

Artificial Selection Optimizes Clonality in Chaya (Cnidoscolus Aconitifolius)

Miguel A. Munguía-Rosas (✉ munguiarma@cinvestav.mx)

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav)

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Abstract

In angiosperms, clonality is a derived trait and is present in several wild and cultivated plant species. Therefore, over time, natural and artificial selection have optimized the novel function of being a propagule in organs whose previous function was exclusively vegetative. Although increased resource storage and resistance to desiccation have been suggested as the main adaptations for clonality in crops, there is little empirical evidence to support this assertion. Here, I assessed the changes that the stems of chaya (*Cnidoscolus aconitifolius*), a clonal crop, have undergone through domestication and evaluated their performance as propagules. To infer which traits have been optimized by artificial selection, I compared stem traits and their performance in root development and clone survival between wild and domesticated plants. I found that, relative to their wild ancestors, the stems of domesticated chaya have a greater storage capacity for water and starch. Additionally, the stems of domesticated plants produced more roots, shoots and leaves, and their clones lived longer. My results strongly suggest that artificial selection has optimized water and starch storage by stems in chaya. Because these traits also confer greater fitness, they could be considered adaptations to clonal propagation.

Introduction

Clonality is a term used to describe asexual reproduction by an individual resulting in a set of genetically identical descendants (except for the appearance of somatic mutations) or clones^{1,2}. In angiosperms this can be achieved through apomictic seeds (*i.e.* seeds produced by unfertilized ovules) or vegetative organs, with the latter by far the most common in this group of plants³. Clonality is a derived trait among angiosperms and has evolved many times^{4,5}. Clonal plants are common in wet, nutrient-poor, cold and shaded habitats^{3,4} where the conditions for sexual reproduction (*e.g.* scarce pollen vectors or mates), seed germination or seedling establishment may be unfavorable³. The majority of clonal plants, if not all, are perennials with relatively reduced resource allocation to sexual organs⁶⁻⁸. The presence of clonality in phylogenetically unrelated species and their ecological distribution, strongly suggest that clonality is adaptive^{3,4,6}.

Clonality is also present in several cultivated plant species. Clonal crops belong to 34 botanical families, are perennials and exhibit a wide range of life forms (trees, shrubs, herbs and vines)^{9,10}. A relevant proportion of clonal crops were domesticated in the wet tropics where today some species continue to coexist with their wild ancestors¹¹. While the relative contribution of sexual reproduction to the fecundity of clonal crops is often limited, the wild relatives of some of these crops are mainly seed reproduced (*e.g.* chaya: *Cnidoscolus aconitifolius*¹² and cassava: *Manihot esculenta*¹³). Therefore, clonality has also evolved from sexually propagated plants by means of artificial selection. The vegetative organs of plants undergo several physical (*e.g.* resistance to desiccation), anatomical (*e.g.* increased thickness) and physiological (*e.g.* resource storage) changes during the transition from sexual to asexual reproduction, and these changes maximize their new function as propagules⁵. Such a complex evolutionary transition was hardly likely to have resulted from a “single event” domestication process—as previously postulated

for clonal crops—that essentially consisted of cloning the wild plants that exhibited the desired phenotype¹⁴. More recently, the domestication of clonal crops has been recognized as a far more complex process^{9,11}. The wild relatives of some clonal crops likely were managed *in situ* before being domesticated and, once brought into agroecosystems, their local adaptation to this new habitat was unconsciously facilitated by humans^{9,11}. Also, as modular organisms, a single plant produces multiple organs that vary in age, size and nutrients,¹⁵ and each module may develop different somatic mutations that are susceptible to selection¹⁶. Therefore, in clonal crops, artificial selection may operate at the level of genotype, ramet or module^{9,17,18}. If the traits exhibited by the vegetative organs that were selected by growers affect their performance as propagules, this function is expected to be optimized by artificial selection^{17,19}.

For some time, it has been claimed that the vegetative organs of clonal crops, such as stems and roots, have been optimized to serve as propagules through artificial selection^{9,11,14,17,20,21}. However, there are few empirical studies explicitly testing this hypothesis. This is probably because our knowledge about the reproductive biology of wild ancestors for most clonal crops is quite limited^{11,13} and, without this information, it is virtually impossible to identify which traits of the vegetative organs used for clonal propagation have been optimized through domestication and to what extent¹³. Much of what we have learned on this topic comes from a series of studies performed on cassava (*M. esculenta*) and its closest wild relatives (*M. esculenta flabellifolia*)^{9,13}. Relative to its wild progenitors, the stems of domesticated cassava exhibited greater starch content and brittleness as well as reduced stiffness and strength¹⁸. While increased starch content in the stems could improve their performance as an organ for clonal propagation, the other traits do not have a clear connection to their function as propagules¹⁸. Although the size of the stems (*i.e.* diameter) of domesticated cassava used as propagules were good predictors of the production of new shoots, roots and the yield (fresh mass of starchy roots) of cassava clones, it is not known whether this was the result of domestication because the study was not replicated with the wild relatives¹⁷. Other authors have suggested that increased resistance to desiccation may be an adaptation of the stems of cultivated cassava to clonal propagation^{13,22,23}. However, to the best of my knowledge, this has not been properly demonstrated for this or any other clonal crop. Thus, more studies addressing the mechanisms underlying the development of propagules from vegetative organs are needed to improve our understanding of how clonality has evolved under domestication.

In this study, I assessed the changes that the stems of chaya (*Cnidoscolus aconitifolius*), a clonal crop with edible leaves, have undergone through domestication. The closest wild relatives of this crop belong to the same species²⁴. In contrast to their wild progenitors, the cultivar produces more, larger, non-stinging leaves, which have thicker stems that are used as propagules²⁵. While the cultivar is exclusively clonally propagated and rarely produces pollen or viable seeds, wild plants only reproduce by seeds in nature¹². The objective of this study was to assess the major changes that vegetative organs (stems) have undergone during domestication and that maximize their novel function as propagules. I approached this by comparing traits previously identified as relevant in clonal propagation (resource storage, resistance to

desiccation, production of new organs^{9,13}) in wild and domesticated chaya. I specifically asked the following questions: What are the changes that the chaya stems have undergone through domestication? Are these changes associated with their performance as propagules? And, have these traits been optimized through domestication; if so, to what extent? I predicted that domestication had optimized clonal propagation in chaya and therefore, the stems of domesticated plants would have greater resource storage capacity and resistance to desiccation, and a greater capacity to produce new organs as well as greater fitness relative to its wild relatives.

Methods

Study species

Cnidoscolus aconitifolius (Euphorbiaceae) is a shrub native to Mexico and Central America^{24,39}. It has been suggested that this plant was domesticated on the Yucatan Peninsula by the Maya where it is called “chaya”^{40,41}. Leaves of the cultivar are edible, and while it is mainly grown in Mesoamerica, it has recently expanded beyond its native distribution range⁴². Across all cultivation areas, chaya is clonally propagated from stem cuttings^{25,40,42}. In its domestication centre, chaya is still cultivated by the Maya in traditional agroecosystems, particularly home gardens where it coexists with its wild relatives^{25,41}. Despite their co-occurrence, there is almost complete reproductive isolation between wild and domesticated forms, mainly due to poor pollen production and reduced fertility of the latter¹². Clonal propagation of the cultivar is, therefore, obligate. The male flowers of wild plants produce abundant pollen, and female flowers set dry fruit with up to three seeds⁴³. Clonal propagation is also possible in the wild relatives but unlikely without human assistance^{25,43}. Domestication syndrome includes the increased production of bigger leaves with significantly fewer trichomes and more succulent stems^{25,44}. Traditional growers usually select the thicker stems of secondary branches from apparently healthy plants for propagation²⁵.

This study complied with relevant institutional, national, and international guidelines and legislation. *Cnidoscolus aconitifolius* is not at risk of extinction or under the protection of international or local authorities. Additionally, plant material (stem cuttings) used in this study was collected in private lands and therefore no permission from local government was required. The identity of the specimens was confirmed by the curator (Dr. J Tun-Garrido) of the herbarium Alfredo Barrera Marín where a specimen was deposited (Voucher: UADY-23474).

Resistance to desiccation

In autumn 2020, I took 60 stem cuttings (35 cm long) from the secondary branches of 60 different plants (30 wild and 30 domesticated) from an experimental orchard established as a part of a bigger project in 2017 in the municipality of Merida in central Yucatan (see reference 25 for details). I used this orchard as source of cuttings to control for environmental and mother effects and thus, isolate the effect of domestication *per se*. All donor plants were exposed to full sunlight, watered evenly once a week; are the

same age and similar in height (2-2.5 m tall). Once in the laboratory, the basal diameter of the cuttings was measured, and a 5 cm segment was cut from the 35-cm-long segment. Both segments (30 cm & 5 cm) were weighed for all cuttings and the small ones were oven-dried at 75 °C for 72 h to estimate water content. The 30 cm segments were placed in a controlled environment chamber (Binder Inc., KBW 240, Tuttlingen, Germany) at a constant temperature of 26 °C with a photoperiod of 12 h light/dark, light was provided by high-pressure sodium lamps ($\text{PFD} = 46.89 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). The only source of water for the cuttings was environmental humidity ($\approx 60\%$) which was homogeneous throughout the chamber. All cuttings were weighed twice a week for a month to estimate the rate of water loss (measured as weight loss). The temperature and photoperiod used resembled the average values observed during the summer on the Yucatan Peninsula.

Resource storage

Using the same source plants and selection criteria outlined above; one month later, I cut another stem section of ca. 10 cm in length to measure soluble sugars and assess the presence of starch. To identify the presence and distribution of starch, I cut a fine slice (3-4 mm thick) from the end opposite the apex of each stem section and immediately added approximately 1 ml of Lugo's solution (5g I_2 + 10g KI + 85ml H_2O) to all the slices simultaneously. After 3 minutes, the slices were washed in distilled water to remove the excess solution. The presence and distribution of starch was easily recognized as it stains dark blue. I indirectly measured total soluble sugars in the remaining portion of the stems by using the method outlined by Okamura and colleagues⁴⁵, which consists of measuring the sugar content of the sap, obtained by squeezing the stem sections, and using a digital refractometer with automatic temperature compensation (HI96801, Hanna Instruments Inc., Rhode Island, USA). Although the refractometer gives the sugar content in Brix units, it is a reliable proxy for % total soluble sugars ($r=0.96$, $P < 0.01$ ⁴⁵). I was able to obtain enough sap to perform the measurements on the stems of 19 wild and 21 domesticated plants; the remaining stems were too hard and/or too dry to obtain enough sap to test.

Shoot production and cutting longevity

Twice a week over four months (120 days), I counted the new shoots sprouting from the 30-cm-long cuttings in the controlled environmental chamber described above in *Resistance to desiccation*. The presence of leaves emerging from the shoots was also recorded. During the experiment, all new shoots were labeled to avoid underestimating the total number of shoots because some of them withered and fell off during the experiment. In addition to shoot emergence time, I recorded the time to cutting death, defined as the time when a cutting showed clear signs of wilting as well as shoot abortion and leaf abscission (when these had developed). I discarded dead cuttings to prevent the proliferation of fungi and bacteria in the chamber, and the potential contamination of the remaining cuttings.

Rooting

In January 2021, I took 57 stem cuttings, 30 cm in length, from 57 plants (26 wild and 31 domesticated) following the same procedure and criteria described above in *Resistance to desiccation*. Two days after collection, the cuttings were planted in 2L plastic pots using a mix of gravel and soil (70:30) as the substrate. Before planting the cuttings, I removed all of the leaves with pruning shears. I left the pots with these cuttings in a plant nursery located next to the experimental orchard mentioned before. All cuttings were exposed to the same light environment (full light exposure) and watered to field capacity once or twice a week during the experiment. After four weeks, I gently removed the cuttings by turning the pot upside down, without pulling on the cutting in order to avoid any damaging the roots. Once the cutting had been removed I washed the part that had been buried to eliminate all traces of soil. I carefully examined the cuttings with a magnifying glass in search of roots. I recorded the presence/absence of roots, the number of main roots (*i.e.* roots that emerged directly from the cuttings) and measured the length of the longest root, if present. Additionally, the presence/absence of any leaves on the aerial part of all cuttings was recorded.

Clone survival

To assess the long-term survivorship of clonally propagated plants (clones), in June 2019 160 stem cuttings (30 cm long) from 40 different mother plants (20 wild and 20 domesticated, four cuttings per plant) were collected using the donors, procedure and selection criteria described above in *Resistance to desiccation*. The cuttings were planted in 20L pots using the same substrate and procedure described in the *Rooting* section. The plants were left in the plant nursery described above, under full light exposure and were watered to field capacity once a week. I checked clone survivorship monthly for nine months (270 days) starting after the fifth month. I did this because survivorship is difficult to assess during the first months of life. For example, cuttings may have no leaves, but may have roots, or may even already have died with no clear signs of wilting because watering keeps the aerial part of the cuttings turgid. A clone was considered dead when it had lost its leaves and the stem presented clear signs of wilting.

Statistical analyses

Resistance to desiccation. I estimated water content by subtracting the final weight of oven-dried cuttings from their initial weight. Water content, expressed as a proportion of total weight, was compared between the stems of wild and domesticated plants (*i.e.* domestication factor) with an ANCOVA, including the diameter of fresh cuttings as a covariable. The domestication x diameter interaction was also included in the model. To improve the normality of the data, the proportion of water was arcsine square root transformed. The rate of water loss was assessed with a mixed-linear model, with weight as the response variable and domestication factor, time and their interaction as fixed effects. Time nested in cuttings was also included in the random part of the model to account for repeated measures.

Resource storage. I assessed differences in total soluble sugar between the stems of wild and domesticated plants with an ANCOVA test, including stem diameter and its interaction with domestication factor in the model.

Shoot production and cutting longevity. The effect of domestication on the incidence and the total number of shoots was assessed using generalized linear models with a binomial (shoot sprouting incidence) and Poisson (number of shoots) error distribution, respectively. In both models, the initial diameter and weight of the cuttings were included as covariables. The effect of domestication on the time when the first shoot was recorded and cutting survival were assessed with time to an event (survival) analyses assuming a Weibull and exponential distribution, respectively. In both models, the initial diameter and weight of cuttings were included as covariables.

Rooting. Rooting incidence, the number of roots and the length of the largest root were compared between cuttings of wild and domesticated plants using generalized linear models (3 models in total) with binomial, Poisson and Gaussian error distributions, respectively. In all models, the initial diameter of the cuttings and its interaction with domestication were included as explanatory variables. The proportion of cuttings that developed leaves was compared between wild and domesticated plants with a proportion test. I assessed whether the number of main roots and the length of the largest root predicted the development of leaves (presence vs. absence of leaves) on cuttings from wild plants using a generalized linear model with a binomial error distribution. I did not include the data for the cuttings of domesticated plants in this analysis because all except one developed at least one leaf.

Clone survival. The survivorship of clones propagated from the cuttings of wild and domesticated plants were compared with survival models assuming a constant (exponential) hazard.

All data analyses were run in R .6.2.⁴⁶

Data availability

The raw data is included as online supplementary material.

Results

Resistance to desiccation

The stem cuttings of domesticated plants ($88.87 \pm 0.99\%$) had a greater water content than those of the wild plants did ($81.23 \pm 1.42\%$) ($F_{1,52} = 24.91$, $P < 0.01$; hereafter comparison wild vs. domesticated will be referred to as the domestication factor). Water content decreased slightly with the diameter of the cutting (Coefficient: -0.12 ± 0.5 ; $F_{1,52} = 15.79$, $P < 0.01$), however, the interaction between the cutting's diameter and domestication was not significant ($F_{1,52} = 0.11$, $P = 0.74$) (Fig. 1A). Stem cuttings of domesticated plants (32.21 ± 1.43 g) were also significantly heavier than those of the wild plants (23.58 ± 1.79 g; $F_{1,56} = 21.04$, $P < 0.01$). Weight loss in stem cuttings was linear (Coefficient: -0.52 ± 0.08 , $F_{1,512} = 67.21$, $P < 0.01$); and the cuttings of wild and domesticated plants lost weight at similar rates (*i.e.* non-significant time x domestication interaction: $F_{1,512} = 0.98$, $P = 0.32$) (Fig. 1B).

Resource storage

Total soluble sugars were very similar in the stems of wild (4.62 ± 0.21 %) and domesticated (4.71 ± 0.18 %) plants. Domestication ($F_{1,36} = 0.09$, $P = 0.78$), stem diameter ($F_{1,36} = 1.39$, $P = 0.24$) and their interaction ($F_{1,36} = 0.95$, $P = 0.33$) had no effect on the percentage of total soluble sugars in the stems. Only a thin ring of starch was detected in the most external parts of cross-sections of the wild plant stems (Fig. 2A), but starch almost completely covered the area of stem sections in domesticated plants (Fig. 2B).

Shoot production and cutting longevity

The vast majority of cuttings of domesticated plants (89.28%) developed at least one shoot during the study. In contrast, only one third of cuttings of wild plants developed at least one shoot (Table 1). The incidence of shoots was statistically different between wild and domesticated plants ($\chi^2_1 = 19.85$, $P < 0.01$); however, it was not affected by the diameter ($\chi^2_1 = 2.93$, $P = 0.11$) or the weight ($\chi^2_1 = 0.59$, $P = 0.44$) of the cuttings at the beginning of the experiment. The number of shoots per cutting was 2.69 times greater in domesticated plants than in the wild plants ($\chi^2_1 = 19.85$, $P < 0.01$) (Table 1). As occurred with shoot incidence, neither the initial diameter ($\chi^2_1 = 0.37$, $P = 0.51$) nor the weight ($\chi^2_1 = 1.71$, $P = 0.17$) of cuttings significantly affected the number of shoots per cutting.

On average, the first shoot was observed ca. 4 days earlier in the cuttings of domesticated plants than in those from the wild ($Z = 5.18$, $P < 0.01$) (Table 1; Fig. 3A). The highest proportion of cuttings with at least one shoot was reached in 15 and 25 days in the stem cuttings of domesticated and wild plants, respectively (Fig. 3A). The time the first shoot was observed was positively (Coefficient = 0.08 ± 0.03) influenced by initial weight ($Z = 2.52$, $P = 0.01$) but negatively influenced (Coefficient = -2.52 ± 0.91) by the initial diameter of the cuttings ($Z = -2.74$, $P < 0.01$). Seventy days after the experiment began, all the cuttings from the wild plants had died. In contrast, four of 28 cuttings (Survivorship = 0.14, 95% CI: 0.06-0.35) from the domesticated plants were still alive after 120 days, when the experiment finished. Mean time to death was 36.63 ± 2.65 and 54.53 ± 6.22 days for cuttings from wild and domesticated plants, respectively. The survivorship curves for the cuttings of wild and domesticated plants were significantly different ($Z = -2.88$, $P < 0.01$) (Fig. 3B). Neither initial diameter ($Z = -1.23$, $P = 0.07$) nor initial weight had a significant effect on the longevity of the cuttings ($Z = 1.76$, $P = 0.08$).

In contrast to the cuttings of wild plants, which produced only poorly developed leaves if any (Fig. 4A), most shoots from the cuttings of domesticated plants developed fully expanded leaves (Fig. 4B). After 120 days, when the experiment finished, the domesticated plant cuttings that survived also had vigorous, fully expanded leaves, and exhibited only minor signs of wilting (Fig. 4C).

Rooting

One month after being planted, cuttings had a rooting incidence of 83% and 76% for wild and domesticated plants, however, this difference was not statistically significant ($\chi^2_1 = 0.41$, $P = 0.52$). All the cuttings from wild plants that did not develop roots showed severe signs of rotting, but in only one third

of the cuttings from domesticated plants that did not develop roots, was there rotting, which was minor, and those cuttings did develop leaves. Cuttings from domesticated plants (12.61 ± 0.32) produced 1.24 times more roots than the cuttings of wild plants did (10.18 ± 0.21) and this difference was statistically significant ($\chi^2_1 = 5.81$, $P = 0.02$) (Fig. 5). The number of roots was also positively (Coefficient = 0.68 ± 0.21) and significantly ($\chi^2_1 = 19.09$, $P < 0.01$) affected by the initial diameter of cuttings, but the interaction between this covariable and domestication was not statistically significant ($\chi^2_1 = 2.51$, $P = 0.11$). The length of the largest root was not statistically different ($F_{1,41} = 0.74$, $P = 0.39$) between cuttings from wild (46.91 ± 4.52) and domesticated plants (53.13 ± 5.52) (Fig. 5). Similarly, neither the effects of initial diameter ($F_{1,41} = 0.22$, $P = 0.63$) nor its interaction with domestication ($F_{1,41} = 1.04$, $P = 0.31$) on root length were statistically significant.

While all rooted cuttings from domesticated plants, except one, developed at least one leaf (97%), only 50% of rooted cuttings from wild plants did ($\chi^2_1 = 9.76$, $P < 0.01$). However, neither the number of roots ($Z = 1.64$, $P = 0.12$) nor the length of the main root ($Z = 1.81$, $P = 0.07$) were reliable predictors of the presence/absence of leaves in the cuttings from the wild plants.

Clone survival

During the experiment, 21 (26.58%) and 49 (60.49%) of the clones from domesticated and wild plants died, respectively. Mean time to death was 211.31 ± 10.06 and 182.41 ± 5.06 days for domesticated and wild clones. At the end of this period, survival was 0.73 (95% CI: 0.64-0.84) for clones from domesticated plants and 0.39 (95% CI: 0.31-0.52) for wild plant clones. Survival curves for the clones of wild and domesticated plants were significantly different ($Z = -3.75$, $P < 0.01$) (Fig. 6).

Discussion

In this study I have shown that the stems of domesticated chaya, a clonally propagated crop, have a greater capacity to store water and starch than its wild relatives does. Once planted, the aerial (greater and faster shoot production and longevity) and the subterranean (greater number of roots) parts of stem cuttings also performed better in the cultivar than in its wild ancestors. Moreover, the cultivar clones had greater survivorship than their wild relatives when grown in a common garden. Given that the environmental variables and biological traits of the mother plants were experimentally controlled, the only relevant source of variation that could explain the differences observed in the stem cuttings from wild and cultivated plants is the domestication process. Therefore, consciously and unconsciously, humans have selected for traits that have improved the performance of stems as propagules in chaya. Since the traits that are selected for also increased the fecundity (*sensu* Elias and colleagues¹⁷) and survivorship of cultivated chaya, they can be considered adaptations to clonal propagation in human-created habitats²⁶.

Resistance to desiccation has been mentioned as an artificially-selected trait that may have contributed to improving the performance of stems as propagules in clonal crops^{13,22,23}. However, my findings for

chaya do not support this idea since the stem cuttings of wild and domesticated plants lost water at a similar rate. On the other hand, even with a similar rate of water loss, the greater water content observed in the cuttings from domesticated plants may delay dehydration relative to that which occurs in wild plant cuttings under similar environmental conditions.

A notable difference in the stems from wild and domesticated plants was starch storage. Starch almost completely covered the internal part of the stems of domesticated chaya, but was restricted to a small area in the most external part of stems in its wild relatives. Starch is accumulated in the sink organs (typically roots and stems) of some plant species, and this is considered a strategy to maintain growth under unpredictable adverse environmental conditions or to restart vegetative growth when adverse conditions are alleviated in seasonal environments²⁷. Molecular evidence suggests that the starch content of sink organs can also be maximized by means of artificial selection in other clonal crops (*e.g.* cassava²⁸, potato²⁹, yam³⁰). A high concentration of starch in stems and/or roots is highly desirable in some clonal crops (*e.g.* sugar cane, potato, cassava), where its presence and concentration is easily detected by humans through the sense of taste and thus, humans consciously have selected for a high starch content^{11,17}. The stems of chaya are not edible, therefore growers probably selected starch-rich stems unconsciously or indirectly by selecting a correlated trait. It is known that growers have selected plants with greater leaf production,²⁵ and these plants may be also more prone to producing an excess of photosynthates under the benign environmental conditions that prevail in agroecosystems and store them in sink organs such as starches^{27,31}. Further research is needed to identify in greater detail the physiological/biochemical mechanism(s) that underlie the greater starch storage observed in the stems of domesticated chaya.

Unrooted cuttings were able to produce shoots and leaves, however, the cultivar's cuttings performed far better than those of the wild plants did. Not only did most cuttings produce shoots but they also produced more, earlier and at a faster rate. Although the longevity of cultivar cuttings increased 1.5 times relative to the wild cuttings, among-cutting variation was notable. The unrooted cuttings of wild plants survived as long as two months, but amazingly, though artificial selection, the longevity of cuttings was double that. For logistical reasons, I had to conclude the experiment after four months, however, four cultivar cuttings were still alive at the end of the experiment. Therefore, the maximum longevity these cuttings is unknown but is longer than four months. This extraordinary longevity of unrooted cuttings is long enough to survive the entire drought season in the study area, and is similar to that of other clonal plants with specialized subterranean storage organs³². Because the cuttings were unrooted and had no source of water or nutrients, the most obvious explanation for the success of the domesticated cuttings, in terms of shoot production and longevity, is their greater internal content of starch and water.

The cuttings of both wild and domesticated chaya have a great (ca. 80%) chance of rooting and develop roots of similar length when planted. Doubtless, this pre-existing condition facilitated the domestication of chaya. The existence of hard-to-root cuttings, even in intensively managed modern crops, suggests that artificial selection has not always led to the optimization of rooting^{33,34}. On the other hand, the

number of roots was greater in the cultivar than in its wild relatives, suggesting that artificial selection has optimized this trait. Again, I suggest that the greater amount of endogenous starch observed in cultivated chaya may explain this result. Agronomic studies have also shown that endogenous sugar positively affects rooting in the cuttings of perennial woody crops³⁵, supporting this idea. Root traits are usually invisible organs for growers; however, previous studies have shown that the traits consciously selected by growers, such as thicker and larger stems, are good predictors of rooting in cassava¹⁷. This seems to be the case for chaya in which growers also select thicker stems as a source of cuttings²⁵ and, as reported in this study, thicker cuttings also produce more roots. An interesting issue to be explored is what contribution the leaves produced prior root development (a condition more frequently seen in the cultivar) may make to the energetic budget and the performance of the cuttings. Cuttings of the cultivar not only produced more roots, but also, rooted clones, with nearly two times greater survivorship than that of its wild counterpart during the first 9 months. Thus, when clonally propagated, cultivated chaya has a greater chance of survival in human-created environments than their wild relatives do. The greater survivorship observed in clones of domesticated plants could be an effect of the greater internal resources and water in the propagules, as well as their greater efficiency in producing roots, shoots and leaves. Rooting success and clone survival are highly relevant in the context of artificial selection because these are considered components of fitness in clonal plants^{17,26}.

In conclusion, I have found strong evidence that artificial selection has optimized chaya stems for clonal propagation. Relative to their wild ancestors, the stems of domesticated chaya have a greater capacity for water and carbohydrate storage. As these stem traits are linked to greater fecundity and clone survival, they can be considered adaptations. In contrast to seeds, the use of vegetative propagules skips the juvenile phase, allows for rapid growth, and reduces mortality^{36,37}. These advantages can easily compensate for some of the associated disadvantages, such as greater desiccation and limited dispersal when grown in agroecosystems tended by humans. These results represent an important advance in our understanding of the evolution under domestication of clonality. In contrast to the traditional view that the domestication of clonal plants was a one-step event^{14,21}, in this study I have shown that a single organ may have undergone several human-driven changes in which major anatomical, physiological and probably many genes are involved. My findings are in line with the more recent view that the domestication of clonal crops has been, instead, a process of adaptation in which numerous selection cycles were probably required^{9,13}. The transition from sexual to asexual reproduction through modified stems (stolons, rhizomes, cladodes) has evolved several times in wild angiosperms⁵. Clonal crops and their wild relatives may also help us understand this process during early species divergence^{8,13}. How does asexual reproduction affect resource allocation patterns? How do clonal plants continue evolving despite reduced recombination, or the lack thereof? What is the relevance of somatic mutation and diplontic selection (*i.e.* competitive interaction among modules with different somatic mutations within the same individual³⁸) in the evolution of clonal plants? These are just a few of the questions that clonal crops may help answer.

Declarations

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Author contributions

MAM-R conceived the experiments, collected and analyzed data and wrote the manuscript.

Competing interests

The author declares no competing interests.

Additional information

Supplementary information is available for this paper.

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Tables

Table 1. Shoot incidence, number (count) and time (days) elapsed after the first shoot was observed in stem cuttings of wild (Wild) and domesticated (Domesticated) chaya (*Cnidoscolus aconitifolius*). Stems were kept under controlled environmental conditions (26° C, 12 h light/dark) and had no external source of water or nutrients. Data are the mean $\pm$ 1 standard error, except for shoot incidence, for which the

proportion of cuttings with at least one shoot at the end of the experiment is shown. Different superscript letters indicate statistically significant differences between the cuttings of wild and domesticated plants.

Trait	Wild	Domesticated
Shoot incidence	0.33 ^a	0.89 ^b
Number of shoots per cutting	0.40 ± 0.11 ^a	2.89 ± 0.41 ^b
Days elapsed until first shoot	13.54 ± 1.76 ^a	9.08 ± 0.75 ^b

Figures

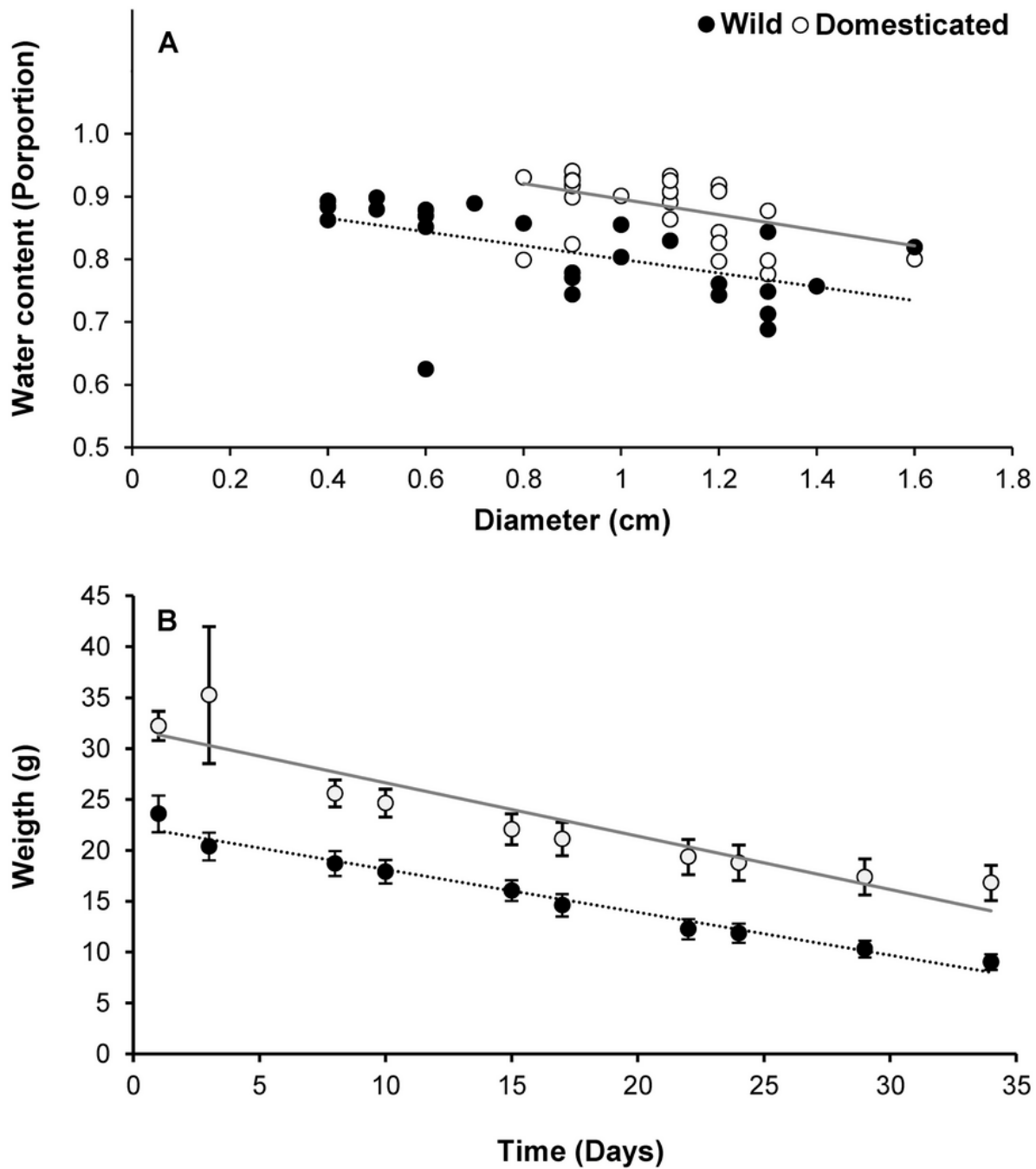


Figure 1

Water content (A) and weight loss (B) in stem cuttings of wild and domesticated *Cnidoscopus aconitifolius*. Circles in A correspond to individual cuttings and in B, correspond to the mean (± 1 SE) of a sample of cuttings. The X axis in A shows the diameter of cuttings and the time elapsed since the stems were cut in B. Gray regression lines correspond to the stems of domesticated plants and black dotted lines correspond to those of wild plants. Water content was assessed by weight differences of a cutting

after being oven dried for 72 h, and weight loss was measured as the weight of a cutting twice a week under constant environmental conditions (26 °C, 12 h dark/light).

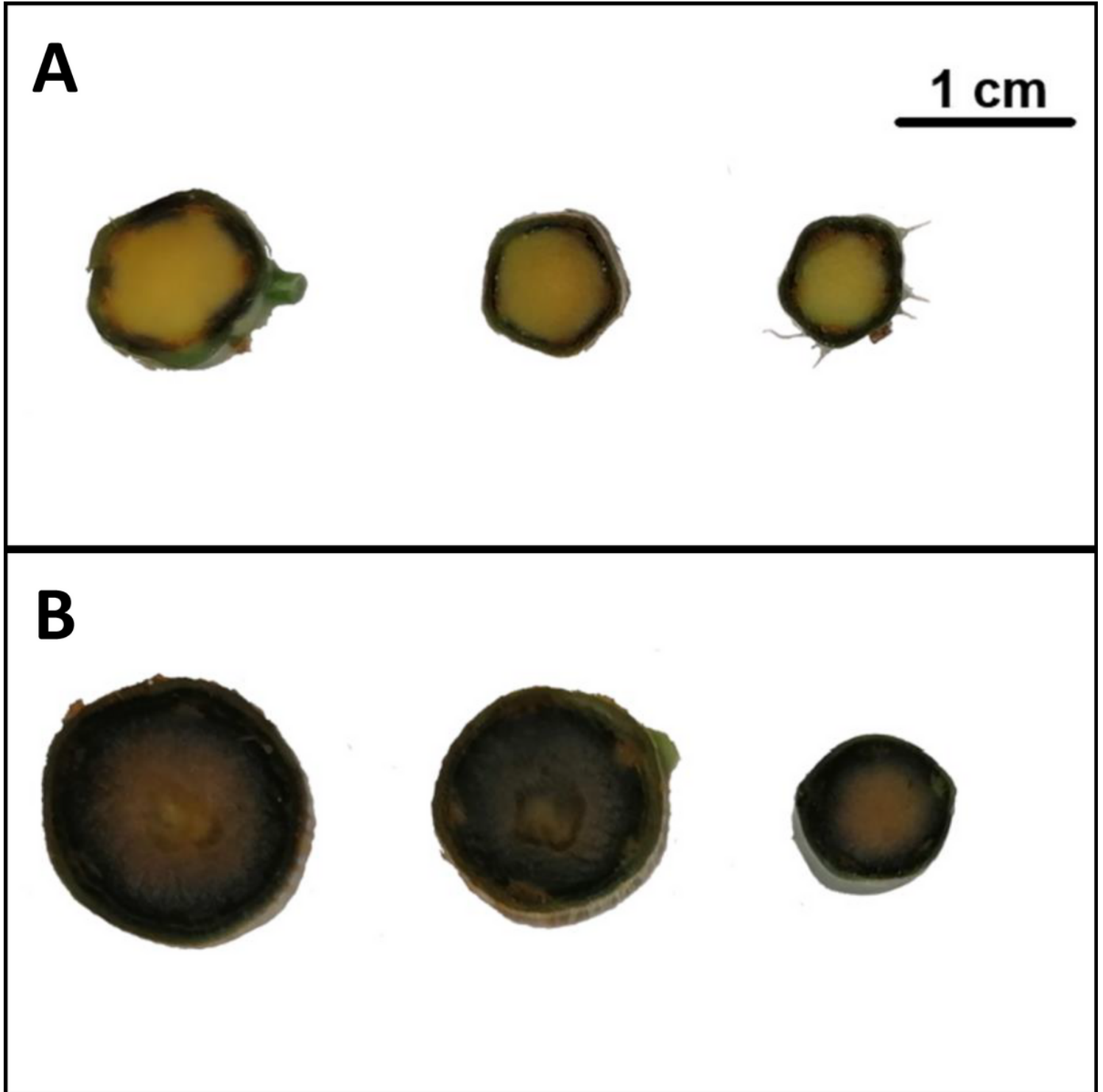


Figure 2

Transverse view of the stems of wild (A) and domesticated (B) plants stained with Lugol's solution. Dark-blue areas indicates the presence of starch. The three stem sections show the variation in terms of size and staining patterns observed within the sample of wild (A) and domesticated plants (B).

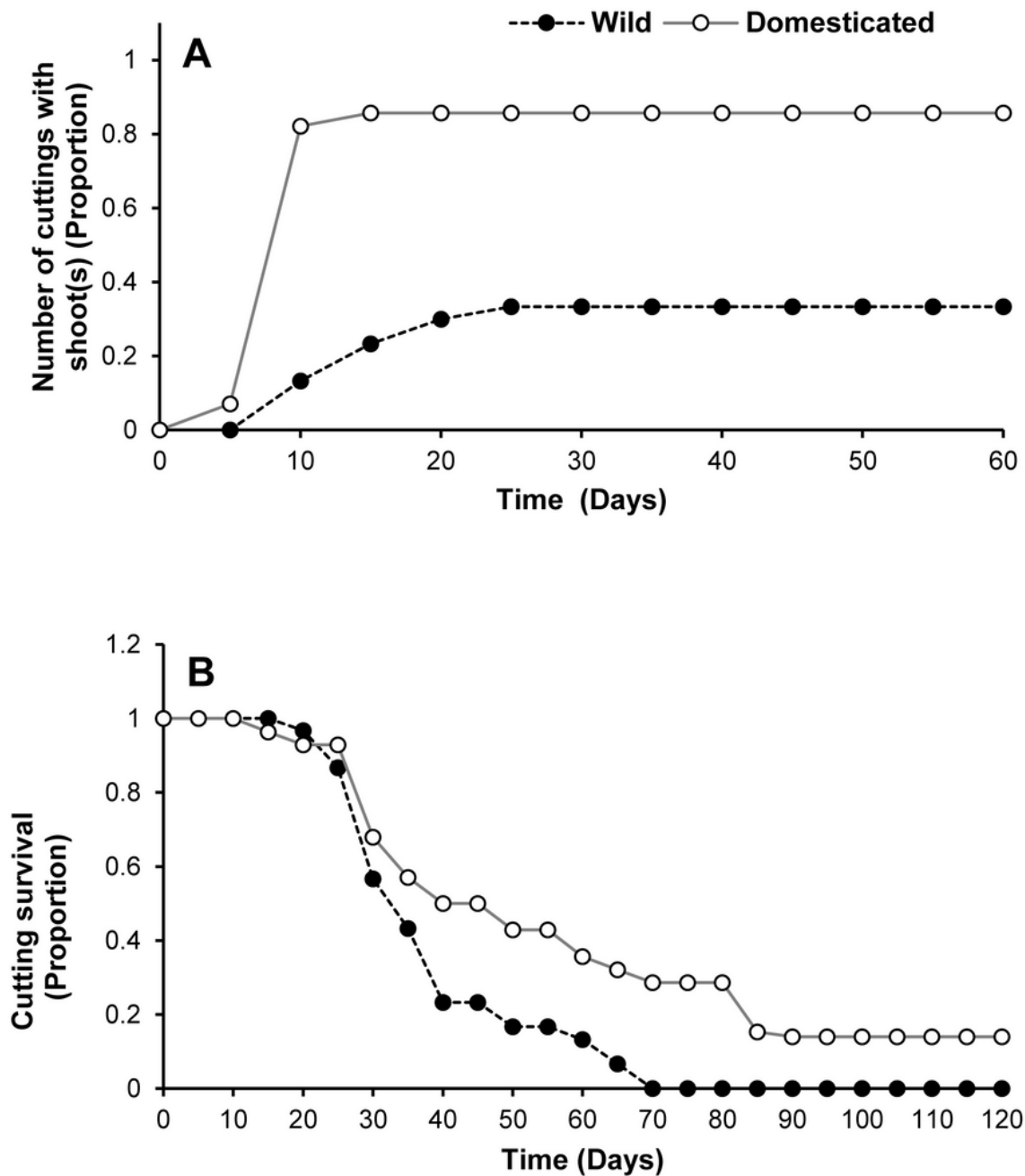


Figure 3

Cumulative proportion of cuttings of wild (Wild) and domesticated (Domesticated) plants of *Cnidoscolus aconitifolius* with at least one shoot (A). Survival curves of stem cuttings for wild and domesticated *C. aconitifolius* after 120 days of monitoring under controlled environmental conditions (B).

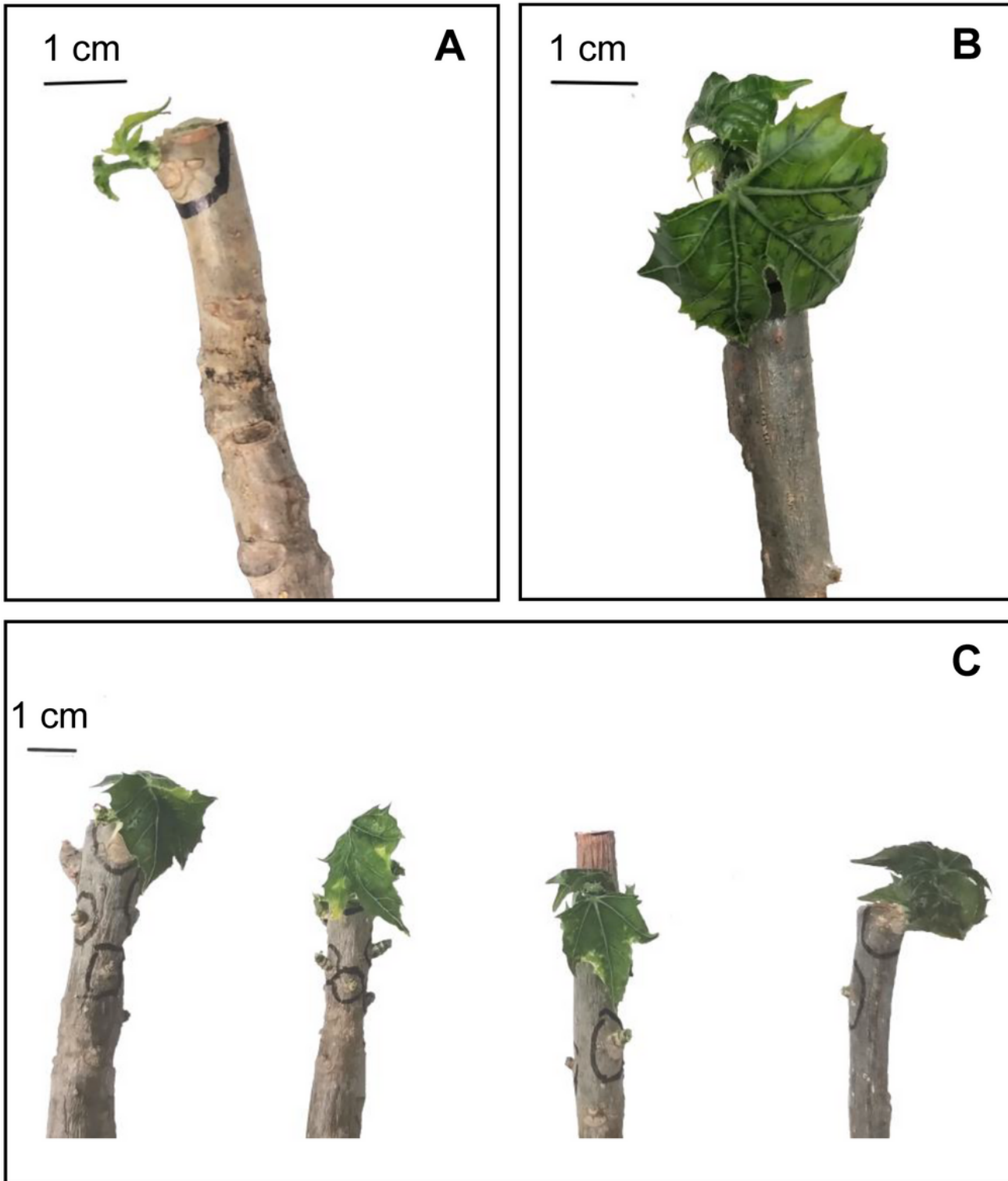


Figure 4

Stem cuttings from a wild (A) and a domesticated (B) *Cnidoscopus aconitifolius* six weeks after having been cut. Although the cuttings in A & B are the same age, the differences in terms of the degree of development of leaves is noteworthy. (C) Surviving cuttings of domesticated plants after 120 days, all of which were obtained from domesticated plants and were completely deprived of substrate and water. Scale bars represent 1 cm in all cases.

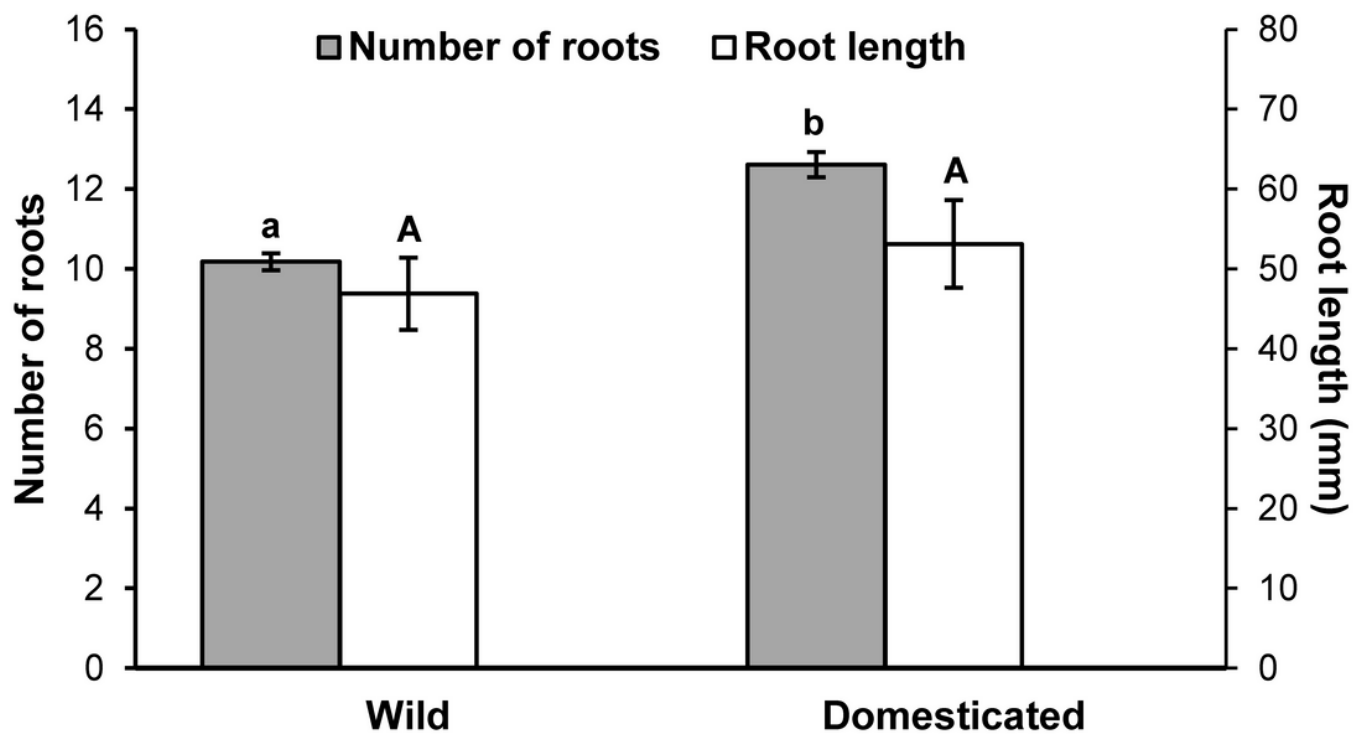


Figure 5

Number of main roots (Number of roots) and length of the largest root (Root length) of stem cuttings of wild and domesticated *Cnidoscolus aconitifolius*. Roots were counted and measured one month after being planted. Data are the mean $\pm$ 1 SE. Different letters indicate statistically significant differences between cuttings from wild and domesticated plants. Lower case letters correspond to the number of roots and upper case letters correspond to the length of the largest root.

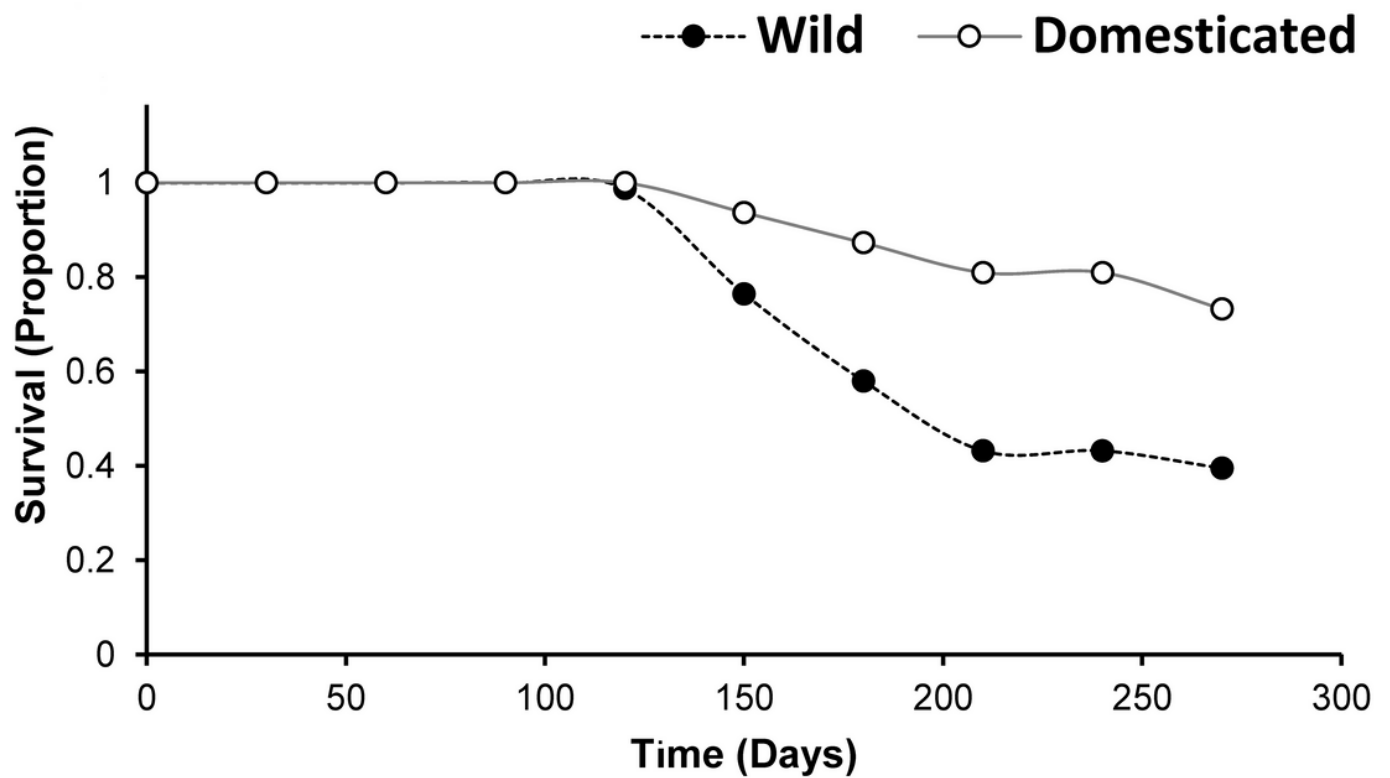


Figure 6

Survival of clones propagated using stem cuttings of wild and domesticated *Cnidoscolus aconitifolius*. Plants were maintained in a plant nursery in a common garden for nine months (270 days).