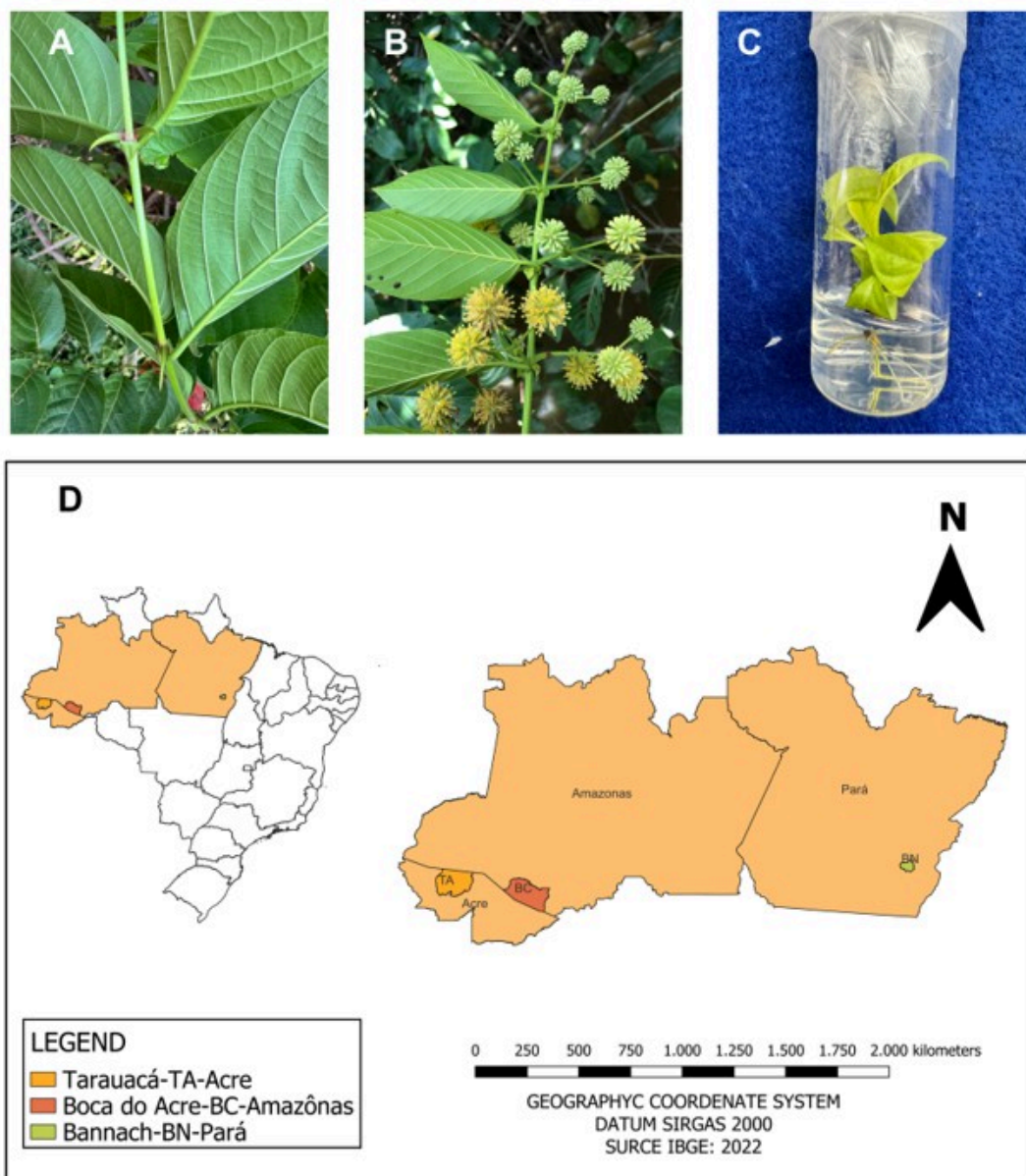
1. Agência Nacional de Vigilância Sanitária (2003) Resolução-RE n. 899, de 29 de maio de 2003. Dispõe sobre o guia para validação de métodos analíticos e bioanalíticos. ANVISA. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2003/res0899_29_05_2003 Accessed 6 February 2026
2. Anglin NL, Wenzl P, Azevedo V, Lusty C, Ellis D, Gao D (2025) Genotyping genebank collections: Strategic approaches and considerations for optimal collection management. *Plants* 14(2):252. <https://doi.org/10.3390/plants14020252>
3. Arado GM, Amatto PPG, Marins M, Rizzi ES, França SC, Coppede JS, Carmona F, Pereira MAS (2024) Anti-inflammatory and/or immunomodulatory activities of *Uncaria tomentosa* (cat's claw) extracts: A systematic review and meta-analysis of *in vivo* studies. *Front Pharmacol* 15:1378408. <https://doi.org/10.3389/fphar.2024.1378408>
4. Bethge H, Mohammadi Nakhjiri Z, Rath T, Winkelmann T (2023) Towards automated detection of hyperhydricity in plant *in vitro* culture. *Plant Cell Tiss Organ Cult* 154:551–573. <https://doi.org/10.1007/s11240-023-02528-0>
5. Blumenthal M (ed) (1997) World Health Organization Medicinal Plants Monographs. *HerbalGram* 40(38)
6. Collard BCY, Mackill DJ (2009) Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol Biol Rep* 27:86–93. <https://doi.org/10.1007/s11105-008-0060-5>
7. Doyle JJ, Doyle JL (1987) Isolation of plant DNA from fresh tissue. *Focus (Rockville)* 12(1):13–15
8. Ersali Y (2024) Control of hyperhydricity of *Pistacia khinjuk* stocks *in vitro* shoots. *BMC Biotechnol* 24:97. <https://doi.org/10.1186/s12896-024-00929-3>
9. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14(8):2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>

10. Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Cienc Agrotec* 35(6):1039–1042. <https://doi.org/10.1590/S1413-70542011000600001>
11. Ferreira SJF, Luizão FJ, Miranda SAF, Silva MSR, Vital ART (2006) Nutrients in soil solution in an upland forest submitted to selective logging in central Amazonia. *Acta Amaz* 36(1):59–68. <https://doi.org/10.1590/S0044-59672006000100008>
12. Gupta SD, Jatothu B (2013) Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnol Rep* 7:211–220. <https://doi.org/10.1007/s11816-013-0277-0>
13. Hampl V, Pavlíček A, Flegr J (2001) Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. *Int J Syst Evol Microbiol* 51:731–735. <https://doi.org/10.1099/00207713-51-3-731>
14. Honório ICG, Bertoni BW, Pereira AMS (2016) *Uncaria tomentosa* and *Uncaria guianensis* an agronomic history to be written. *Cienc Rural* 46(8):1401–1410. <https://doi.org/10.1590/0103-8478cr20150138>
15. Honório ICG, Bertoni BW, Telles MPC, Braga RS, França SC, Coppede JS, Correa VSC, Diniz Filho JAF, Pereira MAS (2017) Genetic and chemical diversity of *Uncaria tomentosa* (Willd. ex. Schult.) DC. in the Brazilian Amazon. *PLoS ONE* 12(5):e0177103. <https://doi.org/10.1371/journal.pone.0177103>
16. Lloyd G, McCown BH (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Combined Proc Intl Plant Prop Soc* 30:421–427
17. Luna-Palencia GR, Huerta-Heredia AA, Cerda-García-Rojas CM, Ramos-Valdivia AC (2013) Differential alkaloid profile in *Uncaria tomentosa* micropropagated plantlets and root cultures. *Biotechnol Lett* 35:791–797. <https://doi.org/10.1007/s10529-012-1128-8>
18. Manchanda P, Sharma D, Kaur G, Kaur H, Vanshika (2025) Exploring the significance of somaclonal variations in horticultural crops. *Mol Biotechnol* 67:2185–2203. <https://doi.org/10.1007/s12033-024-01214-6>
19. Marino LA, Ruffa P, Mozzanini E, Patono DL, Sereno A, Pavese V (2025) LEDs in plant tissue culture: Boosting micropropagation of *Castanea sativa* cultivars. *J Plant Growth Regul* 44:6046–6060. <https://doi.org/10.1007/s00344-025-11812-6>
20. Ministério da Saúde (2013) Relação nacional de medicamentos essenciais (RENAME). Sistema Único de Saúde (SUS). <https://www.gov.br/saude/pt-br/composicao/sectics/renome> Accessed 6 Feb 2026
21. Moraes RM, Cerdeira AL, Lourenço MV (2021) Using micropropagation to develop medicinal plants into crops. *Molecules* 26(6):1752. <https://doi.org/10.3390/molecules26061752>
22. Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* 15:473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
23. Okao M, Bharati R, Fernández-Cusimamani E (2025) Effect of explant physiology and media composition on callogenesis of *Vitellaria paradoxa* leaf explants. *Horticulturae* 11(9):1127. <https://doi.org/10.3390/horticulturae11091127>

24. Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28(19):2537–2539.
<https://doi.org/10.1093/bioinformatics/bts460>
25. Peñaloza EMC, Kaiser S, Resende PE, Pittol V, Carvalho ÂR, Ortega GG (2015) Chemical composition variability in the *Uncaria tomentosa* (cat's claw) wild population Quim Nova. 38(3):378–386.
<https://doi.org/10.5935/0100-4042.20150007>
26. Pereira RCA, Valente LMM, Pinto JEBP, Bertolucci SKV, Bezerra GM, Alves FF, Santos PFP, Benevides PJC, Siani AC, Rosario SL, Mazzei JL, d'Avila LA, Gomes LNF, Aquino-Neto FR, Emmerick ICM, Carvalhaes SF (2008) *In vitro* cultivated *Uncaria tomentosa* and *Uncaria guianensis* with determination of the pentacyclic oxindole alkaloid contents and profiles. *J Braz Chem Soc* 19(6):1193–1200. <https://doi.org/10.1590/S0103-50532008000600021>
27. Pinto GHT, Lopes AA, Morel LJF, Crevelin EJ, Miranda CES, Contini SHT, França SC, Bertoni BW, Pereira AMS (2022) Genetic diversity among genotypes of *Uncaria guianensis* (Aubl.) J.F. Gmel. maintained in an *in vitro* germplasm bank. *3 Biotech* 12:8. <https://doi.org/10.1007/s13205-021-03016-y>
28. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155(2):945–959. <https://doi.org/10.1093/genetics/155.2.945>
29. Rai MK (2023) Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. *Planta* 257:34. <https://doi.org/10.1007/s00425-023-04067-6>
30. Raposo A, Teixeira RB (2011) Metodologia científica: cultivo *in vitro* de Unha-de-gato. Circular Técnica 57. Embrapa Acre, Rio Branco, Brazil.
<https://www.infoteca.cnptia.embrapa.br/bitstream/doc/912085/1/24058.PDF> Accessed 06 February 2026
31. Schenck RU, Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can J Bot* 50(1):199–204.
<https://doi.org/10.1139/b72-026>
32. Singh RK, Chokheli VA (2025) Plant biotechnology: Applications in *in vitro* plant conservation and micropropagation. *Horticulturae* 11(4):358. <https://doi.org/10.3390/horticulturae11040358>
33. Sirikonda A, Jogam P, Mood K, Allini VR (2023) Micropropagation of 2-methoxy-4-vinyl phenol rich *Flemingia strobilifera* and assessment of genetic and biochemical fidelity by SCoT and GC-MS analysis. *Plant Cell Tiss Organ Cult* 154:541–550. <https://doi.org/10.1007/s11240-023-02526-2>
34. Torrejón GD, Martín JJG, Loayza DG, Alanoca R (2010) Contenido de alcaloides en corteza de *Uncaria tomentosa* (Wild.) DC procedente de diferentes hábitats de la región Ucayali-Perú. *Rev Soc Quim Peru* 76(3):271–278
35. United States Pharmacopeial Convention (2022) Cat's Claw (*Uncaria tomentosa* (Willd.) DC.). In: *United States Pharmacopeia and National Formulary (USP–NF)*. United States Pharmacopeial Convention, Rockville

36. Venutolo AS (2010) Establecimiento *in vitro* y cultivo de células de la Uña de gato (*Uncaria tomentosa*). Tecnol Marcha 23(5):24–33
37. Vrudha CPK, Thomas TD (2023) Indirect shoot regeneration from root explants, assessment of clonal fidelity of regenerated plants using SCoT primers and antioxidant analysis in *Thottea siliquosa* (Lamk.) Ding Hou. Plant Cell Tiss Organ Cult. 155:255–266.
<https://doi.org/10.1007/s11240-023-02578-4>
38. Zavala A, Zevallos PP (1996) Taxonomía, distribución geográfica y status del género *Uncaria* en el Perú. Facultad de Ciencias Forestales, Universidad Nacional Agraria La Molina, Lima, Peru. 103 pp

Figures



State	Municipality	Genotype code	Latitude (S)	Longitude (W)
Acre	Tarauacá	TA	7° 54' 51.2"	71° 25' 50.1"
Pará	Bannach	BN	7° 33' 24.0"	50° 37' 12.4"
Amazonas	Boca do Acre	BC	8° 50' 51.1"	67° 18' 23.2"

Figure 1

Uncaria tomentosa: **A** - Aerial parts, **B** – Flowers, and **C** - Plantlet grown *in vitro*;. Panel **D** shows the collection location of individuals in the Brazilian Amazon

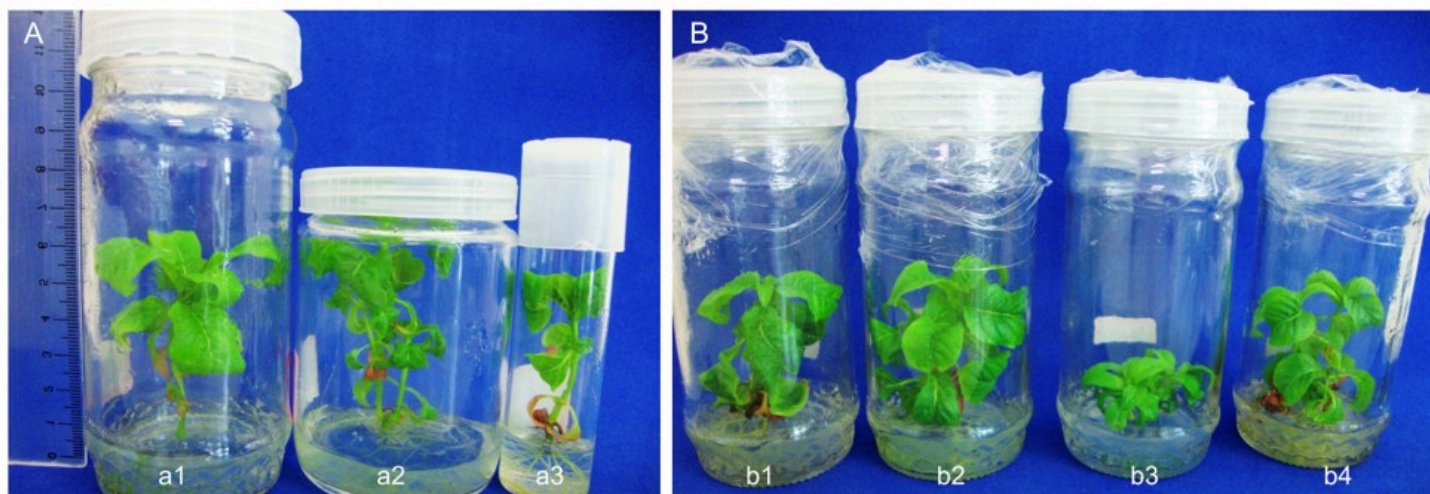


Figure 2

Different treatments employed in optimizing the micropropagation protocol of *Uncaria tomentosa* showing: **A** - Size of glassware (**a1**) large jar, (**a2**) medium jar, (**a3**) small test tube; and **B** - Co-cultivation of explants: (**b1**) one explant/flask, (**b2**) two explants/flask, (**b3**) three explants/flask, (**b4**) four explants/flask



Figure 3

Uncaria tomentosa: **A** - plantlets *in vitro*, **B** - plants acclimatized in the greenhouse, **C** - plants transferred to a natural environment, **D** - plant after eighteen months cultivation in the Amazon rainforest

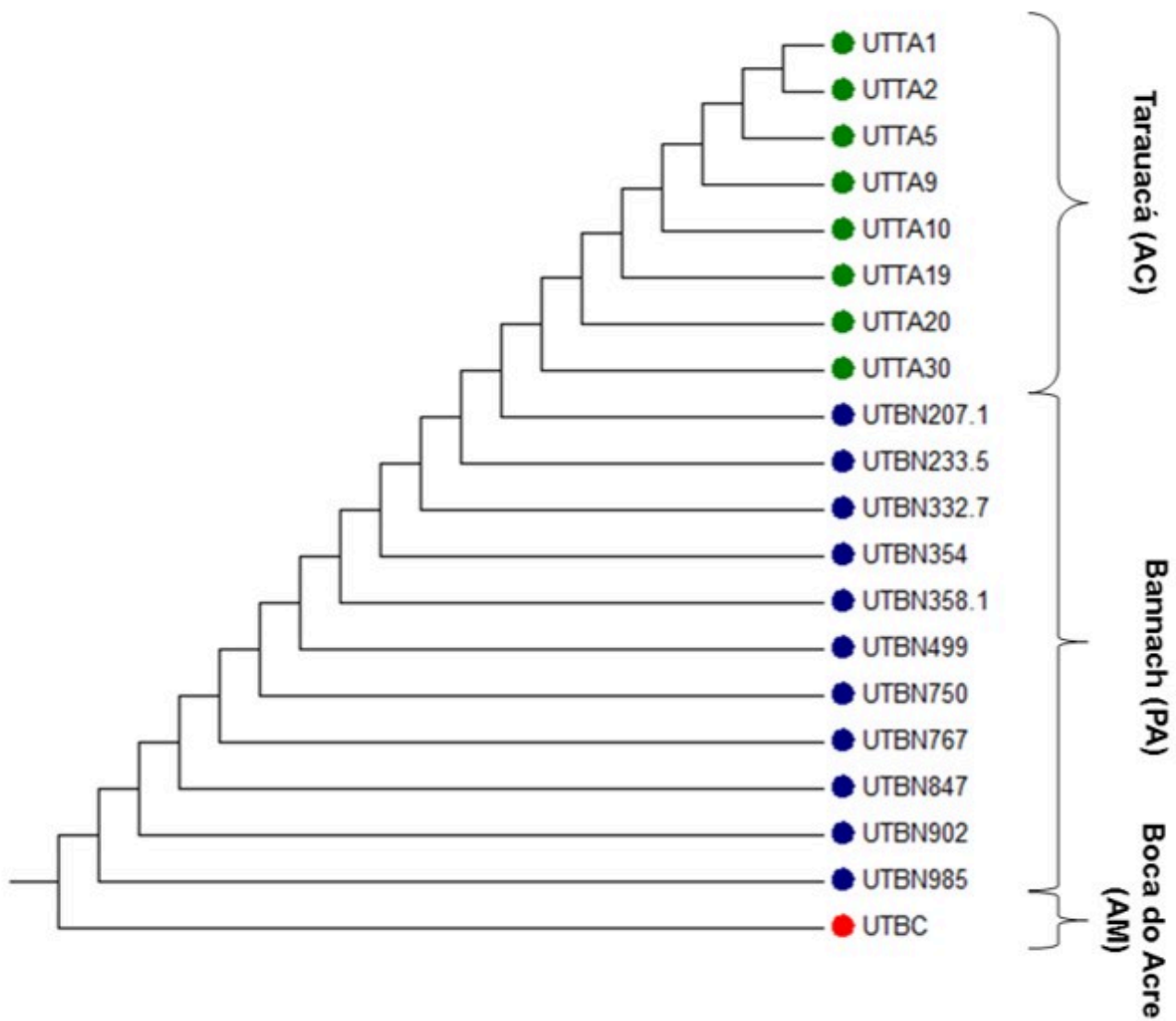


Figure 4

UPGMA dendrogram of 20 selected *Uncaria tomentosa* individuals generated using SCoT markers

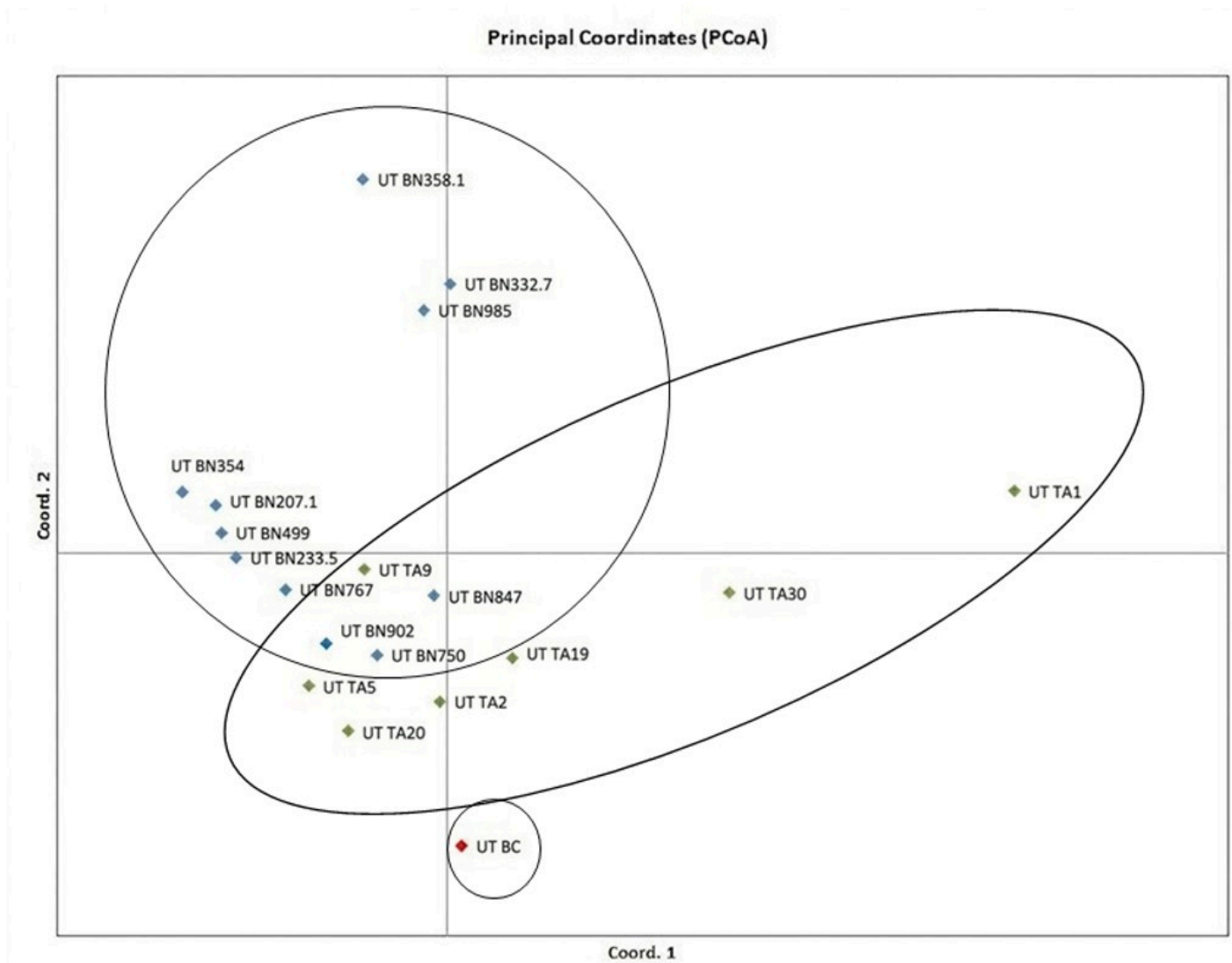


Figure 5

Principal coordinates analysis (PCoA) plot obtained from the Jaccard similarity matrix, generated using SCoT markers, showing dispersion among the 20 selected *Uncaria tomentosa* accessions

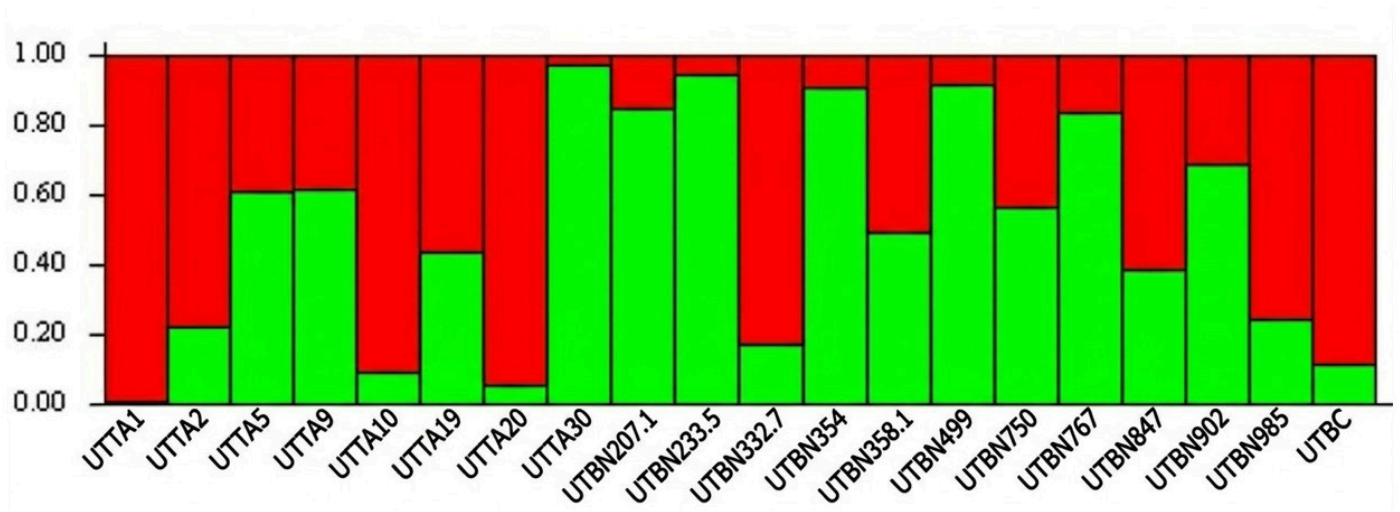


Figure 6

Bayesian clustering analysis of 20 *Uncaria tomentosa* accessions with bar plot constructed using software STRUCTURE. The red and green bars correspond to the proportion of individuals assigned to a hypothetical cluster.