ALBINISM IN *HURA POLYANDRA* BAILL. (EUPHORBIACEAE): MORPHOLOGICAL AND HISTOLOGICAL ASPECTS

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Abstract: Albinism in plants is the inability to produce chlorophylls, limiting the photosynthesis process, which causes abnormal plant development and premature death. Albinism is an unusual phenomenon in plants found in their natural environments, however, it is a frequent phenomenon in the production of haploids by anther culture and somatic hybrids through protoplast fusion. The objective of this study was to determine some morphological characteristics of fruits and seeds and to establish the relationship with the occurrence of albino seedlings, as well as some histological characteristics in this type of seedlings, obtained from a mother plant of 'habilla' *Hura polyandra*, comparing it with normal green seedlings. The parental plant was collected in Salas (Lambayeque, Peru), from an isolated population without other individuals present in the surrounding 10 km. The theory of what was postulated for tropical trees, in that albinism in *H. polyandra* is predominantly caused by self-pollination and genetic inbreeding.

Keywords: albino seedlings, fruits and seeds, green seed-lings, *in vitro* conditions, self-pollination.

ALBINISMO IN *HURA POLYANDRA* BAILL. (EUPHORBIACEAE): ASPECTOS MORFOLÓGICOS E HISTOLÓGICOS

Resumen: El albinismo en plantas es la incapacidad de producir clorofilas, limitando el proceso de fotosíntesis, lo que provoca un desarrollo anómalo de la planta y muerte prematura. En poblaciones naturales el albinismo es un fenómeno raro en plantas. Sin embargo, es frecuente en la producción de haploides por cultivo de anteras e híbridos somáticos por fusión de protoplastos. El objetivo del presente estudio fue determinar algunas características morfológicas de frutos y semillas y establecer la relación con la ocurrencia de plántulas albinas, así como algunas características histológicas en este tipo de plántulas, obtenidas de una planta madre de ‘habilla’ *Hura polyandra*, comparándola con plántulas verdes normales. Este individuo fue localizado en Salas (Lambayeque, Peru) y no se encontró otro individuo en 10 km circundantes. Al igual a lo postulado para los árboles tropicales el albinismo en *H. polyandra* obedecería predominantemente a la autopolinización y la depresión endogámica.

Palabras clave: plántulas albinas, frutos y semillas, plántulas verdes, condiciones *in vitro*, autopolinización.

INTRODUCTION

Hura polyandra Baill., ‘habilla’, ‘habillo’, ‘tereta’, ‘evillo’ is a tree that grows primarily in the wet tropical biome, with its native distribution reaching from Mexico to Central America and Ecuador (Kew, 2023). *H. polyandra* belongs to the Euphorbiaceae, tribe Hureae, with a total of seventeen species allocated and distributed across three genera: *Algernonia Baill*. (11 species), Hura L. (2), and *Ophthalmoblapton* Allemão (4). The South American species are mostly distributed within the Atlantic Forest, particularly in southeastern Brazil (Oliveira et al., 2013), showing a distinct center of diversity. *H. polyandra* is closely related to *Hura crepitans* L., with minor differences present in its vegetative structures such as leaf margin (serrate vs smooth); number of stamen whorls (5-10 whorls); staminate inflorescence; and pistillate white flowers (Oliveira et al., 2013).

Although the literature does not report phytochemical and medicinal studies of *H. polyandra*, it does do so for *H. crepitans* (sandbox tree), where it was reported to have many ethnomedicinal applications for antimicrobial, anti-inflammatory and antihepatotoxic treatment. In this sense, chemical constituents of sandbox tree include: daphnane diterpenes, daphnetoxin acid and huratoxin along, with apocynin and methylpentadecanoate which shown to be effec-tive in antihepatotoxic activity obtained from the leaves and stem bark extracts (Oloyede et al., 2016; Owojuyigbe et al., 2022). However, not only the hepatoprotective potential of the species has been highlighted, but also other ethnomedicinal applications such as a purgative, emetic, and the treatment of leprosy (Owojuyigbe et al., 2020). In contrast, *H. crepitans* is also important for its toxicity, since the latex or sap is known to be a poisonous to fish due to the presence of huratoxin and hexahydrohuratoxin. These are two lectins with haemagglutinating activity that inhibit protein synthesis (Oloyede & Olatinwo, 2011). Likewise, the seed contains a glucosamine with mitogenic properties and two toxic albumins: hurin and creptin, which are distributed in all the organs of the plant (Jaffé & Seidi, 1969; Falasca et al., 1980).

In the field of veterinary medicine, the anticoccidial *effects* of the seeds of *H. crepitans* were evaluated using lambs as an alternative to conventional therapies, in the control of several pathogenic species of *Eimeria as E. ovinoidalis* and *E. crandallis* (Velazquez-Gonzalez et al., 2022). In recent years, important reviews have been carried out on ethnomedical and pharmacological aspects (Owojuyigbe et al., 2020), as well as studies regarding the phytochemical characterization, antioxidant activity and nano-*formulation* (Vasallo et al., 2020) of these plant species. Considering that *H. polyandra* and *H. crepitans* are closely related species, it is highly likely that *H. polyandra* has many of the phytochemical and medicinal characteristics, and thus may carry similar pharmaceutical uses and phytochemical properties.

The partial or complete loss of chlorophyll pigments and incomplete differentiation of chloroplast membranes (hereafter referred to as albinism), is a rare phenomenon in plants (Silva et al., 2020). Although albinism is rare to occur naturally in a tropical forest (Takeuchi et al., 2020), the albino phenotype has been observed in several natural populations across the tropical latitudes (Sasaki, 2008). Causes leading to albinism in tropical trees appear to be preferen-tially via outcross (Ward et al., 2005), self-pollination or inbreeding depression found in isolated populations where genetic interchange is limited, and albinoic differentiation has been found within both the seed and seedling stages (Ismail et al., 2014). In general, this phenomenon negatively affects the photosynthesis and the plants usually die at a young stage without reaching maturity (Kumari et al., 2009). Experiments using *Richetia laxa* (Slooten) P.S. Ashton & J. Heck. (syn. *Shorea laxa* Slooten), however, showed that the albino seedlings survived at least a few months after germination (Takeuchi et al., 2020).

Studies on the occurrence of albinism in plants within their natural environment are scarce, and the initial studies using natural populations were carried out using the 'yellow-monkey flower' *Erythranthe guttata* (DC.) G.L.-Nesom (syn. *Mimulus guttatus* DC.) (Kiang & Libby, 1972). In recent years, albino plants have been studied in some species such as *Agave* L. (Us-Camas et al., 2017), *Sequoia sempervirens* (D.Don) Endl. (Pittermann et al., 2018), and *Pyrola japonica* Alef. (Shutoh et al., 2020), although the motive behind these studies usually revolt around application for ornamental uses. In *Delonix regia* (Bojer ex Hook.) Raf. 'Royal Poinciana', phenotype and albino seedlings were characterized and described for their morpho-physiological changes associated with albinism, in particular those where albino seedlings showed reduced growth; lower chlorophyll and protein content in foliar tissues; and lower concentrations of lipids and carbohydrates stored in cotyledons (Silva et al., 2020). In *S. laxa*, a Dipterocarpaceae tree endemic to Borneo, the high frequency of albinism observed in seedlings as result of inbreeding was investigated and compared using the cross types of albino seedlings versus green-leaf seedlings. Genetic analysis using microsatellites has confirmed that most albino seedlings (98.3%) were derived as the result of self-pollination or inbreeding (Takeuchi et al., 2020).

Albinism is also a common and frequent problem with interspecific crosses. For instance, in the hybridization of *Cicer arietinum* L. with *C. judaicum* Boiss. and *C. pinnatifidum* Jaub. & Spach, only the rescued hybrids survived using *in vitro techniques* and it was identified that poor chloro-plasts formation is the most important barrier to interspecific hybridization success (Clarke et al., 2011). The analysis of albino mutant phenotypes of barley (*Hordeum vulgare* L.), with full or partial chlorophyll deficiency, made possible the identification of nuclear genes controlling chlorophyll synthesis by RNA-seq (Shmakov et al., 2016). Similarly, in the interspecific hybridization among cultivars of Hibiscus species: *H. coccineus* Walter, *H. laevis* All. and *H moscheutos* L., the occurrence of albinism was also an important barrier to successful hybridization (Kuligowska et al., 2016).

In tissue culture experiments such as microspores and anther culture albinism is also identified as being the limiting barrier in the generation of doubled haploids, due to physiological and genetic factors that limit their growth (Kumari et al., 2009). In barley, as well as other cereals such as triticale, wheat, rye and oats, androgenesis is highly useful for plant breeding, however. In all these cereals, albinism often occurs in androgenesis-derived plants and ranges from 1 to 100% depending on the genotypes. It was postulated that the direct cause of albinism is the inability of proplastids to transform into chloroplast (Makowska & Oleszczuk, 2014). Alternatively, several environmental factors, such as the physical and chemical conditions of the population; light and temperature; and culture media composition can determine the frequency of albino plants for-mation. This is the case of albinism in tea plants that lack chlorophylls under certain environmental conditions, constituting two types of albino tea: light- and temperature albino tea cultivars. These mutations are commercially important for their high levels of amino acids and the "umami" taste (Shin et al., 2018).

In recent years, advances in molecular biology have made it possible to achieve a better understanding of albinism in plants on a genetic level. Albino plants that were isolated from regenerated populations of variegated 'Golden Pothos', *Epipremnum aureum* (Linden & André) G.S. Bunting, an *ornametal* Araceae native to Southeast Asia and Papua New Guinea, shown that albinism was established due to impaired expression of EaZIP. It also showed impairments in the encoding of Mg-protoporphyrin IX monomethyl ester cyclase, and the expression of *Arabidopsis* CHL27 (a homolog of EaZIP) that restored green color and chloroplast development (Hung et al., 2021). In 'Huabai 1', a novel natural albino mutant tea germplasm containing high amino acids and catechins contents, a total of 483 DEGs (albino-associated differentially expresses genes) were identified from white shoots compared to its normal green shoots, and 15 DEGs were identified to be involved in phenylpropanoid biosynthesis (Ma et al., 2018).

In this present study, the objective was to describe the morphological and histological changes in albino *Hura polyandra* seedlings, and compare those with normal green seedlings. It is the first report of albinism and focused study to understand this phenomenon using this species.

MATERIAL AND METHODS

PLANT MATERIALS

Fruits and seeds of *Hura polyandra* was collected from two localities: Las Colmenas (Chongoyape District, Lambayeque) (6º36'58.89"S; 79º38'36.38"O; Datum WGS84, 228 m); and Salas (Salas District, Lambayeque) (6º16'59.93"S 79º38'41.14"O; Datum WGS84, 133 m) (Fig. 1). The material was taken from specimens where the adult trees were at least 20 years of age and with a height of 8 and 12 m, respectively (Fig. 2) during the months of December 2022 and January 2023.

In Las Colmenas two adult specimens were located, separated by about 100 m. Only one specimen was found in Salas. In both localities no other specimens of this species were found in the surroundings for more than 10 km.

For each collection site, 20 ripe fruits were collected directly from the tree. In the laboratory, a further six ripe fruits were selected from either collection location, chosen for the best morphological and phytosanitary characteristics.

For every two fruits, around 30 seeds were obtained (each fruit produces an average of 15 seeds). About 20 seeds with similar morphological characteristics were selected from each two fruits, which were then used for *in vitro and ex vitro* germination tests.

In the morphological determination of fruits and seeds, three fruits with the best morphological and phytosanitary characteristics were chosen, collected in the towns of Las Colmenas and Salas. From these fruits, 45 seeds were obtained, of which 20 seeds were selected for the determination of their morphological characters. Fruits and seeds were measured in cm using a Vernier caliper MT-00851 and weighed on an Electronic SF-400 digital scale.

IN VITRO SEEDS GERMINATION

Seeds were washed with detergent for 10 min and then treated with 0.2% Benomyl fungicide for 60 min. In a sterilized air laminar flow chamber, the seeds were disinfested with 70% ethyl alcohol for two minutes and cleansed with a commercial bleach (sodium hypochlorite 1:1) for eight minutes. After removing the disinfectants using sterilized distilled water, the seeds were grown individually in a specially formulated seed germination culture medium. This culture medium was made using mineral salts MS (Murashige & Skoog, 1962), the vitamins m-inositol 100.0 mg.L^{-1} and thiamine HCl 1.0 mg.L^{-1}, sucrose 2.0% and gelled with agar-agar 0.7%. The pH of the culture medium was adjusted to 5.8±0.1 with 0.1 N KOH or HCl before supplementing the agar. Sterilization was performed in an autoclave at 15 lbs.inch^{-2} pressure and a temperature of 121 °C for 20 minutes. Environmental incubation conditions were set at 26±2 °C; with 85% relative humidity; and a photoperiod of 16 h using 1 W.m^{-2} illumination.

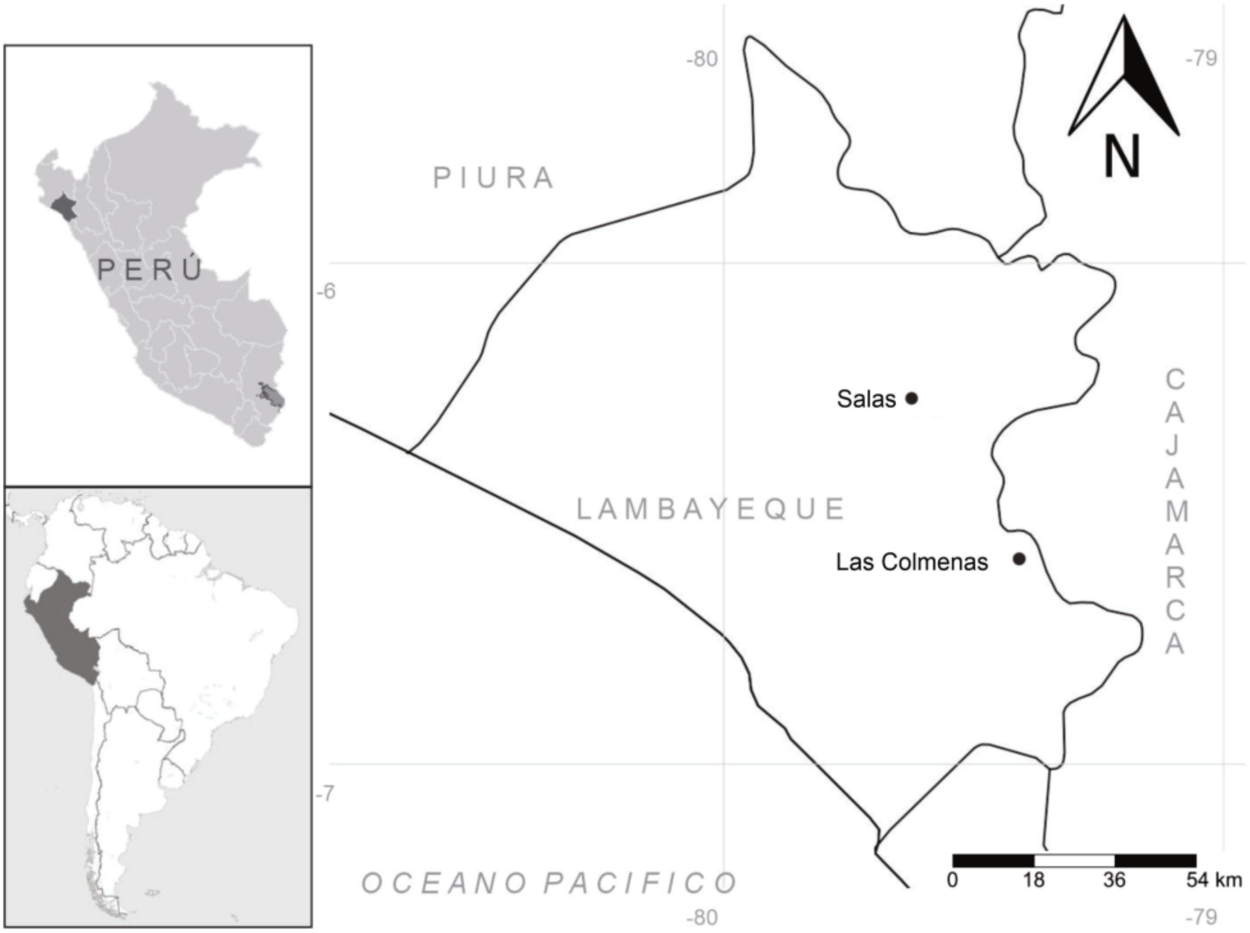


Fig. 1. Collection sites of 'habilla' (Hura polyandra) in Lambayeque Department (Peru).

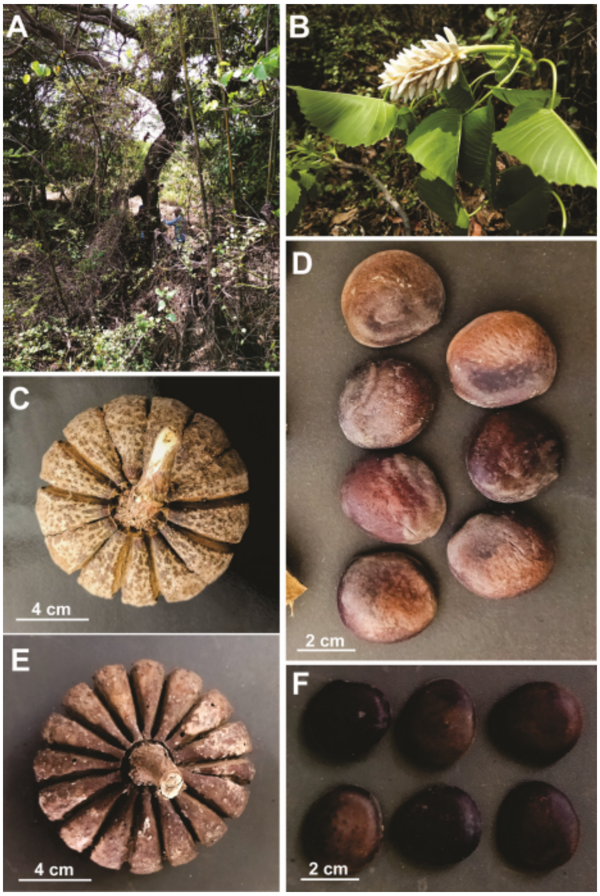


Fig. 2. A. Adult plant of *H. polyandra* in Salas. B. Inflorescence. C. and D. Fruit and seeds (light brown color with scattered whitish spots) of *H. polyandra* from Salas locality. E. and F. Fruit and seeds (uniform dark brown color) of *H. polyandra* from Las Colmenas locality.

A seed was considered germinated when the radicle emerged and reached a size of 5 mm, while a seedling was considered when the cotyledons detached from the integuments inside the seed. The evaluations were carried out after four weeks of culture.

Ex vitro seeds germination

The selected seeds were washed with detergent and treated with a fungicide in a manner similar to the in vitro seed germination. They were conditioned using a humid chamber covered with blotting paper and 90x15 mm glass Petri dishes. Environmental incubation conditions were similar to those established for in vitro cultures. The water supplement was permanent throughout the experiment. The evaluations were carried out after four weeks. The same criteria of evalua-tion previous described were used to determine germination.

The following vegetative structures hypocotyl/epicotyl, cot-yledonary leaves and primary leaves, obtained from five seedlings germinated in vitro and ex vitro, were measured using a ruler graduated in centimeters.

Histological characterization

Green and albino seedlings were selected to make very fine transversal cuts using a freehand method and carrot fragments as a support. Fragments of hypocotyl, epicotyl, petiole, cotyledonary and primary leaves were placed in water in Petri dishes for ten minutes to remove latex with irritant characteristics. The cuts made and epidermal tissue were placed on coverslip slides and their coherent observations made using a trinocular optical microscope Nikon E200 model with a coupled Motic digital camera.

Statistical analysis

The morphological variables of fruits and seeds were presented with the descriptive statistics of averages and standard deviation. Then the averages between green and albino plants were compared, through the Student's T-test, with a significance level of 5%. In the *in vitro* seed germination tests, 60 seeds of six fruits were used, coming from each collection location, Salas and Las Colmenas. Both the fruits and the seeds, at a rate of 20 seeds per two fruits, were taken morphological characteristics. The same procedure was used in the *ex vitro* seed germination tests.

After four weeks of seed culture, in germinated seedlings, both normal green and albino seedlings, the morphological variables evaluated were epicotyl/hypocotyl, cotyledonary leaves (petiole and leaf blade), and primary leaves (petiole and leaf blade) through the X_2 goodness-of-fit test.

Results and Discussion

Frequency of occurrence of albino seedlings

In seeds evaluation, cultivated using the in vitro condition for the three fruits collected in Salas, yielded an average of 41.7% albino seedlings; while in the seeds collected from Las Colmenas there were no seedlings showing albinism (Tab. 1). A similar result was reached in using the *ex vitro* cultivation (Petri dishes), where 46.7% of the seeds collected from Salas produced albino plants; while no albino seedlings from the seeds collected in Las Colmenas (Tab. 2). The goodness-of-fit test (Tab. 3), both in the *in vitro* and *ex vitro* cultures, in the Salas locality the occurrence of green and albino seedlings was indistinct since one green seedling occurred for each albino seedling ($X^2 = 0.51$ and *p-value* = 0.4765), while in the Las Colmenas locality only green seed-lings occurred in all the seeds evaluated. In *S. laxa*, numerous albino seedlings were observed under a mother tree as a consequence of the ecological event called general flowering , a phenomenon unique to lowland seasonal tropical forests in Southeast Asia that occurred between 2013 and 2014 (Sakai, 2002; Takeuchi et al., 2020). However, percentages of green seedlings compared to albino seedlings were not reported. In *D. regia*, the green seedlings identified as wild-type made up 95.0% of the total, while "white plants" identified here as albino seedlings represented 3.0% (Silva et al., 2020). This would make the percentage of albino seedlings compared to normal green seedlings much lower than those obtained in *H. polyandra*. Furthermore, the identification of the albino phenotype in *D. regia* occurred 10-12 days after sowing (Silva et al., 2020); while in *H. polyandra* the identification of albino seedlings occurred 3-5 days after sowing, even when the seed coats partially cover the cotyledons (Fig. 3).

Tab. 1. Frequency of occurrence of green and albino seedlings of *H. polyandra* in *in vitro* culture.

Locality	Experiment replication	Seedling phenotype	
		Green (No/%)	**Albino (No/%)**
Salas	1	13 / 65.0	7 / 35.0
	2	12 / 60.0	8 / 40.0
	3	10 / 50.0	10 / 50.0
Mean		11.7 / 58.3	8.3 / 41.7
Las Colmenas	1	20 / 100.0	0 / 0.0
	2	20 / 100.0	0 / 0.0
	3	20 / 100.0	0 / 0.0
Mean		20 / 100.0	0 / 0.0

Average of six fruits and 20 seeds per replicate.

Tab. 2. Frequency of occurrence of green and albino seedlings of *H. polyandra* in *ex vitro* culture.

Locality	Fruit(No)	Seedling phenotype	
		Green (No/%)	**Albino (No/%)**
Salas	Fr. 1	14 / 70.0	6 / 30.00
	Fr. 2	10 / 50.0	10 / 50.00
	Fr. 3	8 / 40.0	12 / 60.0
Mean		10.7 / 53.3	9.3 / 46.7
Las Colmenas	Fr. 1	20 / 100.0	0 / 0.0
	Fr. 2	20 / 100.0	0 / 0.0
	Fr. 3	20 / 100.0	0 / 0.0
Mean		20 / 100.0	0 / 0.0

Tab. 3. Frequency of occurrence of green and albino seedlings of *H. polyandra* in *in vitro* and *ex vitro* culture (X_2 goodness-of-fittest).

Germination condition	n	Locality	Green seedlings	Albino seedlings	*p-value*
In vitro	60	Las Colmenas	60/60	0/60	0.0000
	60	Salas	35/60	25/60	0.1967
Ex vitro	60	Las Colmenas	60/60	0/60	0.0000
	60	Salas	32/60	28/60	0.6056

Fig. 3. Seedlings of *H. polyandra* 3-5 days after sowing: A. and B. Green seedlings. C and D. Albino seedlings.

As indicated above, in Salas there was only a single specimen of *H. polyandra* present with no other individual specimens observed across more than 10 km of its surroundings. In contrast, in Las Colmenas there were two specimens observed that were separated by a distance of no more than 100 m. For this reason, it is possible that in Salas, although this species predominantly reproduces by outcrossing, the emergence of albino seedlings is the result of selfing taking place on just this single mother tree. This does not appear to have occurred in Las Colmenas where self-crossing would have occurred between the two separate trees, producing 100% green seedlings. In *S. laxa*, most albino seedlings (98.3%) were derived from self-pollination which was confirmed by microsatellite analysis. The overall estimated selfing rate was to be high (61.3%), indicating that reproductive isola-tion occurred in the mother tree and further suggesting the part-cause of albinism as the result of genetic isolation (Takeuchi et al., 2020).

MORPHOLOGICAL CHARACTERISTICS OF FRUITS AND SEEDS OF *H. POLYANDRA*

The fruits of *H. polyandra* collected from Las Colmenas site were slightly larger in length and diameter and the number of seeds it contains in relation to the fruits collected from Salas (Tab. 4). A similar situation was observed with the seeds, where the dimensions of length and width were slightly greater in the seeds collected from Las Colmenas compared to those collected from Salas (Tab. 5). This was further seen in the weight of the seeds individually (2.73 g and 1.99 g, respectively). Additionally, the seeds of fruits collected from Las Colmenas showed a uniform dark brown color (Fig. 2D) while the seeds collected from Salas showed a light brown color with scattered irregularly shaped whitish spots (Fig. 2F).

The literature does not report any relationship between the size and weight of the fruits and the size, weight and characteristics of the seeds with the occurrence of albinism under normal natural conditions, as a being reported in the present study. In *S. laxa,* both natural regenerated green and albino seedlings were studied, but there was no mention on the characteristics of fruits and seeds and their respective relationship with albinism offspring (Takeuchi et al., 2020). In *E. aureum* ‘Golden Pothos’ the albino plantlets were generated from variegated leaves by tissue culture techniques (Hung & Xie, 2009; Hung et al., 2021). Therefore, seeds were not used as starting material and the plants formed mere asexual clones of the mother plant making it unsuitable for the core of this study. In *D. regia*, the seeds were collected from a single mother tree, where albino seed-lings were previously observed near this specimen (Silva et al., 2020). In the mentioned study there was no relationship between the characteristics of the fruits and the albinism of its offspring.

The differences in the size of the hypocotyl/epicotyl, the cotyledonary leaves and the primary leaves between the green and albino seedlings were significant, especially in the size of the hypocotyl/epicotyl, where the green seedlings exceed the size by 10 cm of the albino ones (25.7 and 15.5 cm, respectively) (Tab. 6, Fig. 4A). In *D. regia* albino seedlings showed reduced growth, although during the early survival period the green and albino seedlings did not show discernible differences in growth rate (Silva et al., 2020). In *S. laxa*, even when not

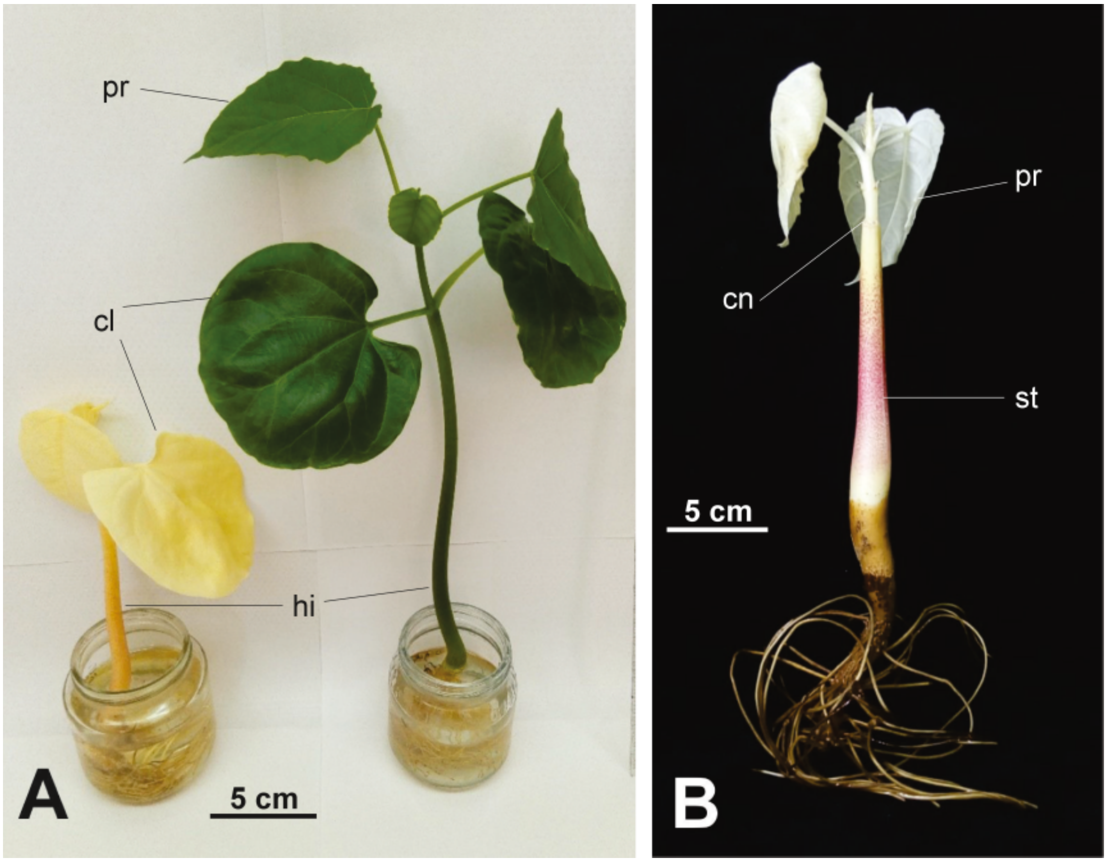


Fig. 4. Seedlings of *H. polyandra* 20 days after seed germination: A. Seedling showing the cotyledonary leaves in green plant with primary leaves (right) and albino plant (left). B. Albino seedling showing reddish pigmentation by anthocyanins on the stem, and the cotyledonary node when the cotyledonary leaves have already died. Structures observed: hypocotyl (hi), cotyledonary leaves (cl), primary leaves (pr), stem (st), cotyledonary node (cn).

was explicity indicated, green seedlings were larger than albino seedlings (~30 and 20 cm, respectively). This could be due to inbreeding depression of selfed seedlings, which resulted in lower seedling survival (Takeuchi et al., 2020). On the other hand, in *H. polyandra* the size of the cotyledonary leaves and the primary leaves also showed differences between the green and albino seedlings, especially in the size of the petiole, although these differences were not significant (*p-value* = 0.4765) in the size of the eventual leaf blade. (Tab. 6, Fig. 4A). In contrast, in *D. regia* the seedlings of both phenotypes presented three pinnate leaves and only from the fourth leaf the typical leaf pattern of the species was observed (Silva et al., 2020). These observations regarding the size of the seedlings were consistent with the results obtained in the study with *H. polyandra*. In the studies using in *S. laxa* (Takeuchi et al., 2020) or *D. regia* (Silva et al., 2020) the size of the hypocotyl/epicotyl axis, petiole and leaf blade of both cotyledonary and primary leaves were not reported.

An important characteristic of albino seedlings is the reddish pigmentation in the hypocotyl, which is due to the presence of anthocyanin pigments stimulated excessively by this mutation (Fig. 3C and 3D, Fig 4B). In numerous studies it has been observed that the majority of albino genotype were deficient in pigments of chlorophyll, xanthophylls and carotenes (Kumar et al., 2022), so the presence of anthocyanin pigments is very distinctive trait observed in the present study. The photoprotector role of the anthocyanin were knowing and the the metabolism of anthocyanin expression is started by phototropins photoreceptors that induce protection from reative species of oxygen under light stressed conditions (Kadomura-Ishikawa et al., 2013; Agati et al., 2021), which indicante that the albino seedlings of *H. polyandra* where under stress.

A relevant characteristic of the albino se-

Tab. 4. Morphological characteristics of fruits of *H. polyandra* collected in the localities of Las Colmenas and Salas (Lambayeque, Peru).

Locality	Morphological characteristics of fruits			
	Height (cm)	Diameter (cm)	Weight (g)	Seeds (No)
Las Colmenas	5.83±0.28	10.30±0.28	139.0±11.5	15.3±0.57
Salas	5.50±0.00	9.17±0.28	107.0±9.85	13.0±1.00

Average of three fruits and 20 seeds per fruit.

Tab. 5. Morphological characteristics of seeds of *H. polyandra* collected in the localities of Las Colmenas and Salas (Lambayeque, Peru).

Locality	Fruit (No)	Morphological characteristics of seeds		
		Diameter (cm)	Width (mm)	Weight (g)
Las Colmenas	1	2.64±0.08	8.13±0.84	2.90±0.12
	2	2.68±0.07	8.72±1.31	2.75±0.68
	3	2.74±0.12	8.88±0.72	2.55±0.13
Mean		2.69	8.58	2.73
Salas	1	2.62±0.21	7.81±1.20	1.80±0.47
	2	2.57±0.09	8.28±1.54	2.15±0.55
	3	2.63±0.06	7.53±0.99	2.01±0.56
Mean		2.61	7.87	1.99

Average of three fruits and 20 seeds per fruit.

edlings is that the majority are usually short-lived even when they can be maintained in in vitro cultures with high sucrose content (García-Alcázar et al., 2017). However, its short-lived nature is a strong limitation in the study of coordinated expressions of nuclear and plastid genes, as these tend to develop as the plant mature past the seedling stage (Hung et al., 2021). In our study the survival of albino *H. polyandra* seedlings under greenhouse conditions was no longer than 4 weeks after seed germination; while in *S. laxa* the survival rate of albino seedlings reaches between 4 and 19 weeks, but was significantly decreased exhibiting a 10-fold higher mortality rate opposed to regular green-leaf seedlings (Takeuchi et al., 2020). In *D. regia*, similar as observed in *H. polyandra*, the white seedlings survived about 3 weeks following germination, till the point where the cotyledons were completely senescent (Silva et al., 2020). This short survival period that last, merely one month to up to a few months is probably due to the fact that the seedlings live on the reserve substances in the endosperm (Sasaki, 2008).

However, in *H. polyandra* the reserve

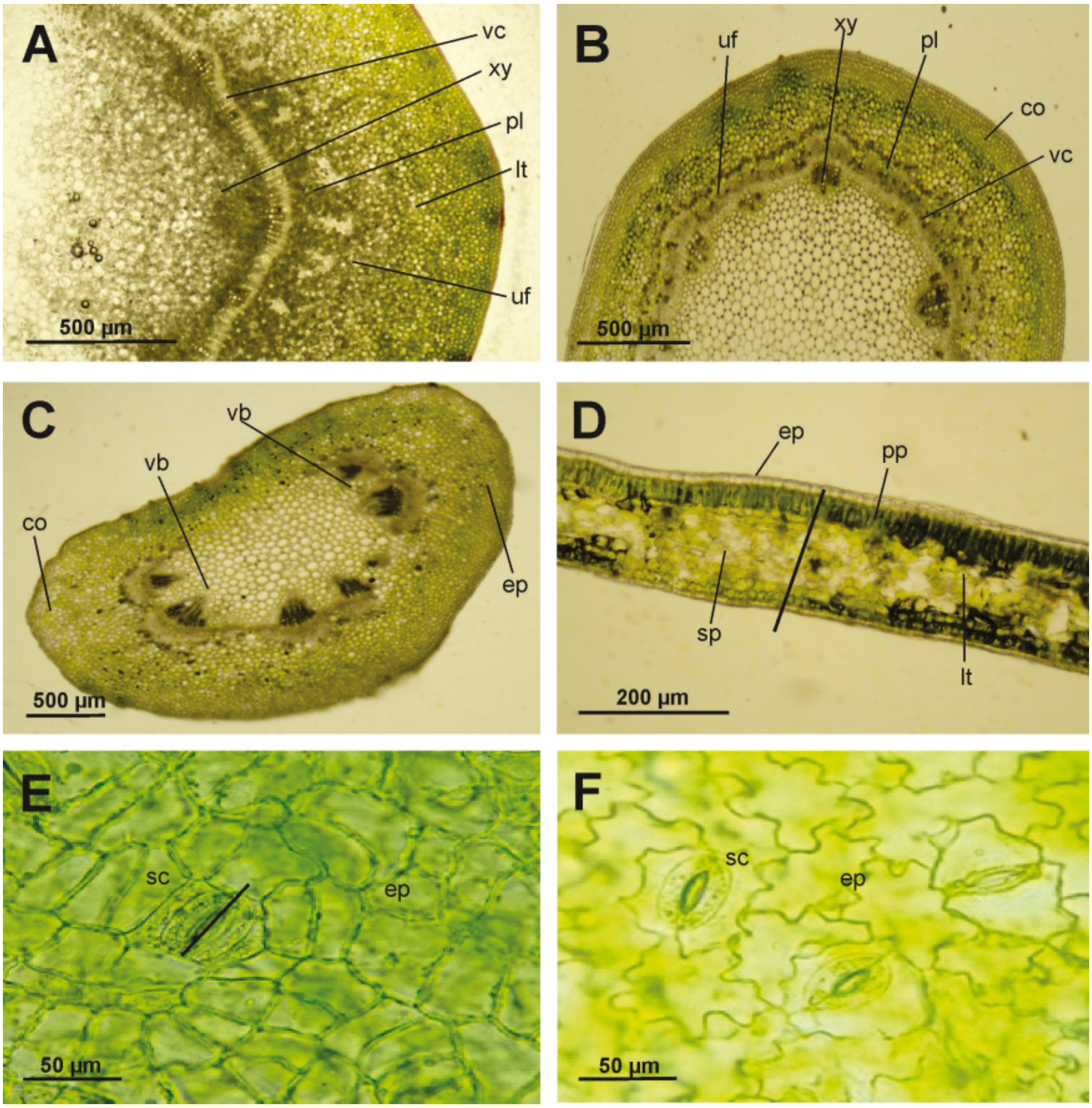


Fig. 5. Histological characterization of green seedlings of *H. polyandra*. A. Hypocotyl, B. Epicotyl, C. Petiole, D. Cotyledonary leaf, E. Epidermis of cotyledonary leaf, F. Epidermis of primary leaf. ep, epidermal cells; sc, stomatic cell; co, collenquima; pl, phloem; xy, xylem; uf, unlignicotyle-donary fied fibers; vc, vascular cambium; vb, vascular bundles; pp, palisade parenchyma; sp, spongy parenchyma; lt, laticiferous cells.

substances are found in the cotyledonary leaves, which are comparable thinner than those found in *S. laxa*. The endospermatic reserves in mature *H. polyandra* seeds are completely consumed in the phase of elongation of the hypocotyl and release of the cotyledonary leaves. Therefore, seedling growth will depend on a small amount of primary metabolites stored in the cotyledonary leaves, which after their expansion will have a source function. But the lower seeds weight from Salas (Tab. 5), also can contributed to lower growth of seedlings, because the reserves were important to seedling in the germination process and initial seedlings development, which were observed in another Euphorbiaceae species (Ribeiro et al., 2014; Aquino-Pereira et al., 2021).

Overall, inbreeding depression of selfed seedlings appears to be the main cause for albinism and photosynthetic disfunction, and would indicate that the population of *H. polyandra*, like the population of *S. laxa*, carries recessive deleterious genes and suffers inbreeding depression in selfed offspring (Takeuchi et al., 2020).

MORPHOLOGICAL AND HISTOLOGICAL CHARACTERIZATION OF GREEN AND ALBINO SEEDLINGS

After 20 days of seed germination, the vegetative structures (hypocotyl, epicotyl, petiole, and mesophyll of the cotyledonary leaf) of

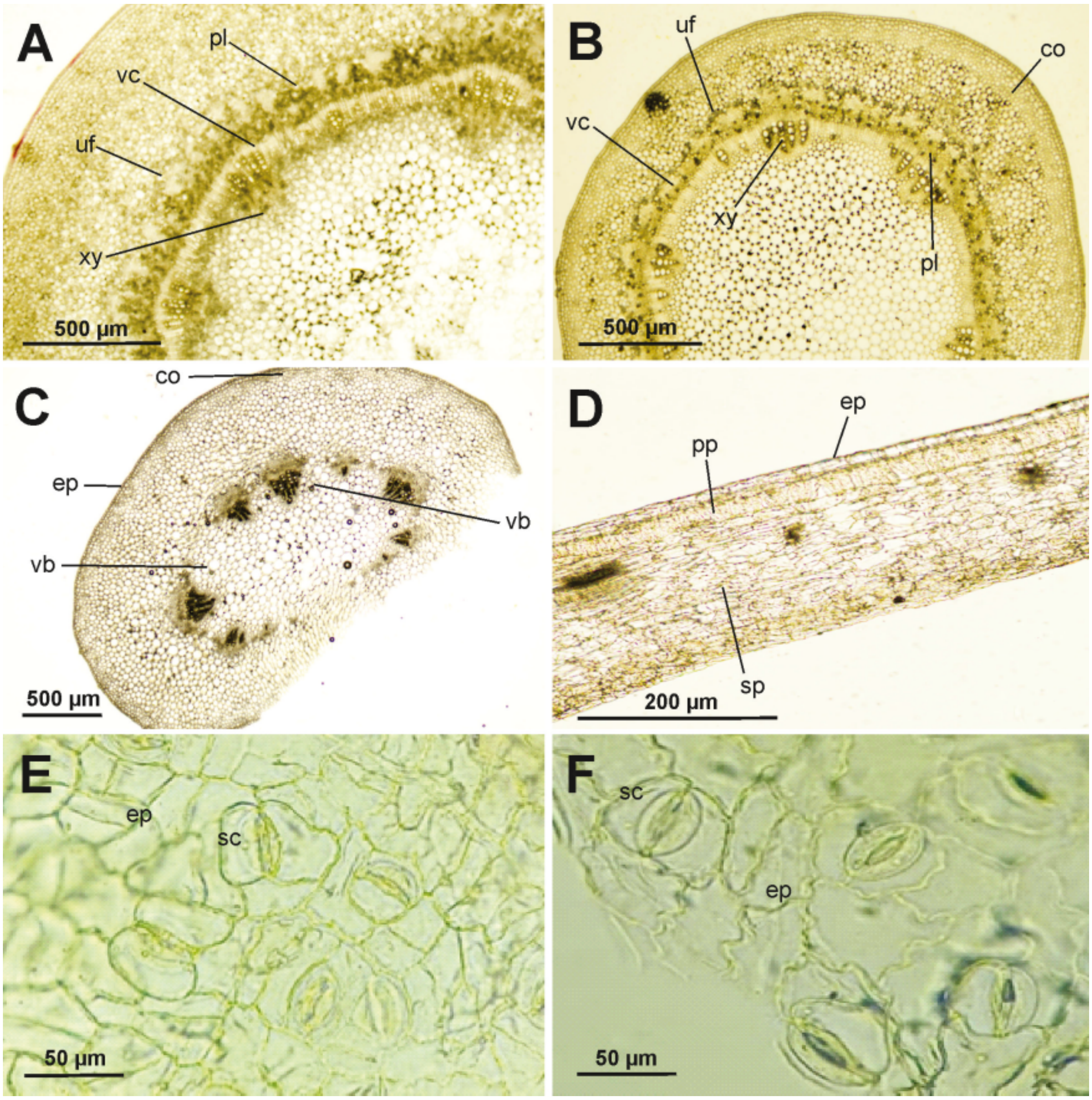
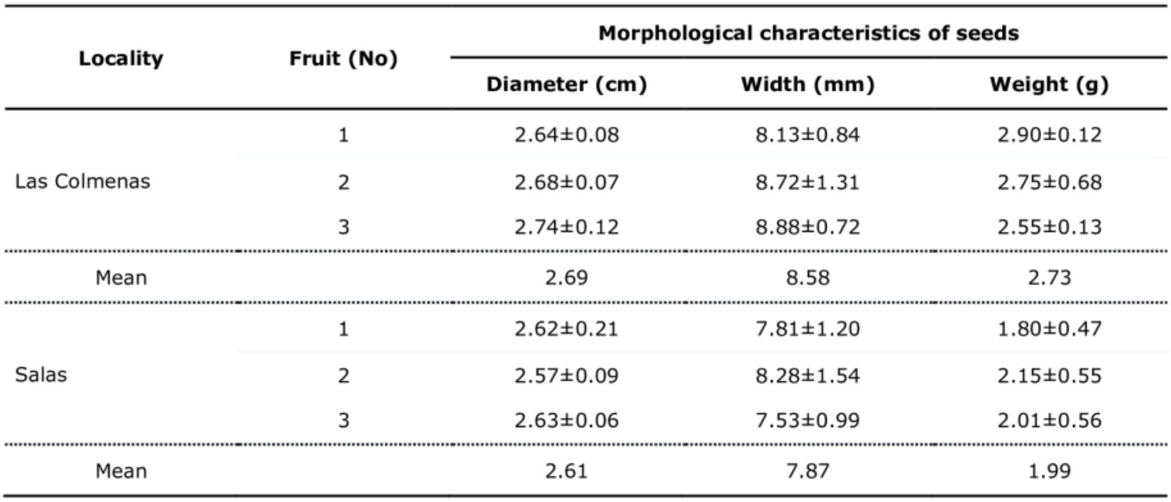


Fig 6 Histological characterization of albino seedlings of *H. polyandra*. A. Hypocotyl, B. Epicotyl, C. Petiole, D. Cotyledonary leaf, E. Epidermis of cotyledonary leaf, F. Epidermis of primary leaf. ep, epidermal cells; sc, stomatic cell; co, collenquima; pl, phloem; xy, xylem; uf, unligni-fied fibers; vc, vascular cambium; vb, vascular bundles; pp, palisade parenchyma; sp, spongy parenchyma.

Tab. 6. Morphological characteristics of green and albino seedlings of *H. polyandra* collected in the locality of Salas (Lambayeque, Peru). Seedlings four weeks after germination.



Locality	Fruit (No)	Morphological characteristics of seeds		
		Diameter (cm)	Width (mm)	Weight (g)
Las Colmenas	1	2.64±0.08	8.13±0.84	2.90±0.12
	2	2.68±0.07	8.72±1.31	2.75±0.68
	3	2.74±0.12	8.88±0.72	2.55±0.13
Mean		2.69	8.58	2.73
Salas	1	2.62±0.21	7.81±1.20	1.80±0.47
	2	2.57±0.09	8.28±1.54	2.15±0.55
	3	2.63±0.06	7.53±0.99	2.01±0.56
Mean		2.61	7.87	1.99

Average of five seedlings followed by error standard. Distinct letters in the same column indicate differences by X^2 test. p-value = 0.4765.

the green seedlings were significantly larg-er than those of the albino seedlings (Fig. 4A). The albino seedlings showed a slightly dark creamy-white coloration, and at the limit of the anatomical neck and the hypocotyl, they presented a reddish pigmentation. This reddish pigmen-tation was not observed in the green seedlings.

Hypocotyl, epicotyl, petiole, cotyledonary and primary leaves from albino seedlings showed the same histological features observed in green seedlings. In green seedlings the hypocotyl tissue showed an uniseriated epidermis, cortex parenchymatous with abundant chloroplasts, presence of un-lignified fibers, primary vascular tissue, and the vascular cambium appears active in the fascicular and interfascicular region (Fig. 5A). Similar histological structures were observed in the epicotyl (Fig. 5B). The petiole showed uniseriate epidermal tissue, and under epidermis, collenchyma and parenchyma, seven collateral bundles were identified (Fig. 5C). The cotyledonary leaf showed starch reserves, a uniseriate epidermis, compact mesophyll, palisade and spongy pa-renchyma, and laticiferous cells (Fig. 5D). Abaxial epider-mal cells with irregular shape and anomocytic stomata cells were observed in both the cotyledonary leaf and the primary leaf (Fig. 5E and 5F). In the albino seedlings the most significant variation is the lack of chloroplasts in all histological samples observed (Fig. 6).

In *D. regia*, the albino leaflets of the seedlings showed the same characteristics observed in wild-type seedlings, however, the albino seedlings showed greater mesophyll density, resulting in a greater thickness of the leaflet. Likewise, albinism in *D. regia* affects micromorphology and stomatal density (Silva et al., 2020). It is possible that, with the help of advanced equipment and techniques, such as the use of scanning electron microscopy, applied to histological studies of *H. polyandra* can find greater differences between albino seedlings compared to their wild-type counterparts. On the other hand, studies on leaf epidermal traits of selected Euphorbiaceae, including *H. crepitans*, studies involving light microscopy and scanning electron microscopy have shown the presence of stomata only on the abaxial surface of the blade, and the absence of glandular trichomes on both sides of the leaf surface (Bahadur et al., 2022). As already mentioned, *H. polyandra* is a species closely related and similar to *H. crepitans* (Oliveira et al., 2013), and thus the same foliar histological characteristics were observed.

Recent studies have proposed alternatives to avoid or overcome the barriers of albinism in plants. In *Pisum sativum* L. the CRISPR/Cas9 genome editing technology was used to edit the pea phytoene desaturase (PsPDS) gene known to cause albinism by triggering *Agrobacterium rhizogenes*-mediated genetic transformations (Li et al., 2023). Another alternative is to identify the RNA editing sites of chloroplast genes, as has occurred with the identification of 39 RNA editing sites in 18 chloroplast genes from the chloroplast genome of *Camellia sinensis* (L.) Kuntze. (Zhao et al., 2023). Furthermore, different degrees of albinism can be determined by methylome and transcriptome analyses, as has been reported in *Malus domestica* (Sukow) Borkh. seedlings where various genes (Sun et al., 2022). Despite all these advanced studies, scientific gaps remain in the knowledge and complete understanding of the causes of albinism in plants.

CONCLUSION

Albinism is a rare phenomenon in plants found in their natural populations and the number of registered albino seedlings grown from natural accessions is relatively low com-pared to green seedlings, however, in an isolated specimen of 'habilla' *Hura polyandra* (Euphorbiaceae), albinism was found in about 50% of the germinated seedlings.

Were observed in the anatomical studies carried out on the seedlings, showing discrepancy in the tissues of the hypocotyl , epicotyl, petiole and cotyledon leaves between green and albino seedlings. In this study the were made the first record of albinism to *H. polyandra* seedlings, although it has been postulated that albinism self-pollination and inbreeding, there remain large gaps in knowledge about physio-logical, biochemical and genetic aspects that causes albinism in plants that must be investigated.

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