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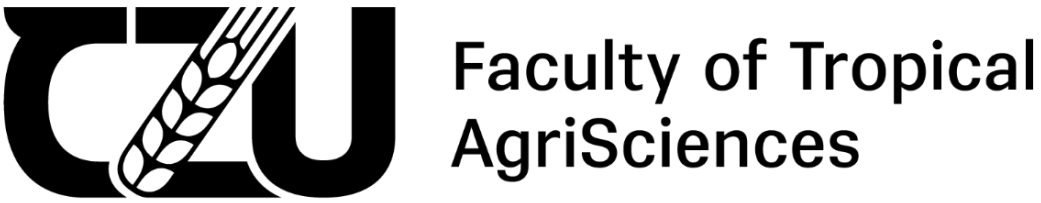

Morphological and nutritional evaluation of the diploid and tetraploid plants of the species *Melothria scabra* Naudin

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Morphological and nutritional evaluation of the diploid and tetraploid plants of the species *Melothria scabra* Naudin independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 10. 4. 2023

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Tereza Anna Javůrková

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Abstract

Melothria scabra Naudin (Cucurbitaceae), known as the cucamelon or Mexican cucumber, is a climbing perennial plant with various culinary and medicinal uses. The aim of this work was to morphologically and nutritionally evaluate new genotypes of *M. scabra*. The plant material was taken from previous laboratory research at the Laboratory of Plant Tissue Cultures, Faculty of Tropical AgroSciences of the Czech University of Life Sciences in Prague. Diploid and polyploid plants of *Melothria scabra* were propagated *in vitro* and the stability of the plant material was verified and evaluated by flow cytometry analysis and chromosome counting. The transfer of plants from *in vivo* to *ex vitro* was 100 % successful. The obtained polyploid and control diploid genotypes were cultivated under greenhouse conditions. After 50 days of cultivation, the plants were gradually harvested. The morphological and biochemical differences between the obtained genotypes were evaluated. Morphological differences in fruits (fruit length and width), flowers (flower width, flower spike width, flower height) and biochemical differences (dry weight and vitamin C) between diploid and autotetraploid *M. scabra* plants were evaluated.

Key words: Cucamelon, flow cytometer, polyploidization, morphological evaluation, vitamin C content

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List of the abbreviations used in the thesis.

MS medium	Murashige a Skoog medium
APM	Amiprophos-methyl
DAPI	4',6-diamidin-2-fenylindol
DH	Double haploid
DNA	Deoxyribonucleic acid
FCM	Flow cytometry analysis
HPLC	High performance liquid chromatography
PAS	Particle Analysing System
PTFE	Polytetrafluoroethylene

1. Introduction

Cucamelon, Mexican miniature watermelon (*Melothria scabra* Naudin) is a diploid species (2n=2x=12) from the family Cucurbitaceae (Bhowmick & Jha 2022). It has its origin from Mexico and Central America but has spread wide across other regions of the world. Cucamelon is grown for its tiny edible fruit that looks like a miniature striped watermelon (*Citrullus lanatus*) (Chomicki et al. 2020). Morphologically, the fruit is oval with average 2.4 cm length and 1.5 cm width (WFO 2023), and is consumed directly or processed as pickles and vegetables. The fruits of *M. scabra* Naudin contains alkaloids, tannins, flavonoids, terpenoids, and saponins (Kamaruddin et al. 2021). The leaves have anti-diabetes activity (Govindula et al. 2019). It is used to reduce blood pressure and it is also applied as a mosquito repellent in some parts of the world (Kamaruddin et al. 2021).

Artificial polyploidy induction is a great breeding strategy in horticultural crops (Niazian et al. 2020), having a promising potential to obtain genotypes with new morphological, physiological, and phytochemical characteristics (Šedivá et al. 2019; Shmeit et al. 2020; Zahumenická et al. 2018). The most common antimitotic agents used to induce autopolyploid plants are colchicine, oryzalin, and trifluralin (Shmeit et al. 2020).

In many previous studies, plants of the genus Cucurbitaceae have been successfully polyploidized.

Species of this genus have been polyploidized in previous studies using typical antimitotic substances, namely oryzalin, colchicine and trifluralin (Cho et al. 2021; Ebrahimzadeh et al. 2018; Zheng et al. 2019).

In most cases, significant morphological and nutritional changes occur. The changes affect both the vegetative and generative parts of the plant (Ravandi et al. 2013; Shmeit et al. 2020; Zheng et al. 2019). The obtained DH plants showed larger leaves, flowers and fruits compared to their haploid relatives and had a higher concentration ratio of existing components in the phytochemical profile of the plants (Ebrahimzadeh et al. 2018).

The Cucurbitaceae family is composed of various species, which are known for their important economic, nutritional, and medicinal value. Autopolyploidy could be successfully applied in the improvement of these species.

2. Literature Review

Melothria scabra Naudin is a plant of the family Cucurbitaceae, native to the Neotropics of Central and South America (Encyclopedia of Life 2023; Hassler 2023).

The raw fruit tastes of cucumber with a hint of lemon or lime. It is not difficult to grow and is a very attractive and easy to grow vegetable. They thrive best in a warm sunny spot with plenty of room for climbing or crawling. Although they are fairly drought tolerant, regular watering will result in a bountiful crop (Biggs 2019).

There are many names for *M. scabra* Naud. It is most called Mexican cucumber or miniature cucumber. In the indigenous region of Mexico, it is known as "sandía de rantón" which translates as watermelon for mouse. The plant may also be known as pepquino, sour gherkin, cucamelon (Encyclopedia of Life 2023; EPPO 2023).

2.1. Taxonomy, botanical, and morphological description

Taxonomically, the plant belongs to the kingdom Plantae, phylum Magnoliophyte, class Angiospermae, category Fabis, order Cucurbitales, family Cucurbitaceae, genus Melothria and species *Melothria scabra* (EPPO 2023).

Melothria scabra Naudin is a climbing plant with bicolateral bundles. The stems are long, narrow, and stubby. The leaves are simple, dissected, palmate and alternate. Leaf margins are undulate or dentate, leaf apex is caudate. Petioles are 1,5 - 6,5 cm long (Figure 1.). The surface of the plant is covered with small trichomes (Figure 2.). The roots are thin, forming white tubers (Novák & Saklický 2012; Tropicos.org 2023).

The plants are monoecious with separate sexes. The flowers are polymetrical, the petals are small, 4-5 in number (Figure 3.). The flowers are entomogamous (Hassler 2023; Novák & Saklický 2012). The fruits are small, oval, 30 mm long and 15 mm wide. They are green in colour with mosaic patterns (Figure 4.). Plants grown from 2.5 to 6 m. The seeds germinate very quickly, in about 10 days. The fruits can be harvested usually 60-75 days after planting (Biggs 2019; Mahr 2023).

They are relatively hardy and low maintenance. Plants are more resistant to pests and diseases that plague Cucurbitaceae such as powdery mildew, whiteflies, aphids (Mahr 2023).

Figure 1. *Melothria scabra* Naudin leaf, **Source:** author

Figure 2. Stem of *Melothria scabra* Naud. with trichomes, **Source:** author

Figure 3. Flower of *Melothria scabra* Naudin, **Source:** author

Figure 4. Ripe fruit of *M. scabra* Naud., **Source:** Author

2.2. Origin and distribution

Melothria scabra Naudin was first described by the French naturalist and botanist Charles Victor Naudin in 1866. The plant is named after this scientist. Consumption and exploitation of the plant dates to pre-colonial times (Mahr 2023).

The plant is native to Colombia, El Salvador, Guatemala, Honduras, Central Mexico, Northeastern Mexico, Northwestern Mexico, Southeastern Mexico, Southwestern Mexico , Nicaragua, Panama, and Venezuela (Figure 5.) (POWO 2023).

Figure 5. Original range of *Melothria scabra* Naudin, **Source:** https://powo.science.kew.org

Currently, the crop is widespread all over the world and is gaining popularity among gardeners (Figure 6.).

Figure 6. Current distribution of *Melothria scabra* Naudin, **Source:** https://www.gbif.org

2.3. Usage of species of Cucurbitaceae family

Plants are used in medicine, food, and many other consumer industries. Species of this genus are used for direct consumption or processed. Some sources point to important physiological properties, such as cardiovascular system, hepatoprotective, immunoregulatory, and anti-inflammatory activities. Plants of the Cucurbitaceae family contain substances such as carotenoids, terpenoids, saponins and phytochemicals. Fruits from this family have positive effects on human health and various studies have clearly shown that cucurbit vegetables have antioxidant, antidiabetic, anti-inflammatory and cleansing properties (Rolnik & Olas 2020).

Species originating from the family Cucurbitaceae are used in Chinese and Ayurvedic systems.

2.3.1. Cucurbitacin

Cucurbitaceae contain take substances that are predominantly characteristic of this family. These are highly oxygenated tetracyclic triterpenes, which we call cucurbitacins. There are more than 18 species (Ujváry 2010). They are present in concentrations of 0.1 to 0.3 in the fruits and roots of plants. They have a strong bitter taste and have a wide range of pharmacological effects, for example, anti-inflammatory, hepatoprotective. In traditional Chinese medicine, this substance is used in the treatment of the liver. Cucurbitacins are constituents in insecticides (Drijfhout & Morgan 2010).

2.3.2. Usage of *Melothria scabra* Naudin

Melothria scabra Naudin is a plant of significant economic, pharmaceutical, and nutritional importance to the local population (Kamaruddin et al. 2021).

The fruit can be eaten raw, for example in salads, cocktails, or sauces. Fruit can be processed into preserves or pickles. In storage they need to be sterilised as they are perishable (Biggs 2019).

The tubers can also be eaten. The tubers are white, elongated and resemble, the shape of a sweet potato or Jerusalem artichoke. They should be processed as soon as possible and are very difficult to store. It is recommended to cook the tubers and add them to porridges, salads, etc. (Kuzmina 2019).

The fruits contain chemical substances alkaloids, flavonoids, terpenoids, tannins and saponins, also contain the carbohydrates fructose, valine, cucurbitacin and other chemical compounds that are nature's powerful radical scavengers. *M. scabra* Naud. fruits could be a natural source of antioxidant compounds (Kamaruddin et al. 2021). There are studies that point to the antidiabetic potential of *M. scabra* Naudin. However, the results of the studies have not yet been examined *in vivo*.

The substances contained in this plant can be used alone in treatment or with other medicinal substances (Anusha et al. 2019). In traditional medicine, this plant is indispensable. Indigenous people use various parts of the plant for its medicinal purposes.

Leaves, roots, or whole plants are used. The leaves are used to treat skin diseases, diarrhoea, fevers, and headaches. The roots are given orally as a solution for constipation (Assefa & Bayu 2018). It is used to treat livestock (Anand et al. 2011).

2.4. Planting

Minimelons are grown in temperate regions mainly in greenhouses or foil greenhouses. The plants are mostly grown annually because they are not frost resistant. For perennial cultivation the plants must be transplanted indoors for the winter.

Melothria scabra Naudin thrives in warm, moist conditions. Plants can be allowed to germinate in sowing pots, trays, or planters. Seeds take approximately 10 days to germinate. Then plant the seedlings in pots indoors and transplant outdoors after the first frost.

The other option is to plant the seeds directly in the greenhouse. The seeds are always planted with the blunt edge down.

Plants should be planted well apart. It is advisable to plant in rows 15 - 40 cm apart. The plants should be given some support during growth and budding. A combination of trellis or mesh with a support bar, preferably bamboo or hazel, is suitable.

Plants need to be watered regularly and can be fertilised. Rot with nitrogen fertiliser. At a height of 2.5 metres the growing tops and side shoots can be cut back, the plant then flowers more and grows less. The plants are resistant to pests and other diseases that attack cucurbits and others.

Plants are very prolific; each can produce over 350 g of fruit. Harvesting begins after 60-75 days and lasts until the first autumn frost. After harvesting, the tuberous root

can be dug up. This can be processed or replanted the following season (Biggs 2019; Mahr 2023).

2.5. Plant breeding of Cucurbitaceae

Plant breeding is carried out to achieve a variety of breeding objectives (Dudley 2002). The main breeding objectives include breeding for reproductive capacity and stability, for quality improvement, for adjustment of the length of the growing season, for stress tolerance, for the creation of plants with new traits and other aspects for plant breeding (Graman & Čurn 1998). The ideal result of breeding is to preserve the natural mating system (Acquaah 2016).

Plant breeding is divided into two basic groups, conventional and unconventional.

Traditional breeding methods, conventional, is based on the natural genetic limits of the species. Conservative tools are used, it is limited in natural biodiversity and the limits are set by the natural genetic material. The whole process is usually very expensive and takes several decades (Acquaah 2016; Bortesi & Fischer 2015). Conventional breeding includes selection, crossbreeding, mutation and polyploidisation (Wan Shafiin et al. 2021).

Unconventional plant breeding involves direct intervention in the genetic information of a given organism using biotechnological and molecular methods. In some cases, a plant can be bred using conventional methods, but unconventional techniques make breeding faster and more efficient (Graman & Čurn 1998).

Methods used in molecular breeding include genetic selection, mutagenic breeding, somaclonal variation, whole genome sequence-based approaches, physical maps and functional genomic tools, genome editing using programmable nucleases, regularly alternating short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) (Ahmar et al. 2020).

Common breeding methods for cross-pollinated spices, which include species in the Cucurbitaceae family, are mass selection, recurrent selection, family selection, and synthetics and polyploidization (Acquaah 2016). Especially of watermelon, the methods are mutation breeding, back cross methods, mass selection and polyploidisation (Wehner 2023).

2.5.1. Polyploidization of Cucurbitaceae

Although most eukaryotic organisms have a diploid set of chromosomes, it is conceivable that the nucleus contains more than two sets of chromosomes. This phenomenon is called polyploidy, or also known as WGD (whole genome duplication) (Bomblies et al. 2016).

Polyploidy occurs naturally in many species of organisms. Natural polyploidization leads to evolution and associated adaptation, natural specialization of species and to increasing genome and genetic diversity (Sattler et al. 2016). This diversity can then seed additional modifications to gene expression, epigenetics, gene networks, the proteome, cell size, and altered responses to stress.

Cells that have three, four or more chromosomes than haploid cells are polyploid (Graman & Čurn 1998). A polyploid that has three pairs of chromosomes is called a triploid. It is followed by tetraploid with four pairs of chromosomes, pentaploid with five, and then hexaploid, heptaploid, and others with increasing numbers of chromosomes (Ranney 2006).

Polyploids usually differ from diploids in their morphological, ecological, physiological, and cytological characteristics. The differences are mostly due to increases in cell size, gene expression and diversity (Ramsey & Schemske 1998).

Polyploid organisms are often more resistant to environmental factors and can cope better with biotic and abiotic stresses. In the modern commercial world, polyploid organisms are in demand because of their enlarged plant organs, the so-called “gigas” effect, their greater resistance, and the production of seedless fruits (Sattler et al. 2016).

Seedless fruits are often produced by plants that have an odd number of chromosome sets, resulting in meiotic errors that prevent seed production (Eigsti 1957).

The specific effects of polyploidy vary depending on the type of organism. There are a variety of aspects, but among the most important are the degree of heterozygosity, the level of ploidy, and the mechanisms that relate to gene silencing, gene interactions, gene dose effects, and regulation of specific traits and processes (Ranney 2006).

Polyploidy consists in the disruption of metaphase in mitotic division. This is induced by antimitotic agents such as oryzalin, colchicine and others (Graman & Čurn 1998).

In Cucurbitaceae, natural polyploidy occurs in some cases. Already in the 1970s, species with natural polyploidy were discovered (Singh 1979). For example, the species

Telfairia occidentalis Hook F., *Momordica Dioica* Roxb., *Echinocystis macrocarpa*, *Trichosantes* sp. (Ghosh et al. 2021; Uguru & Onovo 2008).

Several attempts towards polyploid induction in plants from Cucurbitaceae family has been made. For instance, in *Cucumis melo* L., polyploids were successfully induced using oryzalin. The obtained polyploid plants exhibited substantial morphological changes in seeds, leaves, and stomata when comparison with the diploid plant (Cho et al. 2021). Additionally, a similar study on the same species reported a higher total soluble solid content in tetraploid fruits than diploid fruits (Wang et al. 2015).

In *Cucumis sativus* L., polyploids were produced by using colchicine that displayed larger leaf size, flower diameter, stoma size, pollen grains and more chloroplast number in guard cell (Zheng et al. 2019). In the same species, doubled haploid (DH) plants were obtained using colchicine, trifluralin and oryzalin. The DH plants obtained exhibited larger leaves, flower and fruit size compared to their haploid relatives (Ebrahimzadeh et al. 2018).

Polyploidy was also induced in *Citrullus lanatus* (Thunb.) Matsum et Nakai by colchicine treatment where the obtained polyploid plants had broader and thicker leaves of dark green colour, larger stomata, pollen grain, seeds, and fruits than their respective diploids (Gaikwad et al. 2009; Zhang et al. 2019).

Along with the morphological parameters, polyploidization could also influence ratio of existing components in the phytochemical profile of the plants. For instance, autotetraploidy in *Cichorium intybus* L. had a significant effect on total phenolic compound and chlorogenic acid concentrations in leaves (Ravandi et al. 2013), the essential oil yield and thymol content was increased in *Thymus vulgaris* L. (Shmeit et al. 2020) and macro and micronutrients increased in the *Callisia fragrans* (Lindl.) Woodson (Beranová et al. 2022).

3. Aims of the Thesis

The main objective of this work is a morphological and nutritional comparison of diploid plants (2n = 12) and polyploidized plants (2n = 24) of the species *Melothria scabra* Naudin grown under greenhouse conditions.

In vitro polyploidization could cause different morphological characteristics that could yield higher productivity.

The aim of this work was established according to the following hypotheses:

H1: There is a morphological difference between diploid and autotetraploid plants.

H2: There is a difference in nutrient content between diploid and autotetraploid plants.

4. Materials and methods

4.1. Plant material

The plant material, diploid (2n=2x=12) and autetraploid (2n=2x=24) plants were obtained from the collection of plant material maintained in the plant explants laboratory of FTA, CZU.

Autotetraploid plants of *M. scabra* Naudin, were obtained by induced polyploidy *in vitro* (Kuzmina 2019). For the actual research, a sufficient number of plants were propagated using nodal cultures. Explants were grown at 25/20 ± 0.5 C° under a 16-h light/8-h dark photoperiod, controlled automatically. The illumination intensity was 3800 lux (51.3 μmol m^2s^2) from cool white fluorescent lamps (121.36 cm long). To determine ploidy stability, ploidy levels in diploid and tetraploid plants were determined by chromosome counting and flow cytometry.

4.2. Verification of the ploidy level

4.2.1. Flow cytometry

The flow cytometry analysis was performed according to Shmeit et al. (2020). Small parts of leaf tissue were chopped using a razor blade in a Petri dish containing 500 μL of Otto I buffer (0.1 M $C_6H_8O_7$, 0.5 % Tween 20). Samples of crude suspension containing the isolated nuclei were subsequently filtered through a 50 μM nylon mesh. The second step was to dye the nuclei; as such, 1 mL of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12 H_2O$) containing fluorescent dye DAPI in 2 μg/mL concentration was added to the filtered samples. All measurements to detect ploidy levels were executed in relative fluorescence intensity of at least 3000 nuclei and were recorded using a Partec PAS flow cytometer (Partec GmbH, Münster, DE) equipped with a high-pressure mercury arc lamp. Histograms of DNA content were evaluated using the Flomax software package.

4.2.2. Chromosome counting

From diploid and tetraploid plants, root tips about 1 cm long were taken and were soaked in saturated paradichlorobenzene solution for 3 hours. This was followed by washing the material with distilled water, which was repeated three times. The root tips

were immediately placed in a freshly prepared solution of ethanol (96 %) and acetic acid (99 %) (3:1) for 1 hour at room temperature. Hydrolysis and staining followed. The tips were incubated in 1N HCl at 60 °C for 15 min, in triplicate. Staining was performed with Schiff's reagent for 1 hour and then washed again. The root tips thus prepared were made into approximately 0.2 cm sized preparations, placed on a laboratory slide with a drop of 2 % orcein-acetic salt. Chromosomes were imaged on an Olympus BX51 light microscope (Olympus Optical Co., Tokyo, Japan) at 100× magnification (Beranova et al. 2022).

4.3. Transfer to *ex vitro* condition

Nodal segments of diploid and tetraploid plantlets were cultivated on MS medium free of plant growth regulators for eight months with subculture once every one month.

The acclimatization of the plantlets was performed in greenhouse conditions. The Plantlets with well-developed root systems were removed from MS medium and transferred to plastic pots (5 × 5 cm) containing a sand: soil: peat moss: vermiculite (1:1:1:1; v/v) mixture. The plants were maintained for 1 week covered with polythene bags under high humidity, and then the humidity was gradually lowered. The percentage of *ex vitro* survival was evaluated after 4 weeks.

4.4. Quantitative and morphological evaluation

In the autotetraploid and diploid (control) plants, the weight, width, length, shape and colour were evaluated in the fruit. In the seed, the weight (weight of a thousand seeds) and the shape were evaluated. In the flower the size, number of petals and colour were analysed. The leaves were evaluated for shape and colour.

4.5. Nutritional evaluation

4.5.1. Determination of dry matter content

Dry matter determination was performed based on two 5 g weights of each sample into pre-dried aluminium trays, which were then placed in an oven (Memmert, Germany) heated to 103 °C for 4 h. After drying, the trays were allowed to cool in a desiccator and then weighed.

4.5.2. Determination of vitamin C

The determination of vitamin C was carried out in several steps. The mobile phase, extraction reagent, standard solutions, and the sample itself were prepared.

To prepare the mobile phase, 0.2 µl of H_3PO_4 was added to a solution of methanol and demineralized water, 40:760 ml, to adjust the pH to 3. Then the mobile phase was placed in an ultrasonic bath (Elma, Germany) for 30 min. All demineralized water was prepared by Milli Q Plus (Millipore, Germany).

This was followed by the preparation of the extraction reagent. The extraction reagent was a 3 % metaphosphoric acid solution where 15 g of metaphosphoric acid was dissolved in 500 ml of demineralized water.

Before preparing the plant sample, a standard solution was made. A stock solution of ascorbic acid at a concentration of 1 g/l was prepared by dissolving 25 mg of ascorbic acid standard in a 25 ml volumetric flask using an extraction reagent. Subsequently, a calibration series of ascorbic acid at concentrations of 1, 5, 10, 20, 60 and 100 mg/l were prepared from the stock solution and diluted with the extraction reagent.

Whole fruits of *Melothria scabra* Naudin were used to prepare the sample for vitamin C measurement. 2.5 g of the sample was weighed into a beaker and poured over about 15 ml of extraction reagent. The sample with the extraction reagent was homogenized using a stick blender (IKA Ultra-Turrax, Germany). The beaker was placed subsequently on an ultrasonic bath (Elma, Germany) for 5 min. After 5 min, the sample was filtered through filter paper into a 25 ml volumetric flask, which was filled to the brim with extraction reagent. Finally, the sample was injected onto an HPLC syringe microfilter with PTFE membrane (0.45 µm) and analysed by HPLC using a 1360 Infinity II chromatography system (Agilent, USA) and a DAD WR detector. The HPLC conditions for the determination of ascorbic acid were water and methanol in a 760:40

ratio for the mobile phase, pH 3 and isocratic elution. The injection volume was 20 µl at a flow rate of 0.3 ml/min. The separation medium was in Infinity Lab Poroshell 120 columns, 2.7 µm C 18, size 150 x 3 mm (Agilent, USA) at 25°C and wavelength 254 nm. Three parallel determinations were performed for each sample.

4.6. Statistic

The data was taken during flowering, mainly in the fruit harvest and were performed by using STATISTICA software. Quantitative measurements were assessed by the Kruskal Wallis test. To refine the analysis, the multiple comparison test of the mean of the rank order for all of groups. A statistically significant difference was considered if $p < 0.05$.

5. Results

5.1. Verification of ploidy level and stability

Plant polyploidy has been verified by flow cytometry (FCM) (Figure 6.) and chromosome counting (Figure 8). In Figure 7 (a and b), a histogram generated by FCM shows the relative DNA content between diploid and tetraploid plants. The highest number of polyploids was obtained when explants were treated with 80 µM oryzalin for 40 h (6 tetraploids) and these plants were selected for the experiment. The results from chromosome counting confirmed the results obtained by FCM. The chromosome counts obtained were 2n=2x=12 in diploid (control) plants and 2n=2x=24 in tetraploid plants (Fig. 8, a and b).

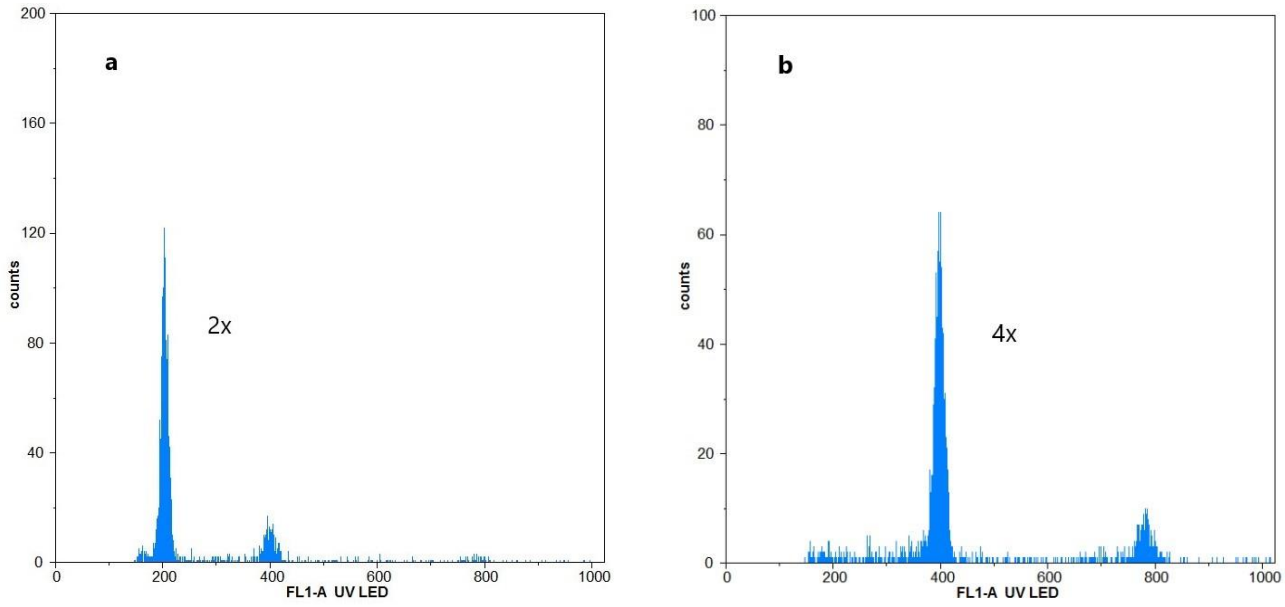

Figure 7. Histogram of flow cytometry analysis from Melothria scabra Naudin: (a) histogram of control plant (diploid) and (b) polyploid plants (tetraploid).
Source: Author

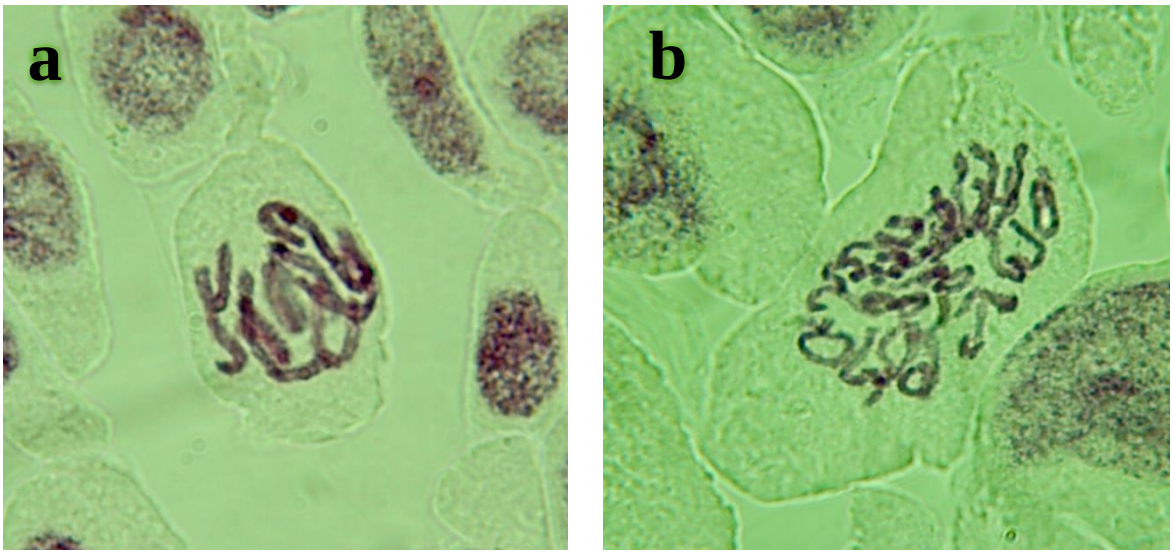

Figure 8. Chromosome counting of *Melothria scabra* Naudin: (a) Chromosome numbers in diploid plant (2n = 2x = 12) and (b) Chromosome number in induced tetraploid plant (2n = 4x = 24), under 100× magnification.
Source: Author

5.2. Morphological comparisons between diploid and autotetraploid *Melothria scabra* Naudin plants

The plants were cultivated in greenhouse conditions (after transfer to *ex vitro* conditions). Polyploid plants had a vegetation period of 155 days. Cultivation of diploid (control) plants was interrupted after 120 days of cultivation due to fungal infection by *Sphaerotheca fuliginea* and *Erysiphe cichoracearum*.

Melothria scabra Naudin flowers gradually, but the first flowers in diploid plants were observed after 50 days of cultivation and in polypoid plants after 57 (for genotype 31) and 60 (for genotype 52) days of cultivation. The flowers from diploid and tetraploid genotypes varied significantly (Table 1 and Figure 9). The flowers varied in the width of the entire flower. Polyploid plants had larger flowers (36 % on average) and a greater number of petals (5 petals) than diploid plants (4.6 ± 0.7 petals) (Table 1).

Table 1. Morphological evaluation of the flower

Plants tested	Flower width (mm)	Receptacle width (mm)	Flower height (mm)	Number of petals
Control	8.46 ± 1.74 [a]	2.28 ± 0.71 [a]	2.89 ± 0.57 [a]	4.6 ± 0.7 [a]
Genotype 52	11.5 ± 1.29 [b]	2.69 ± 0.57 [a]	3.33 ± 0.45 [a]	5 ± 0 [a]
Genotype 31	11.18 ± 1.24 [b]	2.83 ± 0.35 [a]	3.05 ± 0.64 [a]	5 ± 0 [a]

Data were tested by Kruskal Wallis test with a p-value of 0.05. **Source**: Author

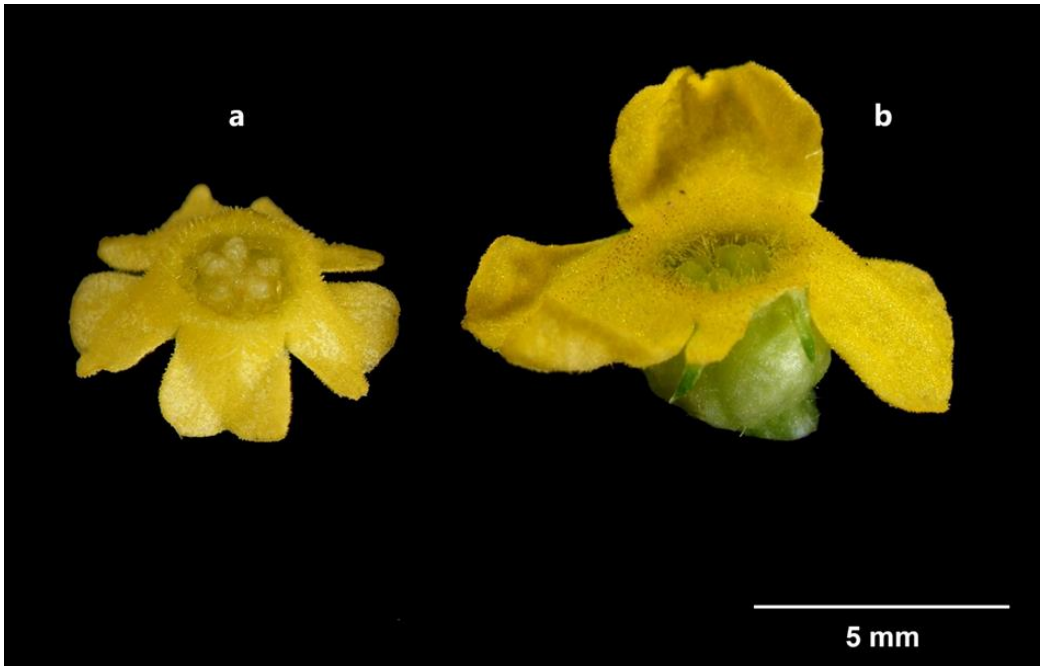

Figure 9. Morphological variation between diploid (**a**) and induced tetraploid (**b**) flowers of *Melothria scabra* Naudin cultivated in greenhouse conditions.
Source: Ing. Miroslav Klíma, Ph.D.

The development of fruits till maturity took on average 17 days in control, 20 and 25 days in the genotypes 52 and 31, respectively from the beginning of flowering. There is a statical difference in the length, width, and weight of the fruit (Table 2 and Figure 10). Fruits of polyploid plants (genotype 52) were on average 10 % shorter than those of diploid plants, but all polyploid plants had wider (13-15 %) fruit with significantly higher weight (14-23 %). The fruits also varied in colour and pattern.

Table 2. Morphological evaluation of the fruit

Plants tested	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)
Control	27.12 ± 1.63 [a]	14.99 ± 0.64 [a]	3.47 ± 0.45 [a]
Genotype 52	24.55 ± 2.01 [b]	17.29 ± 0.91 [b]	3.97 ± 0.39 [b]
Genotype 31	27.78 ± 2.42 [a]	17.03 ± 0.97 [b]	4.28 ± 0.58 [b]

Data were tested by Kruskal Wallis test with a p-value of 0.05.
Source: Author

Figure 10. Morphological variation between diploid (**2x**) and induced tetraploid (**4x**, 31 and 52 genotype) fruit of *Melothria scabra* Naudin.
Source: Ing. Miroslav Klíma, Ph.D.

Tetraploid induction also had a significant effect on morphological traits of the leaf (Figure 11).

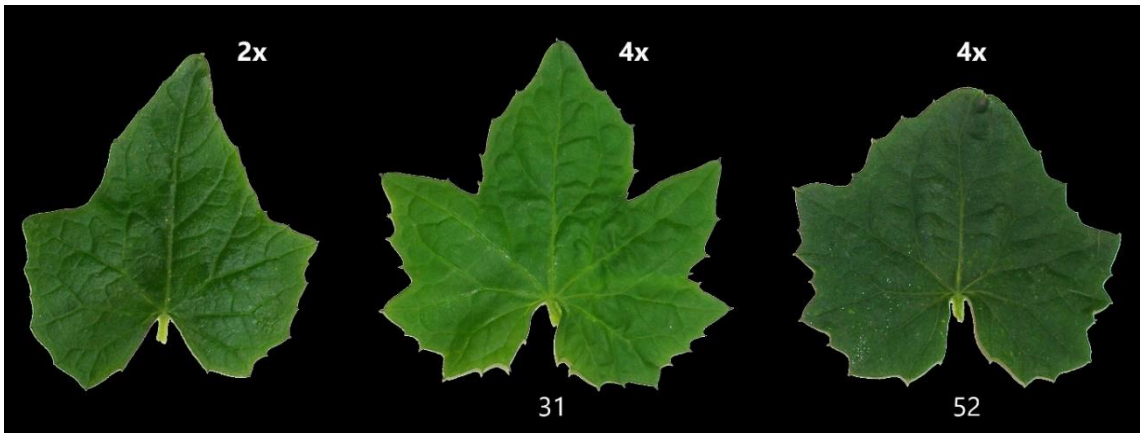

Figure 11. Morphological variation between diploid (**2x**) and induced tetraploid (**4x)** (31 and 52 genotype) leaves of *Melothria scabra* Naudin.
Source: Ing. Miroslav Klíma, Ph.D.

Leaf blade margins of polyploid plants were more dentate than those of diploid plants. Changes in the colour of the leaves were also distinctly different. The leaves of genotype 31 had a light green coloration and genotype 52 showed a dark green coloration compared to their diploid forms.

The seeds are similar in shape and colour (Figure 12). Germination varies between species. In the control plant it is almost 100 %, in the polyploid it is less. Genotype 31 is between 50 % and genotype 52 is slightly less.

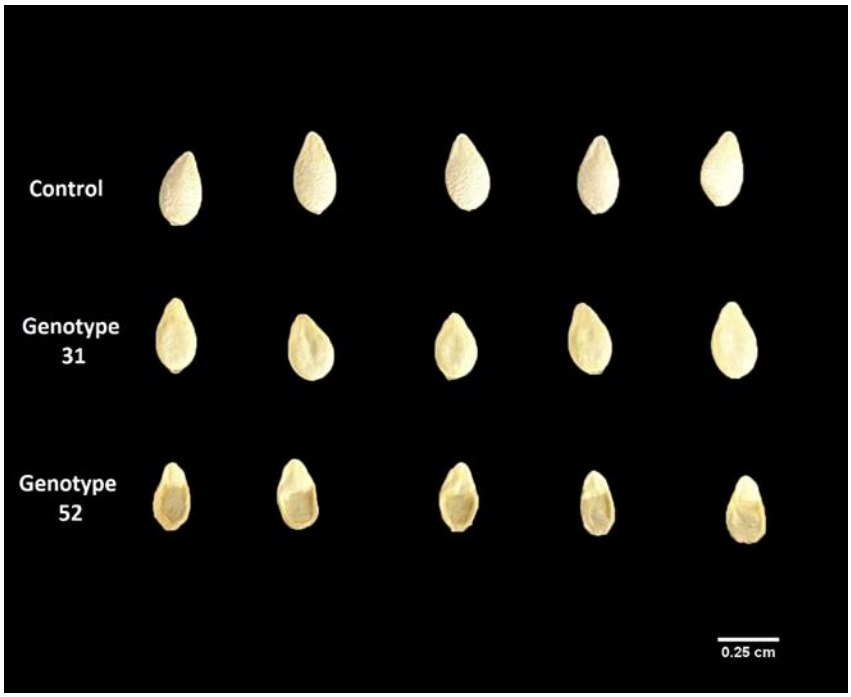

Figure 12. Comparison of seed morphology among diploid and polyploid genotypes. **Source:** Ing. Miroslav Klíma, Ph.D.

5.3. Nutritional evaluation between diploid and autotetraploid *Melothria scabra* Naudin plants

Nutritional evaluation was performed in the second half of the culture, at approximately day 100. Ripe *Melothria scabra* Naudin fruits were collected and analysed immediately after harvesting, within a few hours. All fruits were harvested and processed under the same conditions.

There is a statical difference in the dry matter in each type of genotype (Table 3.). The dry matter in the polyploid fruit was a quarter (22-25 %) less than in the control. This may be caused by a higher ability to retain water.

The vitamin C content was statistically different. Control plants contained 13 - 21 % more vitamin C (Table 3.). Due to the higher water content of the fruit, it is possible that the vitamin C has been diluted.

Table 3. Nutritional evaluation of *Melothria scabra* Naudin

Plants tested	DM g/100 g FW	Vitamin C mg/kg DM
Control	10,30 ±0,07 [a]	14,5 ±0,72 [a]
Genotype 52	7,73 ±0,13 [b]	12,78 ±0,67 [b]
Genotype 31	7,99 ±0,05 [c]	11,97 ±0,21 [b]

DM = dry matter, FW = fresh weight
Source: Author

6. Discussion

Through polyploidization, new genetic material can be obtained, with new characteristics than its diploid forms, thus the variability of the species can be enriched. Usually, polyploid organisms exhibit heterosis, increased vigour, higher yield, produce higher quality products and are more tolerant to both biotic and abiotic stresses than their diploid counterparts (Bae et al. 2020).

For the induction of autopolyploids, the most widely used antimitotic agent is colchicine (Shariat & Sefidkonb 2022; Tsai et al. 2021). But in recent years, for chromosome duplication, oryzalin is being used with great success, including in species of the Cucurbitaceae family (Bae et al. 2020; Cho et al. 2021; Ebrahimzadeh et al. 2018).

The polyploidization efficiency was up to 15 %. The present results are the first results of *ex vitro* cultivation and evaluation of polyploidized *M. scabra* Naud. A study by Wang et al. (2015) reported a higher efficiency of tetraploid induction (up 14 %) in *Cucumis melo* var. Makuwa, using oryzalin and amiprophos-methyl (APM) in comparation with colchicine. Similarly, Ebrahimzadeh et al. (2018) reported a higher effective chromosome doubling in *Cucumis sativus* L. using oryzalin compared to trifluralin and colchicine used the study. Through this technique they obtained up to 92.31 % of regenerated doubled haploid plants.

For the morphological evaluation of the flowers and leaves, and for the morphological and nutritional evaluation of the fruit, two new autopolyploid genotypes (31 and 52) were chosen, that showed good development and a great visual difference under *in vitro* culture conditions. This study confirms the significant changes in morphological and size parameters observed, caused by the doubling of the number of chromosomes in somatic cells. Previously, it has been elucidated that artificial induction of autopolyploidy in family Cucurbitaceae triggers gigantism. For instance, in *Citrullus lanatus* (Zhang et al. 2019) and *Cucumis melo* (Cho et al. 2021) induced tetraploid plants exhibited significantly larger flower, leaves, seeds, and fruit size than diploid plants. Although, a study by Bae et al. (2020) is not in line with these findings in tetraploid plants ($2n = 4x = 44$) of *Citrullus lanatus*. The morphological changes observed in the tetraploid plants showed small, thick, and crumpled leaves. Morphological parameters are often used as the first primary screening criteria for polyploids but are generally not completely

reliable. Hence, the flow cytometric analysis and chromosome counting are the most direct and accurate method to identify polyploids (Zhang et al. 2019).

Fruits of newly obtained tetraploid genotypes and control diploids were nutritionally evaluated. Polyploid fruits contained less dry matter, suggesting that they are able to fix more water. Vitamin C concentration also decreased because of the higher water content. It is possible, therefore, that the concentration of the vitamin was not increased, but not decreased either, it was merely diluted. Plant polyploidization is frequently associated with changes in nutrient contents. For example, in *Oryza sativa* L. (W. Wang et al. 2022) tetraploid species showed higher lipid content, mainly unsaturated fatty acids and phospholipids. Ascorbic acid, titratable acids, soluble sugar, and cyclic adenosine monophosphate were significantly higher in polyploid fruits of *Ziziphus jujuba* Mill. (L. Wang et al. 2019) or the tetraploid *Moringa oleifera* Lam. (Zhang et al. 2020) had a higher content of forage-related nutrients than the diploids, although to varying degrees.

Another advantage of polyploidy is that it leads to novel genotypes resistant to various biotic and abiotic stresses (Tossi et al. 2022). There are studies that report that polyploid plants have a greater resistance to biotic and abiotic stress factors. For example, in apple (*Malus x domestica* Borkh) the resistance of autotetraploids to *Venturia inaequalis* (Hias 2018), *Alternaria alternata* and *Colletotrichum gloeosporioides* (Chen et al. 2017) infection is reported in comparison to their diploid forms. Similar results are presented by Šedivá et al. (2019) in *Anemone sylvestris*, where the autotetraploids had a better response to *Phytophthora plurivora* infection. In case of *Melothria scabra* Naudin in the current study, the cultivation of diploid (control) plants was interrupted after 120 days of cultivation due to fungal infection of Cucurbit Powdery Mildew (*Sphaerotheca fuliginea* and *Erysiphe cichoracearum*). Towards the end of the growing season, the polyploid plants were only slightly attacked by these fungi, even though they were growing close to the diploid plants. Considering these previous findings and preliminary observation from the current study, it would not be baseless to assume that polyploid plants of *Melothria scabra* Naud. could be more tolerant to biotic factors compared to their diploid forms. However, a systematic study to confirm this hypothesis is necessary to be carried. The research on the effects of polyploidy on biotic and abiotic factors could help to obtain new genotypes of horticultural crops with greater resistance, and thus obtain high quantitative and qualitative yields.

7. Conclusions

The effect of polyploidy on various agronomic and nutritional characteristics of *Melothria scabra Naudin* was evaluated.

Genotypes with novel morphological and phytochemical characteristics were generated in plants in which polyploidy was induced.

In terms of morphology, there were changes in the size of vegetative and generative organs. From the results obtained, hypothesis number one was confirmed, that there are morphological differences between diploid and autotetraploid plants.

In terms of nutrition, there was an increase in the water content of the fruits and subsequently of the whole plant. The plant needs to be evaluated in more detail from a nutritional point of view, but from these results hypothesis number two, that there is a difference in nutrient content between diploid and autotetraploid plants, has already been confirmed.

Other agronomic characteristics of autotetraploid plants, such as resistance to biotic and abiotic factors, need to be evaluated in future studies.

Polyploidisation is a promising tool for obtaining new genetic material for crops in the Cucurbitaceae and other families.

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