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Towards Conserving Crop Wild Relatives along the Texas–Mexico Border: The Case of *Manihot walkerae*

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Abstract: Walker's Manihot, *Manihot walkerae*, is an endangered species endemic to south Texas and northeastern Mexico and is a Crop Wild Relative (CWR) of the international and economically important crop cassava (*M. esculenta*). *Manihot walkerae* is globally endangered (IUCN's Redlist, Texas list, USA); however, it is not recognized on the Mexican list of endangered species (NOM-059-SEMARNAT). We assessed the status of *M. walkerae* in Mexico and re-evaluated its global status. According to our analysis, *M. walkerae* should be considered an endangered species based on the IUCN's assessment method and a threatened species in Mexico based on the Mexican criteria. Our findings encourage the establishment of sound conservation plans for *M. walkerae* along the Texas–Mexico border.

Keywords: endangered; crop wild relative; extinction risk assessments; area of occupancy; extent of occurrence

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1. Introduction

A globally recognized and necessary component of maintaining food security as human populations continue to expand is the conservation of crop wild relatives (CWR) [1,2]. CWR are crucial for sustainable agricultural production because they are sources of important genetic material for closely related crop species. They can provide beneficial traits such as increased resistance to abiotic and biotic stressors (e.g., temperature, disease, and herbivory) to crops [2,3]. As is true of many of the world's species, the diversity of CWR is threatened because of anthropogenic disturbances that contribute to habitat destruction, climate change, overexploitation, and the introduction of invasive species [2,4]. In efforts to conserve this diversity, both in situ (e.g., land protection) and ex situ (e.g., seed banks) methods provide solutions to these challenges. In situ methods, which conserve CWR species in their natural habitat, provide the added benefit of allowing the CWR to continue serving its ecological role as well as continuing the dynamic evolution of the species in its natural environment [1]. The use of the IUCN's Redlist criteria is a means of promoting in situ conservation for CWR by assessing their extinction risk. Unfortunately, many of these species remain unassessed and for this reason, it is important to undertake threat assessments for as many CWR as possible, especially for those that are priority CWR species [1,2].

The International Union for Conservation of Nature (IUCN) Redlist provides a comprehensive and reliable method for assessing the extinction risk of species that can be used at a global level [5]. The IUCN's method considers five criteria that include a species' population size and geographical range, as well as an extinction probability analysis. These values are then used to assess a species' extinction risk and to classify species into one of seven risk categories, from species of least concern to those that are extinct [5]. However, a disadvantage of the IUCN's method is that, in some cases, the data needed to assess a species' extinction risk are often not readily available. This scenario can then make it difficult or impossible to use the five criteria method effectively. In these cases, species are referred to as data-deficient by the IUCN [6–8]. Species distribution modeling (SDM) can be used when assessing a species' extinction risks, specifically to support the extent of occurrence (EOO) and area of occupancy (AOO), which are both measures of a species' geographic range [7–11]. SDM is especially helpful when species are data-deficient because it can compensate for a lack of distributional data [8]. An added benefit of this tool is its capacity for a more rapid assessment of a species' extinction risk by not having to spend additional time gathering new occurrence data [12]. This type of rapid assessment allows for the timely reinforcement of a species' predicament and, as a result, may potentially improve its chance of survival since there is more time to address extinction threats [13].

Cassava, *Manihot esculenta* Crantz, is a widely used food source, with approximately 200 million people worldwide dependent on this crop [14]. As such, it is of great importance for food security and is considered a priority crop [1,15]. A concern for cassava is that many of its CWR have been negatively affected by anthropogenic activities, and the natural occurrence of these species has diminished significantly (e.g., in Brazil) [16]. Consequently, there is a demonstrated need for the conservation of cassava CWR, especially since they provide genetic source material for improving yield, drought resistance, and disease resistance, among other benefits [1]. Walker's Manihot, *Manihot walkerae* Croizat (Euphorbiaceae) is a cassava CWR and is an endemic species of the Tamaulipan thornscrub ecoregion that exists in the transboundary region of southern Texas and northeastern Tamaulipas, Mexico. It is designated as an endangered species by the IUCN and, in Texas, under the U.S. Endangered Species Act (ESA), but is not nationally listed in Mexico's Norma Oficial Mexicana-059-SEMARNAT-2010 (NOM-059), also known as Mexico's list of endangered species [17–19]. Studies have found that *M. walkerae* features genes that can help prevent post-harvest deterioration, a condition that significantly limits cassava consumption. Hybrids of these two species have been found to have greater longevity and be more resistant to post-harvest deterioration [15,20,21]. Additionally, *M. walkerae* may possess genes that confer resistance to some of the prominent diseases of cassava (e.g., cassava brown streak and bacterial blight) and could also contain genes for increased cold resistance [22]. Overall, *M. walkerae* offers much promise for improving cassava's role in attaining food security; thus, its conservation exemplifies pre-emptive actions that can advance ecological, economic, and social purposes on multiple levels.

In the case of *M. walkerae*, the lack of official protection in Mexico may impede advocacy efforts for the establishment and/or expansion of protected areas that promote in situ conservation for this CWR. Another problem that may result from this missing designation is vulnerability to exploitation. Additionally, universal threats facing *M. walkerae* throughout its bi-national distribution include habitat destruction, population fragmentation, small population sizes, herbicide application, overgrazing, herbivory by native and introduced wildlife, surface mining (caliche, petroleum, and natural gas), residential development, and competition by invasive plant species [17,22,23].

Designating a species' extinction risk category both globally and nationally is of critical importance for conserving species and is often a first step to developing conservation plans [5]. The benefits of global listing, especially using the IUCN's Redlist framework, are those of a credible, reliable, and comprehensive tool for stakeholders when developing conservation strategies. Perhaps most importantly, the Redlist is readily available to the public, thus allowing for global education concerning species that are at risk of extinction at all levels [5,24]. An additional benefit provided by national and regional assessment methods is heightened awareness at the community level of the need to protect particular species. These actions can result in the conservation of a species' genetic diversity through the creation of protected areas and agreements between agencies, non-profits, and private landowners to protect the species in situ [5]. There has been a debate over whether the results of IUCN threat rankings significantly conflict with the outcomes of national ranking methods. However, previous studies have revealed that the IUCN and Mexico's National Risk Assessment Method (MER) provide similar results when assessing the conservation status of a species [7]. Nonetheless, species that are IUCN-listed but that remain unlisted in Mexico need to be assessed using MER to determine whether they meet national listing criteria. This process then facilitates the development of sound conservation plans for that species [6,7].

The goals of this paper were twofold. First, we re-assessed the international conservation status of this species using its historic and potential distribution. Second, we assessed the Mexican national conservation status of the species using the MER to propose listing it as endangered throughout its Mexican distribution. Our larger objective is to help secure the multi-faceted benefits that this species could bring to crop production, therefore contributing to sustainability and food security.

2. Materials and Methods

2.1. Model of Potential Distribution

The bi-national occurrence data used to create the geographic species distribution model (SDM) for *M. walkerae* were obtained from historical population records provided by the Texas Parks and Wildlife Department (e.g., Texas Natural Diversity Database). Although the total number of individual occurrences for *M. walkerae* was 399, most of these were in proximity to one another, which could cause geographic autocorrelation. We reduced this bias by using the "spatially rarefy occurrence data" tool in the SDM toolbox version 2.2 at a minimal distance of 4 km [25]. The resulting number of spatially rarefied occurrences was 19, and these were used to generate models through the maximum entropy algorithm (MaxEnt) [26,27].

The entire Tamaulipan thornscrub ecoregion basemap was chosen as our study area since it represents the natural landscape boundaries that *M. walkerae* is endemic to and contains the full extent of this species' occurrences. The environmental variables used to construct the geographic distribution model for *M. walkerae* were those that most influence its growth and physiology and were recommended by rare plant experts. Collectively, there were 15 not highly correlated environmental variables used to construct the model (Table 1). These environmental variables can be separated into two categories: continuous and categorical. Continuous variables are typically seen in raster format, contain cells or pixels with gradually changing data, and exhibit no distinct boundaries, such as in temperature or elevation. Categorical variables, on the other hand, have clear boundaries that show a change in characteristics, such as in soil type or hydrological sub-basins.

Variables were chosen to generate the distribution models based on the values of the variance inflation factor (VIF = $1/(1 - r2)$), which allows for excluding redundant variation among them [25]. The correlation coefficient (r) was obtained from multiple regression using the variable with the highest correlation coefficient as the predictor variable and the rest as independent variables. The excluded variables were those that had a VIF greater than 5.0, because their variation was contained in the other independent variables [25]. The procedure was repeated until no variable had a VIF value greater than 5.0.

The 19 rarefied occurrences of *M. walkerae* were then uploaded into MaxEnt along with the environmental variables (Table 1) and run to produce 50 replicates at a random test percentage of 30. This means that 70% of them were used to construct the model, and 30% were used to check the model's accuracy. The 50 resulting models were then visualized in ArcGIS, and a consensus model was constructed using the raster calculator spatial analyst tool [28].

Table 1. The environmental variables used to construct *Manihot walkerae*'s potential distribution model and their percent contribution to the model.

Environmental Variable Name	Type	% Contribution
Biomes	Categorical	6.7
Water basins	Categorical	59.7
Soil Type	Categorical	9.4
Percentage of water per km^2	Continuous	0.1
Canopy height	Continuous	0
Percentage of bushes per km^2	Continuous	3.2
Percentage of deciduous broadleaf trees per km^2	Continuous	1.3
Ecoregion	Continuous	0.5
Elevation	Continuous	11
Herbaceous percentage per km^2	Continuous	0.2
Humidity index	Continuous	1.3
Maximum temperature in the coldest four-month period (°C)	Continuous	4.1
Total annual precipitation	Continuous	0.1
Solar radiation for the month of June	Continuous	0.3

To determine how much of our study area in km^2 was predicted as the potential geographic distribution, we used the "Reclassify" tool in the SDM toolbox to convert it into a binary model that showed both areas that are suitable and unsuitable areas [25]. The consensus model was reclassified using the "Maximum training sensitivity plus specificity Cloglog threshold" value obtained from MaxEnt.

We then quantitatively evaluated the accuracy of this geographic distribution consensus model using the Area Under the ROC curve (AUC) and partial ROC. AUC values ranged between 0.5 and 1. A value of 0.5 is equivalent to a random prediction, while a value of 1 indicates a perfect prediction [29] where maps are accurate, robust, or statistically good. Because there has been some criticism of the AUC [30], we also used an alternative method to evaluate the accuracy of the models, called the partial Receiver Operating Characteristic (partial ROC). This assessment method is more statistic-specific because it only considers regions where data have been observed [31]. Partial ROC values that are significantly higher than 1 and closer to 2 signify a good prediction and a more accurate map [32].

2.2. IUCN Assessment

The IUCN's risk assessment method uses five different criteria (A–E) to designate a species into one of seven categories: least concern (LC), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CE), extinct in the wild (EW), and extinct (EX). Criteria A, C, and D are associated with population sizes, while criterion B is based on geographic range. Criterion A specifically focuses on population reduction or decline, criterion C focuses on small population size and decline, and criterion D on very small or restricted populations. Criterion E is a quantitative analysis of a species' extinction risk in the wild [5]. For a species to be listed as threatened in the IUCN Redlist, it must meet at least one of the five criteria [5].

Criterion A can be further divided into four categories (A1–A4), where A1 states that there is an observed, estimated, inferred, or suspected population reduction in the past where the causes of the reduction are reversible, understood, and have stopped. On the other hand, A2 states that the causes of the reduction have not stopped, may not be understood, and are not reversible [5]. A3 focuses on a projected reduction in population in the future for a maximum of 100 years, and A4 can be applied only when there is an observed, estimated, inferred, or suspected population reduction both in the past and future, where the causes of the reduction have not stopped, may not be understood, and are not reversible [5]. These four criteria of the A category can be assessed in the following ways: (a) direct observation, (b) an index of abundance of the appropriate taxon, (c) a decline in the AOO, EOO, and/or habitat quality, (d) actual or potential levels of exploitation, and (e) effects of introduced taxa, hybridization, pathogens, pollutants, predators, and parasites [5]. Species or taxa are then designated as either critically endangered, endangered, or vulnerable based on threshold values. *M. walkerae* was assessed through category A2, using SDM, to calculate a decline in the AOO, EOO, and/or habitat quality by subtracting transformed habitats into both cropland and urban/built-up land based on a land use raster from the area predicted as the potential geographic distribution [7]. Criterion B assesses a species' geographic range in the form of the extent of occurrence (EOO) and the area of occupancy (AOO) along with at least two of the following conditions: fragmentation, an observed or predicted decline and fluctuation in either the EOO or the AOO, the quality of habitat, the number of locations or subpopulations, and the number of mature individuals [5]. The EOO is defined as the area within an imaginary boundary that is drawn to include all occurrences for a species, while the AOO is the area within the EOO that is occupied by the species [5]. The distribution area of *M. walkerae* was used to designate it as critically endangered (CE), endangered (EN), or vulnerable (VU) based on criterion B threshold values (Table 2).

Table 2. IUCN criterion B threshold values.

	Critically Endangered	Endangered	Vulnerable
Extent of Occurrence (EOO)	<100 km^2	<5000 km^2	<20,000 km^2
Area of Occupancy (AOO)	<10 km^2	<500 km^2	<2000 km^2

We used the Geospatial Conservation Assessment Tool (GeoCAT) to acquire the EOO and AOO by inputting all 399 historical occurrence records for *M. walkerae* [31]. The area that the model predicted as the potential geographic distribution (PGD) for *M. walkerae* was also compared to criterion B EOO threshold values (Table 2).

Criterion C is used to assess small population size and decline of a species, where a species with a collective population of 250 individuals or less is designated as CE, less than 2500 individuals as EN, and less than 10,000 as VU. Criterion C can be divided into two categories (C1 and C2), where C1 states that there is an observed, estimated, or projected decline of at least 25% in 3 years or 1 generation (CR), 20% in 5 years or 2 generations (EN), or 10% in 10 years or 3 generations (VU). For C2, there is an observed, estimated, or projected decline where (a) the number of mature individuals in each subpopulation is assessed along with the percentage of mature individuals in one subpopulation, and (b) extreme fluctuations occur in the number of mature individuals [5]. The threshold values for C2a are less than 50 mature individuals in a subpopulation (CE), less than 250 (EN), and less than 1000 mature individuals (VU), with 90–100% of mature individuals in one subpopulation designating the species as CE, 95–100% as EN, and 100% as VU [5]. *M. walkerae* was assessed using criterion C2, considering that there was an estimated number of 1000 mature individuals collectively existing in its populations [5].

Under criterion D, which assesses very small or restricted populations, *M. walkerae* could be classified as vulnerable under D1 because it does have an estimated number of 1000 mature individuals. However, it does not meet D2 (extremely restricted AOO) since the AOO for the species is greater than 20 km^2. Criterion E (the extinction probability analysis) could not be conducted in our assessment since the generation time for *M. walkerae* is not yet known. It is for these reasons that *M. walkerae* was assessed using criteria A2ac, B1, B2ab, and C2a.

2.3. Mexico's National Risk Assessment Method (MER)

Conversely, Mexico's National Risk Assessment Method (MER) uses four different criteria to designate species into one of four risk categories ranging from species of least concern to those that are believed to be extinct in the wild [32,33]. The first is criterion A, where the extent of a species' occurrence in Mexico is assessed by comparing the species' geographic distribution in Mexico with the total area of Mexico, which is 1,964,375 km^2 [32].

Values that can be used for this assessment are the species' EOO, AOO, and predicted potential geographic distribution [7]. Threshold percentages are used to classify species into one of four categories and are as follows: widely distributed species cover >40% of Mexico's area, semi-restricted or "vast" species cover >15% of Mexico's area but less than 40%, restricted species cover 5% to 15% of Mexico's area, and very restricted species cover less than 5% of Mexico's area. Each of the categories is assigned a value from 1 to 4, with species that are widely distributed having a value of 1 and species that are very restricted having a value of 4.

We used the EOO, the AOO, and the predicted potential geographic distribution (PGD) from the model that was constructed earlier to assess *M. walkerae* with criterion A. The EOO and AOO values were calculated with only the historical occurrences for *M. walkerae* that are distributed in Mexico (33 out of 399 total), since the MER is an assessment method specific to Mexico. We then uploaded the 33 occurrences into the Geospatial Conservation Assessment Tool (GeoCAT) to acquire the EOO and AOO [33]. The decision was made to crop the pre-existing geographic distribution model to exclude Texas instead of running an additional Mexico-specific model, as the latter approach could cause a false prediction of suitable habitat (overprediction or underprediction when the species' complete range is not considered). The percentage of the area covered by the species' distribution was calculated by dividing the EOO (1223 km^2), AOO (76 km^2), and PGD (9986 km^2) by Mexico's total area (1,964,375 km^2) and multiplying by 100.

Criterion B is an assessment of the species' natural habitat requirements and can be classified into three categories. The first is a slightly or not limiting habitat, which is assigned a value of 1, the second is an intermediate or limiting habitat, which is assigned a value of 2, and the third is a hostile or highly limiting, which is assigned a value of 3 [34,35]. Criterion C is an assessment of the intrinsic biological viability of the species, which includes factors such as reproductive strategy, population demography, phenology, genetic variation, and recruitment rate. These factors are then used to classify a species into one of three categories based on the predicted vulnerability of the species to extinction, ranging from those that have high vulnerability (value of 3) to species that have low vulnerability (value of 1) [34,35].

Finally, criterion D is an assessment of the existing degree of human impact on the species. Examples of human impact include habitat destruction, fragmentation, land use change, pollution, use in trade and trafficking, and the introduction of invasive species [34,35]. Species that are highly impacted are assigned a value of 4, species with a medium degree of human impact are assigned a value of 3, and species with a low level of human impact are assigned a value of 2. Once a species is assessed with all four criteria, the values assigned to the species in each criterion are summed and then used to classify the species into one of four categories. Species with a summed value of 9 or less are designated as least concern, species with a value of 10–11 are threatened, and those with a value of 12–14 are considered endangered. Further, a species that is believed to be extinct in the wild has a value of 14 [34,35].

Criteria B (species' natural habitat status) and D (degree of human impact) for *M. walkerae* were assessed using the human footprint raster layer, a dataset produced by compiling scores from population density, land transformation, accessibility, and electrical power infrastructure data to yield an estimate of human impact ranging from 0 to 100 [36]. Human footprint ranges were categorized in the following manner: 50 or above (B: hostile or highly limiting, value of 3; D: high, value of 4), 25–49 (B: intermediate to limiting, value of 2; D: medium, value of 3), and 0–24 (B: slightly or not limiting, value of 1; D: low, value of 2) [7]. The human footprint values for *M. walkerae*'s range in Mexico were extracted from ArcGIS to construct a table of values using the 33 national occurrences. The values were then averaged and compared to the threshold values for criterion B.

3. Results

3.1. Result: Manihot Walkerae's Potential Geographic Distribution Model

The potential geographic distribution consensus model for *M. walkerae* produced from 50 replicates had a statistically relevant AUC value of 0.93 and a partial ROC value greater than 1.80 (Figure 1). This indicates that the map is statistically accurate and can be used for assessing the extinction risk of *M. walkerae* with both the IUCN and MER assessment methods.

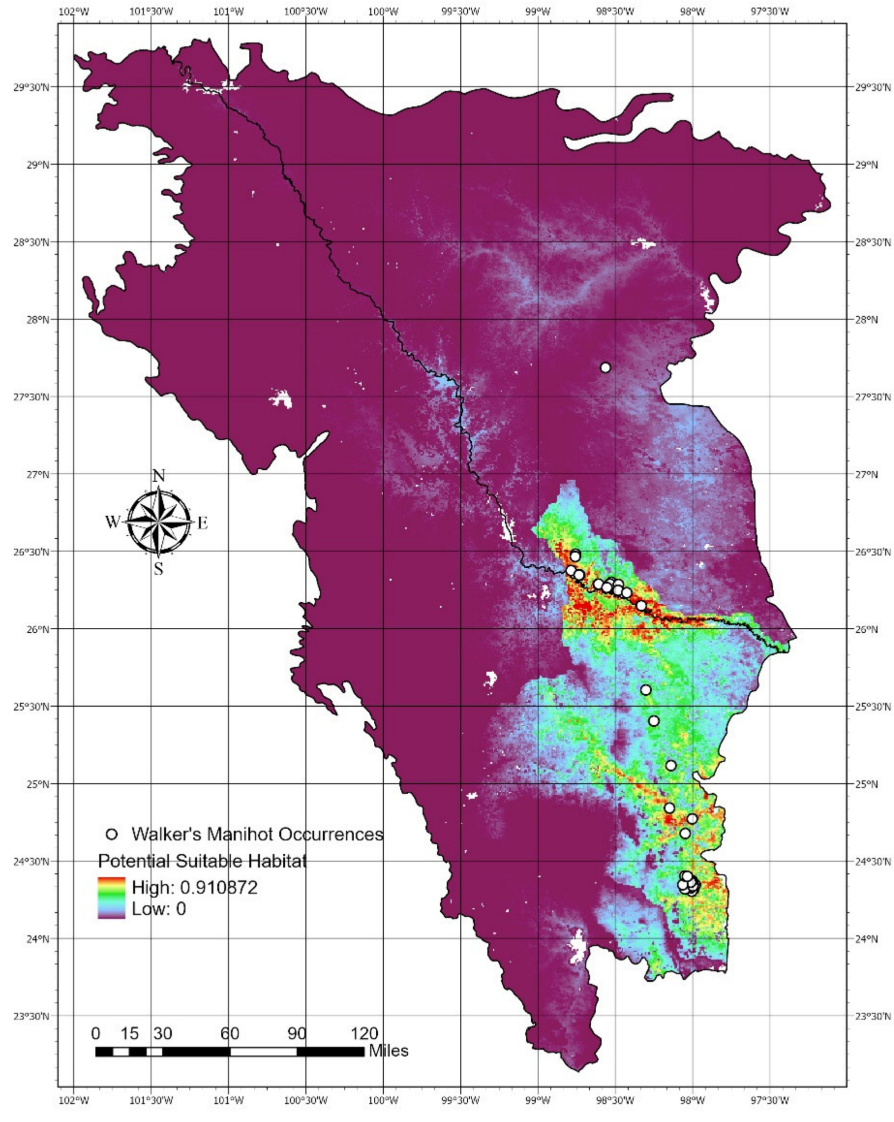

Figure 1. Map of the potential suitable habitat/potential geographic distribution (PGD) for *Manihot walkerae* in the Tamaulipan thornscrub ecoregion study area. The color scale ranges from blue to red, with blue depicting areas of unsuitable habitat, green neutrally suitable habitat, and red the area of highest suitable habitat. The final AUC value for the consensus map was calculated as 0.93 with a partial ROC value > 1.80.

The environmental variables that contributed most to the model were water basins, soil type, and elevation (Table 1). The variables that contributed least include canopy height, herbaceous percentage, and percentage of water per km^2. Most records occur in areas that are predicted as being highly suitable (shown in red), except for one historical record north of this distribution. Highly suitable areas are prominent along the lower reaches of the Rio Grande/Rio Bravo River and in the southeastern portion of the study area, which corresponds primarily to inland sections of Tamaulipas and Nuevo Leon, Mexico (Figure 1).

3.2. Result: IUCN Assessment Method

After assessing *M. walkerae* with IUCN criteria A2c, B1, B2ab, and C2a, we found that two of these criteria essentially designated the species as endangered, while the other two categorized it as vulnerable based on threshold values. Criterion A2c, which specifically addresses a species' population reductions that are observed, estimated, or inferred and where the causes of the reduction have not stopped and are not reversible, designated *M. walkerae* as a vulnerable species since there is an estimated 48% decline in available geographic distribution based on the land use map (Figures 2 and 3). The percentage of potential geographic distribution loss was calculated by subtracting habitats transformed into cropland and urban and built-up land, based on a land use raster, from the area predicted as the potential geographic distribution for *M. walkerae.* When looking at the composition of land use types in the Tamaulipan thornscrub ecoregion, the predominant land use types are tropical or subtropical shrubland (55.31%), followed by cropland (20.77%), and tropical or subtropical grassland (15.02%) (Table 3).

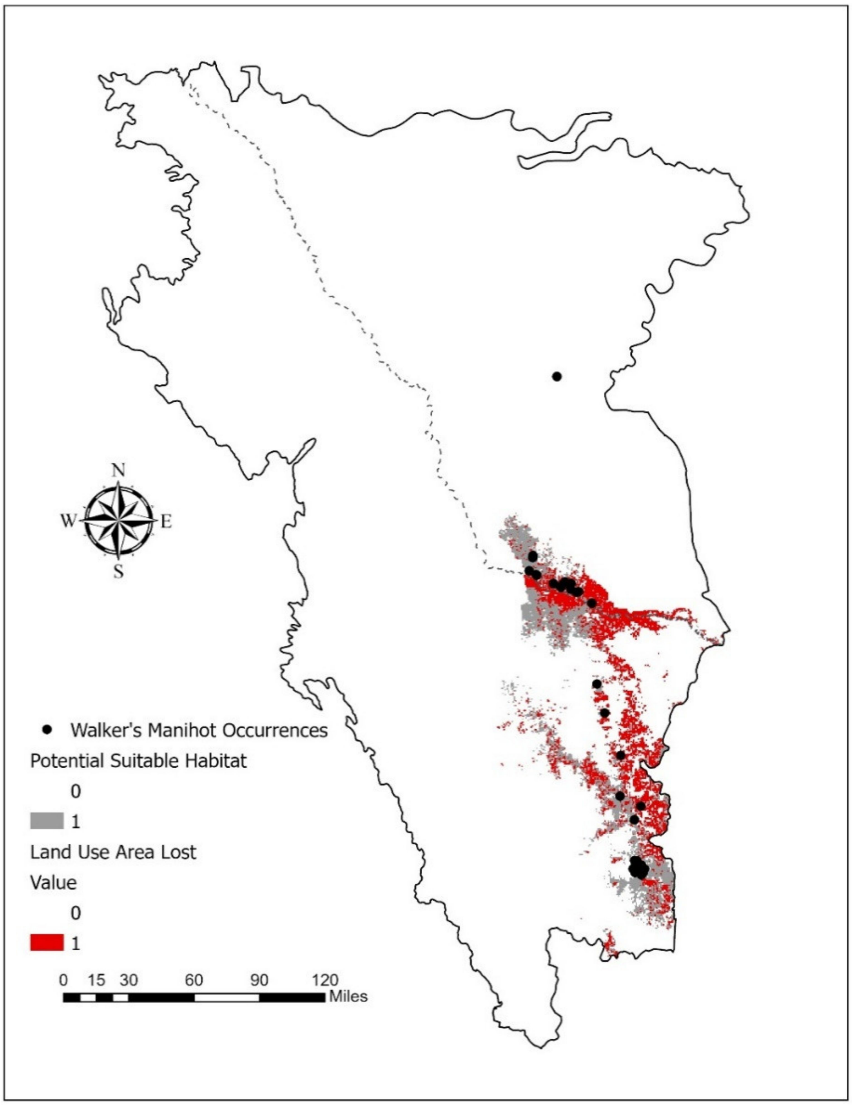

Figure 2. Map showing *Manihot walkerae*'s potential suitable habitat (gray) lost due to transformation into cropland and urban and other built-up areas (red). The area lost (5938 km^2) is approximately 48% of the predicted geographic distribution (12,274 km^2). These data were used to assess *M. walkerae* under criterion A2c of the IUCN, where there is an estimated population size reduction based on a decline in the AOO, EOO, and/or habitat quality. This criterion categorizes *M. walkerae* as vulnerable since the predicted population reduction is less than 50%.

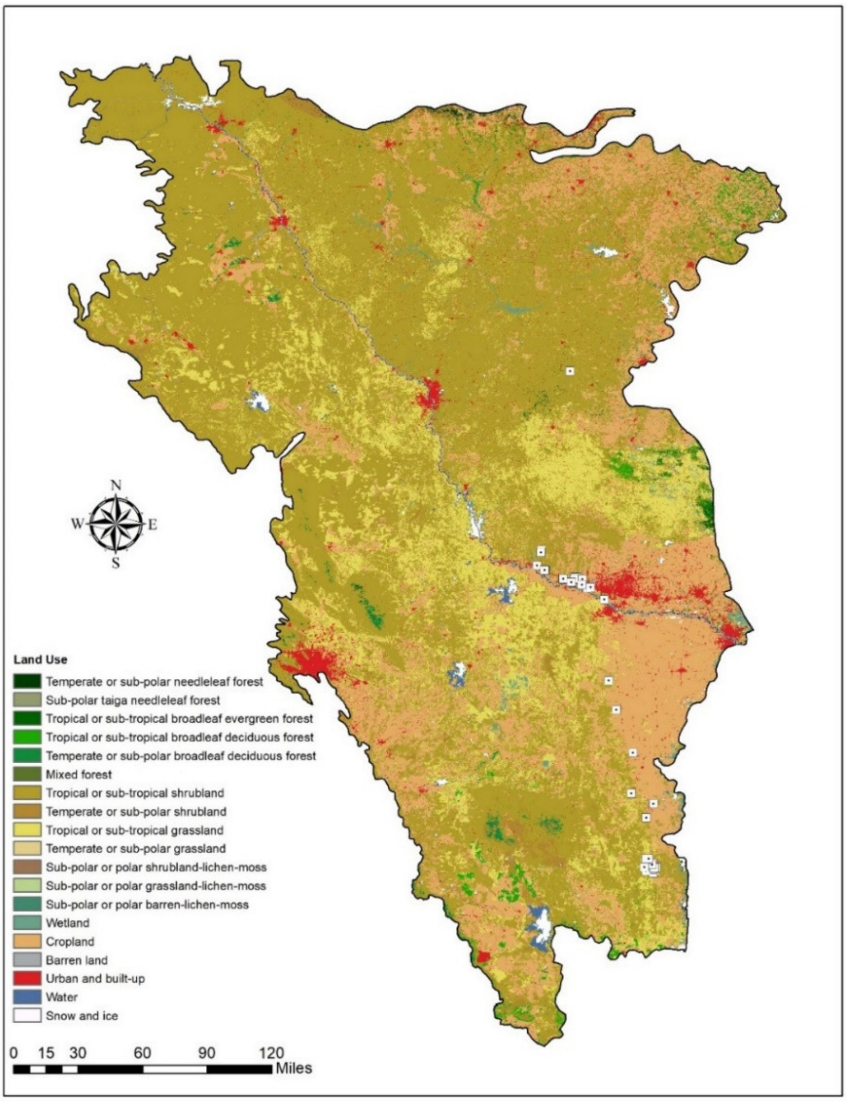

Figure 3. Land use in the Tamaulipan thornscrub study area, where the native vegetation types are primarily tropical or subtropical shrubland (mustard brown) and temperate or subtropical grassland (yellow). Many of the areas within *Manihot walkerae*'s historical distribution have since been converted to cropland (peach orange) and urban/built-up land (red).

Table 3. Composition of land use types in the Tamaulipan thornscrub ecoregion study area.

Land Use Type	Area (ha)	% Area
Temperate or sub-polar needleleaf forest	15,168	0.08
Tropical or sub-tropical broadleaf evergreen forest	39,389	0.21
Tropical or sub-tropical broadleaf deciduous forest	177,755	0.95
Temperate or sub-polar broadleaf deciduous forest	62,914	0.33
Mixed forest	57,111	0.30
Tropical or sub-tropical shrubland	**10,400,941**	**55.31**
Temperate or sub-polar shrubland	251,426	1.34
Tropical or sub-tropical grassland	**2,824,264**	**15.02**
Temperate or sub-polar grassland	107,256	0.57
Wetland	186,422	0.99
Cropland	**3,906,422**	**20.77**
Barren land	46,973	0.25
Urban and built-up	**525,708**	**2.80**
Water	203,952	1.08

Criterion B1, which was used to assess *M. walkerae*'s extent of occurrence, also designated the species as vulnerable according to both the EOO (10,363 km^2) and the predicted geographic distribution (12,274 km^2) since these values were less than the 20,000 km^2 vulnerable threshold value but greater than the 5000 km^2 endangered threshold value (Table 4, Figure 4). However, criterion B2ab, which was used to assess *M. walkerae*'s restricted AOO, designated the species as endangered since this metric was less than the 500 km^2 endangered threshold value (132 km^2), and the species also met the condition of existing in fragmented populations that have declined (Table 4, Figure 4).

Table 4. Assessment of IUCN criterion B: restricted geographic range for *Manihot walkerae* through its extent of occurrence, area of occupancy, and predicted geographic distribution values in its complete range of distribution (South Texas and Tamaulipas, Mexico). Both the EOO and the predicted geographic distribution categorize this species as vulnerable (less than 20,000 km^2), while the AOO designates it as endangered since it is less than 500 km^2.

Criterion B	Values (km^2)	Criterion B Category
EOO	10,363 km^2	Vulnerable
AOO	132.00 km^2	Endangered
Potential Geographic Distribution	12,274 km^2	Vulnerable

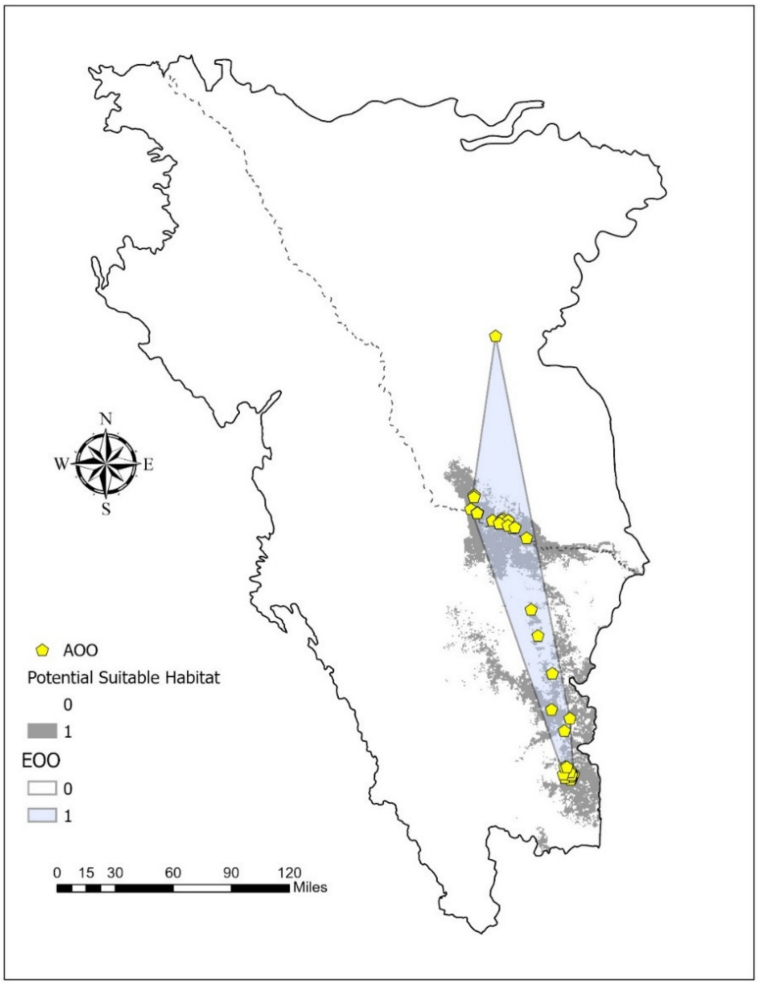

Figure 4. Map showing *Manihot walkerae*'s extent of occurrence (EOO), area of occupancy (AOO), and potential suitable habitat or predicted geographic distribution (PGD). The areas covered by each of these values are: EOO 10,363 km^2, AOO 132 km^2, and PGD 12,274 km^2. Both the EOO and PGD designate this species as vulnerable (less than 20,000 km^2), while the AOO designates it as endangered (less than 500 km^2).

Criterion C2a was used to assess *M. walkerae*'s small population size and decline. Here, *M. walkerae* also met the endangered threshold by having less than 2500 individuals (i.e., an estimated 1000). Additional support for the endangered classification is based on the number of mature individuals in each subpopulation (<250) and the fact that the percentage of mature individuals in a subpopulation is between 95% and 100% of the species. Ultimately, *M. walkerae* can be considered an endangered species based on the IUCN's assessment method under criteria B2ac and C2a.

3.3. Result: Mexico's Risk Assessment Method (MER)

M. walkerae was assessed using all four of the MER's criteria and ultimately met the conditions for being designated as a threatened species. Criterion A was used to assess the extent of *M. walkerae*'s occurrence in Mexico by comparing the species' EOO, AOO, and PGD in Mexico with the total area of Mexico (1,964,375 km^2). All three values (EOO: 1223 km^2, AOO: 76 km^2, and PGD: 9986 km^2) indicate that the species is very restricted (value of 4) and that it covers less than 5% of Mexico's area: 0.062%, 0.0038%, and 0.51% (Table 5).

Table 5. Assessment of MER criterion A: extent of species distribution in Mexico for *Manihot walkerae* through its extent of occurrence, area of occupancy, and predicted geographic distribution. All three values categorize *M. walkerae* as a very restricted species in Mexico with an assigned value of 4 since it covers less than 5% of Mexico's total area.

Criterion A	Values (km^2)	% of Mexico's Area (1,964,375 km^2)	Criterion A Category	Criterion A Value
EOO Mexico	1223 km^2	0.062%	Very restricted species	4
AOO Mexico	76 km^2	0.0038%	Very restricted species	4
Potential Geographic Distribution in Mexico	9986 km^2	0.51%	Very restricted species	4

Criteria B (the species' natural habitat status) and D (the degree of human impact) in Mexico were assessed using the human footprint raster layer and by assigning the species a value of 24 throughout its distribution in Mexico. This resulted in a categorization of slightly or not limiting under criterion B (value of 1) and as having a low degree of human impact under criterion D (value of 2) (Table 6).

Table 6. Assessment of MER criteria B (the species' natural habitat status) and D (the degree of human impact) for *Manihot walkerae* in Mexico using the human footprint raster layer, a data set produced by compiling scores from population density, land transformation, accessibility, and electrical power infrastructure data to yield an estimate of human impact ranging from 0 to 100. Human footprint ranges were categorized in the following manner: 50 or above (B: hostile or highly limiting, value of 3; D: high, value of 4), 25–49 (B: intermediate to limiting, value of 2; D: medium, value of 3), and 0–24 (B: slightly or not limiting, value of 1; D: low, value of 2). The human footprint raster layer assigned *M. walkerae* a value of 24 throughout its Mexican distribution; therefore, it was categorized as slightly or not limiting under criterion B and as having a low degree of human impact under criterion D.

Criterion	Human Footprint Value	Category Designation	Category Value
B	24	Slightly or not limiting	1
D	24	Low degree of human impact	2

Finally, when assessing criterion C, the intrinsic biological viability of the species that makes it vulnerable to extinction, we considered the genetic variation and recruitment rate in populations of *M. walkerae*. Since this species has small populations that are fragmented and disconnected from each other, there may be very low genetic variation and diversity [17]. These conditions could, in turn, make it more vulnerable to extinction due to an anthropogenic or environmental disturbance event [34,35]. Additionally, since *M. walkerae*'s population trend is declining and because recent field visits in Mexico (2019 to 2021) have revealed that some historical populations no longer exist, the recruitment rate for this species is considered low. Collectively, the low genetic variation/diversity along with its low recruitment rate led to our assignment of a value (3) that is consistent with high vulnerability to extinction. The values from all four criteria were then summed (criterion A (4) + criterion B (1) + criterion C (3) + criterion D (2) = 10), and the resulting total designated the species as threatened in Mexico.

4. Discussion

Extinction risk assessments are crucial tools for the conservation of species at both the global and national levels. In this study, we used the IUCN's risk assessment method and Mexico's risk assessment method (MER) to assign an extinction risk category to Walker's Manihot, *Manihot walkerae,* an important crop wild relative of cassava. We incorporated a model of *M. walkerae*'s predicted geographic distribution (PGD) alongside the extent of occurrence (EOO) and area of occupancy (AOO) to assess this species with criterion B of the IUCN and criterion A of the MER (Figures 1 and 4; Tables 4 and 5). This is useful because the EOO has previously been regarded as a value that potentially overestimates a species' distribution, while the AOO can underestimate it. Maps that show what areas are suitable and unsuitable for a species can be used in conjunction with these two values to provide a more reliable assessment [7]. Criterion B of the IUCN revealed that, based on the EOO and the PGD, *M. walkerae* can be considered a vulnerable species because these areas cover more than 5000 km^2 but less than 20,000 km^2 (Figure 4, Table 4). However, since the AOO was found to be 132 km^2, which is less than the 500 km^2 endangered threshold, the species can be designated as endangered under criterion B2. When comparing all the geographic range values to the MER criterion A thresholds, we found that *M. walkerae* is a very restricted species in Mexico, covering less than 5% of Mexico's area (0.062%, 0.0038%, and 0.51%) (Table 5).

Urbanization and the transformation of lands for agricultural use are some of the leading causes of the loss of biodiversity in crop wild relatives [37]. In this study, we used the PGD model to estimate *M. walkerae*'s population decline due to land use transformation, and these values informed criterion A of the IUCN [7]. This revealed that approximately 48% of *M. walkerae*'s predicted PGD could be disrupted because of land use change. These pressures are clearly a leading cause of the past, present, and potentially future population losses in the species (Figure 2). If the remaining unaltered PGD area could be assessed against EOO threshold values (5938 km^2), it would be very close to meeting the endangered threshold value under criterion B1 (Figure 2). Although this value did not meet criterion A2′s endangered threshold of 50% loss, it is important to note that 48% is much closer to the endangered threshold value than it is to the 30% vulnerable threshold value. If another measure of criterion A2 (e.g., direct observations of population reduction) were also included, this species could be designated as endangered.

Upon a closer look at the land use maps, one can see that the dominant land use type in proximity to historical occurrences in Mexico is croplands (Figure 3; Table 2). This raises a concern in terms of whether designating protected areas for this species is a viable option, since many of these croplands are privately owned. These circumstances could also put *M. walkerae* at greater risk of national extinction in Mexico if it is not listed in the NOM-059. A previous study in another semiarid region showed that some plants in the Euphorbiaceae family are tolerant to anthropogenic disturbance; however, no research has yet looked at this tolerance level in *M. walkerae* [38]. Field work visits to *M. walkerae* populations on private property have alluded to this species having some tolerance to anthropogenic disturbance, as it has been observed recovering from periodic grazing and being surrounded by trash. This raises the possibility that, in cases where the creation of protected areas for this species is not feasible, in situ conservation of private property through easements, agreements, and other methods could be a viable alternative.

Observations at the population level that could allow for direct assessments of *M. walkerae*'s population decline in Mexico are now in progress. Recent field visits have revealed that, compared to previous surveys conducted in the late 1990s and early 2000s, there are fewer *M. walkerae* individuals; this concurs with the declining trend listed on the IUCN website [14]. Additionally, natural history observations that examine the symbiosis between *M. walkerae* and other species (e.g., pollinators and nurse plants) are important for conserving this rare plant and are critical components of successful strategies for future conservation plans (e.g., reintroduction). Natural history studies could also potentially allow for an extinction risk probability analysis to be performed on *M. walkerae*, thereby allowing the species to be assessed by IUCN criterion E.

Assessments of crop wild relatives (CWR) are crucial for providing solutions to food security efforts and magnifying the connectivity between ecological and social welfare that we so often take for granted. Species populations must be protected across their entire range of distribution to preserve genetic diversity that could make good on expanding beneficial traits in many agricultural crops. These efforts become increasingly critical in light of an expanding world population and the growing number of threats that face agricultural commodity producers in a changing climate (e.g., increased flood frequency, pest invasions, cold snaps, etc.). Thus, including *M. walkerae* in the Mexican list of endangered species could be key to preserving the genetic diversity of this CWR. Moreover, the results of future natural history studies will help to establish long-term sound conservation plans in this region [39]. Ultimately, conserving *M. walkerae* would not only benefit the food security provided by cassava but would also maintain the ecological role that this species serves in its native ecosystem. A limitation of this study is that we relied solely on geographic range data and used species distribution modeling to accomplish our assessment. These methods do not consider species interactions such as pollinators, predators, and nurse plants; however, ongoing research is focused on documenting this information.

Our findings were that *M. walkerae* should be designated as endangered based on the IUCN assessment method and should also receive threatened status under the MER criteria. The IUCN assessment performed here supports the previous IUCN assessment done by Vera-Sanchez and Nassar in 2019 [14], while in Mexico, no previous assessment for this species has been attempted. The findings of this work may help advocate for the listing of *M. walkerae* as a threatened species under Mexico's NOM-059 and could benefit both the conservation of biodiversity and food security.

5. Conclusions

We used the IUCN's global risk assessment method and Mexico's National Risk Assessment method (MER) to classify cassava's crop wild relative, *Manihot walkerae*, into a global and national risk category in Mexico. This species had previously been assessed with the IUCN's method, but no attempts had yet been made to assess *M. walkerae* using the MER. Our results validate the previous IUCN designation of *M. walkerae* as an endangered species based on criterion B2ac, a restricted area of occurrence with fragmented and declining populations, and by criterion C2a, a small population size in decline. Although the predicted geographic distribution model (PGD) for *M. walkerae* did not meet the IUCN's criterion B1 for designation as endangered, it met the EOO vulnerable threshold of having a restricted distribution of lower than 20,000 km^2. However, if the total PGD area that remains after subtracting transformed habitats due to land use change were to be assessed against EOO threshold values (5938 km^2) it would be very close to meeting the 5000 km^2 endangered threshold value, and we advise that this be considered in future assessments. Under the MER, *M. walkerae* was designated as a threatened species with a summed value of 10 from the four different criteria. All three of the geographic range measurements, AOO, EOO, and PGD, designate *M. walkerae* as a very restricted species in Mexico. This factor, along with a high vulnerability to extinction based on its low genetic variation and recruitment rate, leads us to advocate for its inclusion as a threatened species under Mexico's NORMA Oficial Mexicana, NOM-059. We recommend that future studies of *M. walkerae* focus on incorporating natural history data into extinction risk assessments that will enable the development of sound conservation plans for this important CWR.

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