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Faculty of Tropical AgriSciences

Induction of polyploidy in *Hedera helix* L. by oryzalin treatment

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Induction of polyploidy in *Hedera helix* L. by oryzalin treatment” 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, 18.4. 2024

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Jáchym Rauš

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My wholehearted thanks belong to Professor Eloy Fernández for his unwithering support throughout the whole duration of my exploits in researching and writing this thesis. I command both his extensive knowledge and his kind guidance.

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Abstract

Hedera Helix L. is a perennial wood vine of the *Araliaceae* family. It is the most prominent member of the *Hedera* genus as it has substantial horticultural and ecological significance. This thesis conducted induction of autopolyploidy through oryzalin treatment of diploid (2n = 2x = 48) *hedera helix* cuttings. To induce autopolyploidy two concentrations of oryzalin were used: 100 and 200 µM, and exposure periods of 24 and 48 h. Flow cytometry was used to establish ploidy levels in studied specimens. This research has succeeded in producing two autotetraploid plants (2n = 4x = 96) along with one mixoploid. This thesis presents a new method of polyploidy induction which is both cost effective and fast. The autotetraploid plants could serve as a valuable reference point for future morphological and genetic studies of *Hedera* species. Future research is needed to investigate the biochemical and morphological potential of autotetraploid *Hedera helix* plants.

Key words: Oryzalin, *Ex vitro* polyploidy, Mutation, Flow cytometry.

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LIST OF THE ABBREVIATIONS USED IN THE THESIS.

FCM: FLOW CYTOMETRY

ER: ENDOPLASMIC RITICULUM

1. Introduction

Hedera helix L., commonly known as English ivy, is a prominent member of the family *Araliaceae*. Recent taxonomic revisions have identified 12 distinct species within the genus *Hedera*, underscoring its diversity (CABI, 2019). As a diploid species, *Hedera helix* displays a typical chromosome set that forms the genetic baseline for studies in polyploidy (Green et al., 2013). This perennial climber is adept at reaching heights up to 30 meters, often forming dense, expansive carpets on forest floors when not climbing (CABI, 2019). Its robust growth is supported by aged stems that can grow up to 25 cm in diameter. The foliage of *Hedera helix* is evergreen, featuring 3-5 lobed leaves. The leaves on climbing stems are notably larger, an adaptation that may play a role in its climbing efficacy.

The genus *Hedera* exhibits variations in ploidy levels, notably between *Hedera helix* and *Hedera hibernica*, which are diploid and tetraploid, respectively (Green et al., 2013). The presence of multiple ploidy levels within closely related species provides a unique opportunity to explore the induction of polyploidy as a mechanism for speciation and the emergence of new traits. Polyploidy in plants not only contributes to genetic diversity and species evolution but also enhances stress resistance, vigor, and adaptability. This thesis aims to delve into the mechanisms and outcomes of polyploidy induction in *Hedera helix*, with a focus on its potential to modify plant morphology and resilience to stress.

Through experimantal idnuction of polyloidy in *Hedera helix* this research seeks to contribute to our understanding of polyploidy's role in morphology of said plant. This thesis also investigates a novel approach to polyploidy induction which is both fast and cost effective. The autopolyploid plants resulting from this experimnt can serve as a valuable reference point for future studies of polyploidy and genetics of the *Hedera* genus.

2. Literature Review

2.1. Morphology and diversity

English Ivy (Hedera helix) is a perennial woody vine of the Araliaceae family. Taxonomically this plant belongs to the kingdom: *Planteae*, phylum *Magnoliophyta*, class *Magnoliopsida*, order *Apiales*, family *Araliaceae*, genus *Hedera*, species *Hedera helix* L. (USDA 2024)

Hedera helix is native to Europe, Western Asia, and North Africa as well as North America (Metcalfe 2005). This species is diploid 2n = 48 (Obermayer & Greilhube, 2000). Native to various regions across Europe, including England, Ireland, and the Mediterranean, *Hedera helix* has successfully established itself globally (Okerman, 2000). Despite its widespread distribution, the ecological role of *Hedera helix* remains a subject of debate. It provides significant cover during winter months, offering potential benefits such as protection for soil and food sources for non-migratory birds. However, this same coverage is criticized for offering minimal soil enhancement and negligible nutritional value to wildlife (Okerman, 2000). More critically, *Hedera helix* poses ecological challenges by competing with trees for sunlight, particularly notable during the winter months when it remains photosynthetically active and many trees are dormant (Thomas 1980; Ackerfield & Wen 2002).

2.2. Ploidy levels in *Hedera* species

Hedera is a very large and complex genus. The stated number of species contained within the *Hedera* genus differs from study to study. Some authors claim that there are as many as 15 species (Pojarkova 1951) within the genus while others put the number as low as 9 (Rose 1996). Most studies put that number somewhere in the middle. (Vargas et al. 1999; Ackerfield & Wen 2003; Khailenko et al. 2021). Notably, Green et al. (2011) claim that the most commonly recognized number is 13. Their study compiled genetical and morphological data from 27 specimens from all 13 species. They confirmed that the speciation of hedera is very complex and that the number of species could be even higher. Based on their results from sequencing genomes of the studied specimens, the authors argue in favor of splitting several taxa. Among these taxa is Hedera canariensis which appears to be a polyphyletic taxon. Furthermore, hedera cypria and hedera pastuchowi also display genetic variation and should be recognized as separate taxa. The well-studied and controversial hedera Hibernica was recently recognized as a separate species even though historically it was thought to be a subspecies of H. helix. This is mainly due to the morphological and ecological similarities between the two taxa. In this study authors were mainly focused on genomic analyses. They admitted that a possible shortcoming could be their small sample size (27 specimens).

On the other hand, Valcárcel & Vargas (2010) compiled large amounts of data not only when it comes to genetics but also through morphometric analyses. Even though the authors focused mainly on quantitative morphology, their study also included rigorous genetic research. The authors also used a substantially larger sample size than Green et al., more than 600 specimens. They argued that *H. cypria* should not be granted the status of a separate species. Rather, they proposed that *H. cypria* remain a subspecies of *H. pastuchovii.* Authors therefore conclude that there should be only 12 species recognized within the *Hedera* genus. Furthermore, they argue that polyphyly alone is not a sufficient reason to split a taxon as they found almost all species within *Hedera* to be polyphyletic. Morphological characteristics should therefore play an equally important role in taxon definitions. (Valcárcel & Vargas 2010).

According to aformentioned authors, Hedera species are as follows:

- *H. algeriensis* – 2n = 96 (4x)
- *H. azorica* – 2n = 48 (2x)
- *H. canariensis* – 2n = 48 (2x)
- *H. colchica* – 2n = 192 (8x)
- *H. helix* – 2n = 48 (2x)
- *H. hibernica* - 2n = 96 (4x)
- *H. iberica* - 2n = 144 (6x)
- *H. madarensis* - 2n = 144 (6x)
- *H. maroccana* - 2n = 48 (2x)
- *H. nepalensis* – 2n = 48 (2x)
- *H. pastuchovii* – 2n = 144 (6x)
- *H. rhombea* – 2n = 48 (2x)

There are also numerous cultivars as the plant has been bred for esthetical purposes for more than three centuries (Rose 1996; Khailenko et al. 2021). The Hedera genus has spread all across the globe from the west coast of northern America to Southeast Asia. The ploidy levels are almost as diverse as the species itself. Fortunately, the more recent scientific literature is consistent when it comes to ploidy levels of the species. (Vargas et al. 1999; Ackerfield & Wen 2003; Valcárcel & Vargas 2010; Green et al. 2011; Khailenko et al. 2021)

The generic base number has been established at x = 24.(Vargas et al. 1999; Metcalfe 2005; Valcárcel & Vargas 2010). This is supported by the fact that all ploidy levels within the *Hedera* genus are multiples of 24. There are 4 distinct ploidy levels: Diploid, tetraploid, hexaploidy, and octoploid (as listed above). *H colchica* has the highest ploidy level and a chromosome count of 192 making it octoploid. (Vargas et al. 1999)

This thesis aims to research *H. helix* as it has the lowest observed number of chromosomes but unlike other diploid species of *Hedera*, it is well accustomed to the local climate. Because 48 chromosomes is still a lot compared to other plants it is less likely to exhibit the gigas effect when made artificially tetraploid. This is supported by the fact that the already existing plants of the species that are tetraploid (like *H. hibernica*) don´t seem to differ morphologically in a way that would be consistent with the gigas

effect. On the other hand, *H. colchica* is morphologically very distinct from its diploid and tetraploid counterparts. Its leaves have no lobes and are elongated. It doesn't climb and often forms a shrub. But it is hard to say whether these differences are caused by polyploidy or substantially different ecological conditions this species has adapted to. Furthermore, other species like *H. maderensis* and *H. maroccana* differ greatly when it comes to ploidy levels (6x and 2x respectively). Yet they are morphologically very similar to one another. Morphological and ecological diversity along with vigour were found to be unrelated to ploidy level (Vargas et al. 1999). These findings form an interesting opportunity to investigate whether the conclusions of Vargas et. al will hold even for artificially polyploid plants.

2.2.1. Ploidy level of *hedera helix* L. and genome size

The ploidy levels within Hedera Helix L. have been long debated and studied. Some older studies suggested that this plant exhibits different ploidy levels throughout its life cycle. Juveniles and adults were thought to have different ploidy levels (Schaffner & Nagl 1979; Metcalfe 2005). Juveniles are characterized by having less differentiated lobes and they typically grow in darker places. This is because the plant doesn't form adhesive roots. When the plant receives sufficient light and has grown as a juvenile for as many as 10 years, it undergoes a phase change into an adult form. (Schaffner & Nagl 1979)

That being said, the aforementioned authors claim that adult plant cells have 71% more DNA than juvenile cells. This has been disproved by Obermayer & Greilhuber (2000). What also stands unsupported is the claim that there is a great level of diversity within the species when it comes to genome size and ploidy levels. New revised research into this topic suggests that the opposite is true. Hedera helix seems to have a remarkable consistency when it comes to variation of genome size in its subspecies. This finding merits attention because *Hedera helix* is a polyphyletic taxon.

Hedera helix L, *Hedera canariensis,* and *Hedera helix* ssp. *arborescens* all seem to have a genome size of 2C = 2.80 pg and the same chromosome count of 48. All these taxa are diploids. (Obermayer & Greilhuber 2000) This concurs with the studies on polyploidy variation in the *Hedera* genus.(Ackerfield & Wen 2003; Valcárcel & Vargas 2010; Green et al. 2011)

2.3. Polyploidy induction in *Hedera helix* L.

To the author's knowledge, there are currently no studies that would investigate any form of artificial polyploidy induction in the *Hedera helix*. This is likely because plant species are often subjected to polyploidy induction with the aims of raising their yields and boosting the enzymatic processes that drive the creation of secondary metabolites (Mohammadi et al. 2023) That's why polyploidy studies mostly focus on plants that have the potential to be valuable in agriculture. (Mondin et al. 2018; Bharati et al. 2023; Fernández-Cusimamani et al. 2024). Since hedera helix is an invasive species and its utility is limited to esthetical uses, conventional breeding by selection is more sensible. Studies on hedera mostly focus on the medicinal focus on genetics, systematic botany and morphology.

Hedera helix is a very resilient plant and many species from the *Hedera* genus are polyploid. Therefore, the plant can serve as a model specimen on which investigations in new polyploidy induction methods can be conducted. Furthermore, an artificially polyploid species could serve as a valuable reference point for future studies on the genetic a morphological diversity of the *Hedera* genus.

2.4. Polyploidy induction: history and mechanisms

The first ever article describing the means through which autopolyploid plants can be obtained was published in December of 1937. The authors used colchicine to obtain tetraploid plants of *Datura*. (Blakeslee & Avery). Carried on simultaneously and published only six weeks later was O. J. Eigsti's article describing the ability of colchicine to induce polyploidy on a cellular level. He conducted several studies in ex vitro conditions by applying varying amounts of colchicine to seedlings of radish and corn and testing its application on root tips of onion too. Eigsti succeeded in creating polyploid cells. He didn´t concern himself with studying whole tissues or even the entire plants like Blakeslee and Avery. Rather he focused on studying individual cells and describing the process through which polyploidy occurs. Eigtsi found that colchicine inhibits the mitotic spindle formation and therefore prevents the cells from splitting their nuclei in two during mitosis (Eigsti, 1938). It wasn't until 2006 that his findings were disproven by a study that found that spindle disruption alone cannot explain the emergence of polyploid cells. This

is because low concentrations of colchicine disrupt the formation of microtubule skeleton in all phases of the cellular cycle and yet do not produce viable cells. Many abnormalities such as irregular nuclei are created at low concentrations. It is only in 10 times greater concentrations of colchicine that we can observe the formation of polyploid cells. In these amounts, colchicine aids the creation of new tubulin-containing structures during c-metaphase. And it is only in these amounts that the formation of polyploid cells occurs. These findings strongly support the notion that there are more mechanisms at play aside form spindle formation disruption during the process of polyploidization. (Caperta et al. 2006).

When in vitro cultivation became mainstream and economically viable in the 1960s so did the in vitro application of colchicine for polyploidy induction. Today colchicine is predominantly used in vitro, although there are a few studies still using the soaking of seeds in ex vitro conditions (Eng & Ho 2019).

Oryzalin is a synthetic herbicide of the dinitroaniline class. Like colchicine it disrupts the formation of mitotic spindle. It has a higher affinity to plant microtubules than colchicine and is a more potent inhibitor of their function. (Morejohn et al. 1987) But as Carpeta et al. demonstrated in their study, the depolymerization of the microtubules alone cannot explain the process of polyploidization. In 2009 Langhans et al. discovered that high concentrations of oryzalin cause the formation of nodules in the endoplasmic reticulum. These nodules, also called oryzalin bodies, are formed from tubules in the ER undergoing anastomosis. The authors conclude that this is evidence for microtubule depolymerization not being the sole explanation for oryzalin´s effect on the plant cell.

Until recently the influence of membrane organelles, such as the ER, on mitotic spindle wasn´t clearly understood. But today there is evidence that the ER exerts a direct influence on the formation of the mitotic spindle and its functionality (Araújo et al. 2022). This could potentially indicate that similarly to colchicine which in high concentration stimulates the formation of new structures in the mitotic spindle, the formation of oryzalin bodies in the ER could play a similar role in modifying the process of spindle formation. Whether these compact nodules disrupt the ER or reinforce it remains to be seen. What is clear is that disruption of the ER inhibits the proper formation of the mitotic spindle and slows down the process of chromosome migration during mitosis (Araújo et al. 2022).

Eng & Ho (2019) in their review of polyploidy induction studies performed with colchicine establish 3 categories of polyploidy induction methods: *In vitro, Ex vitro, In vivo.* According to their definition, ex vitro polyploidy is conducted using either seeds or somatic parts of the plant. According to this definition, this thesis aims to induce polyploidy in *ex vitro* conditions as cuttings will be soaked in oryzalin. Very few studies have been conducted in *ex vitro* conditions using oryzalin. (Bae et al. 2020; Mori et al. 2021) To the author's knowledge, these studies resorted to using seeds as the plant material. One study attempted to induce polyploidy in grapevine rootstock cuttings using a combination of oryzalin and nitrous oxide (NO2). Although some of the affected rootstock cuttings exhibited larger stoma, thicker leaves, and increased chloroplast numbers, they were found to have normal ploidy levels. (Kara & Doğan 2022).

3. Aims of the Thesis

This thesis aims to obtain autotetraploid plants of *Hedera helix* L. 2n = 4x = 96 from diploid plants 2n = 2x = 48 using oryzalin in *ex vitro* conditions.

H1: It is possible to obtain autotetraploid plants of *Hedera helix* L using oryzalin in *ex vitro* conditions.

H2: Polyploid plants will have distinct morphological characteristics.

4. Methods

4.1. Plant material

The plant material was acquiered from the ČZU garden store. The cultivar of the plant is suited to outdoor growing and is characterized by small leaves and great number of thin branches. This makes the plant suited for the experiment as the outdoor cultivars are more resilient and less prone to abiotic stress than indoor cultivars. As the plant will be cultivated in university greenhouse where it will grow through out winter selecting an Ivy strain that is meant to be cultivated indoors would be unwise. There is also another reason why this cultivar might prove to be better suited for in vivo polyploidy induction. The process of polyploidization requires that the meristematic tissues be saturated by the antimitotic agent. Although we used organic solvent in the mutagenic solution, oryzalin is unlikely to penetrate very deep into the hardy stems of an in vivo cultivated plant. That is why in vitro polyploidy is usually viewed as a more reliable way for creating autopolyploid plants. And as the volume of meristematic tissue rises with the cube of its radius the surface only increases with its square. That means that a small increase in the thickness of the stem will create large gap between the volume and the surface of that stem. This will lead to a much smaller portion of the tissue being exposed to the mutagen as the substance only enters through the surface and has a finite depth into which it can penetrate.

The plant material was than cut up using autoclaved scalpel into clones having 3 nodes each. About 2/3 of the foliage was than removed from these clones to reduce the rate of evaporation. A total number 120 clones were used in the experiment. 40 for each concentration and 40 for a control group. The 40 clones for each concentration were than split in half so that each concentration would have 2 exposure periods. Therefore 4 flasks each containing a litre of oryzalin solution, and 20 clones were created. These clones were than rinsed in distilled water and placed in the mutagenic solutions.

4.2. Oryzalin solution

We have prepared an Oryzalin solution and used 1% dimethylsulfoxide as solvevnt. We then diluted the solution to obtain 2 liters with a concentration of oryzalin 100 μM/l and 2 liters of solution with a concentration of 200 μM/l.

4.3. Polyploidy induction

We first rinsed the clones in distilled water, and then placed them in the flasks with oryzalin solution. Then we covered the flasks with two layers of aluminum foil. We then proceeded to place them on a shaker for one hour. After that, the flasks were placed in the in vitro grow room and covered with aluminum foil to prevent the breakdown of oryzalin.

After a total of 24 hours of exposure to the oryzalin solution, we took out the two flasks each having a different concentration of the agent and 20 cuttings. Then we proceeded to rinse them in three flasks of distilled water.

The same process was repeated 24 hours later with the second group making the total exposure time for the second group 48 hours.

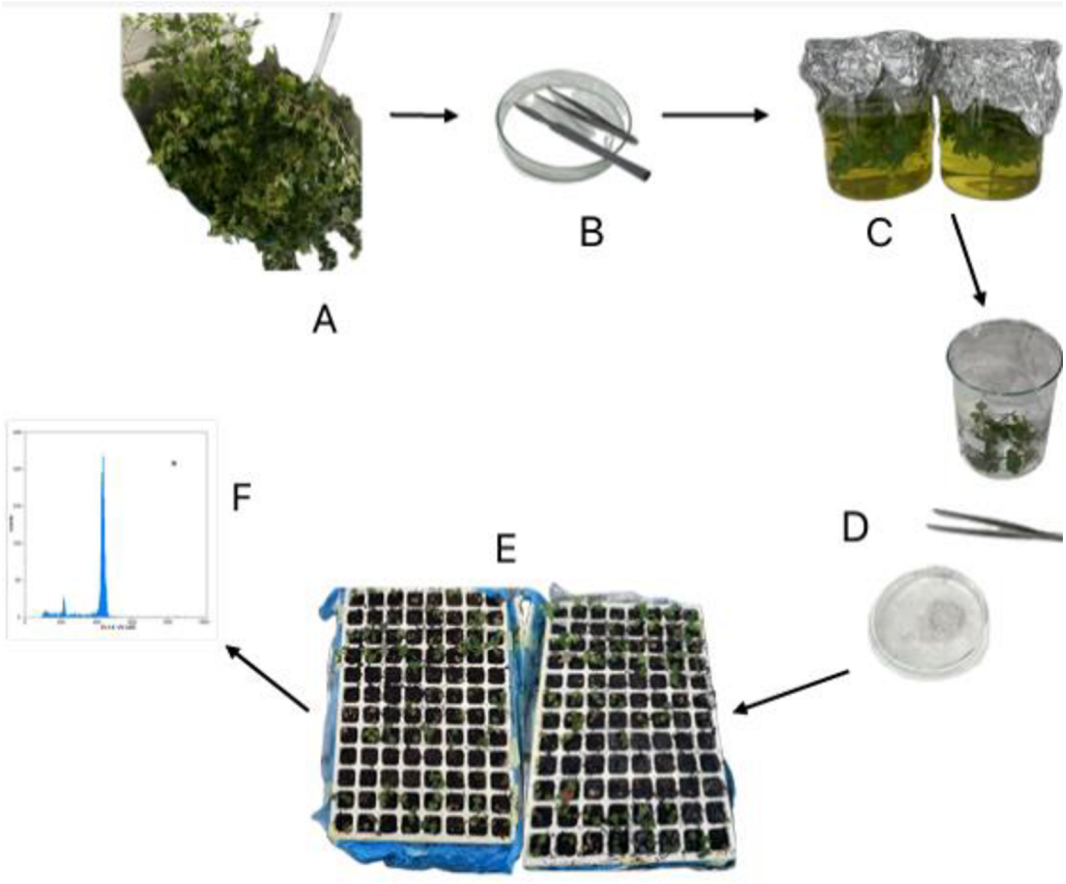

Figure 2: Polyploidization scheme: a) plant material, b) making cuttings with a scalpel, c) oryzalin solution, d) washing and dipping in stimulax I, e) growing, f) FCM polyploidy assessment

Source: author

4.4. Planting

The clones were planted in a garden substrate after being dipped in Stimulax I rooting accelerant. We used two trays of plantlets each having total number of 80 plantlets. One tray was created on the first day of the experiment with the 24 hour exposure group and on the second day of the experiment, a tray containing plants exposed for 48 hours was planted.

4.5. Growing

The 24 hour and 48 exposure trays were marked as Tray A and Tray B respectively. Both trays were placed in FTA greenhouse immediately after planting all of the 60 clones. 20 for each of the two concentrations and 20 in the control group. The plants were watered 1-2 times a week, depending on the speed of evaporation. They were watered only when the surface of the medium dried to prevent overwatering.

4.6. Flow cytometry analysis

For accurately detecting polyploid plants we performed two sets of measurements using flow cytometry. During the first measurement, all plants were tested. The second measurement was performed only on plants that showed up as polyploid during the first measurement or were morphologically divergent form the control group. To conduct the measurement, we used the leaves of the affected plants as well as the plants from the control groups. We used razorblades to cut up the leaves on a small petri dish and added 1 ml of the solution Otto 1 (0.1 M citric acid, 0.5% Tween 20). The mixture was then poured through a nylon filter into a flask. Otto II solution (0.4 M Na2HPO4.12H2O) was then added to the flask along with fluorescent dye DAPI (4´,6-diamidin-2-fenylindol). The sample was then measured in Partec PAS flow cytometry.

5. Results

5.1. Survival rates

Surviving plants were counted at the end of the experiment and rates of survival along with polyploidization effectiveness were determined.

Table 1: Survival counts and rates at the end of the experiment. * Plants were counted at the end of experiment

Source: Author

Concentration of oryzalin (µL)	Duration of exposure (h)	Number of cuttings	Number of surviving plants*	Rate of survival (%)	Number of tetraploids	Number of mixoploids	Effectivness of polyploidization (%)
200	24	20	9	45	1	0	5
200	48	20	7	35	0	0	0
100	24	20	9	45	1	1	10
100	48	20	7	35	0	0	0
0	0	40	15	37.5	0	0	0

5.2. Flow cytometry results

All surviving plant cuttings were tested. The results of flow cytometry are represented by a histograms which capture the relative amount of DNA in cell nuclei. As a reference point, sample from the control group is taken to establish the amount of DNA in diploid plants. All specimens are then measured against the reference sample.

5.2.1. First measurement

On 22.1. 2024 we conducted the first measurement using FCM. All plants that exhibited abnormal ploidy levels were found in Tray A (24 h exposure). 3 plants were found to be polyploid.

5.2.2. Second measurement

On 12. 3. 2024 we performed a second measurement using FCM. This was done to confirm polyploidy in plants that were already established by polyploids in the first measurement and to check if any new polyploids had emerged. Aside from established polyploids, we measured only those plants that were morphologically divergent and those that were marked for further observation. We confirmed that we have obtained 3 polyploid plants. 2 autotetraploid plants and one mixoploid. One mixoploid and one autotetraploid plant were discovered in 100 µg/l concentration and the other autotetraploid plant was discovered in 200 µg concentration. All of these specimens were in 24 hour exposure group.

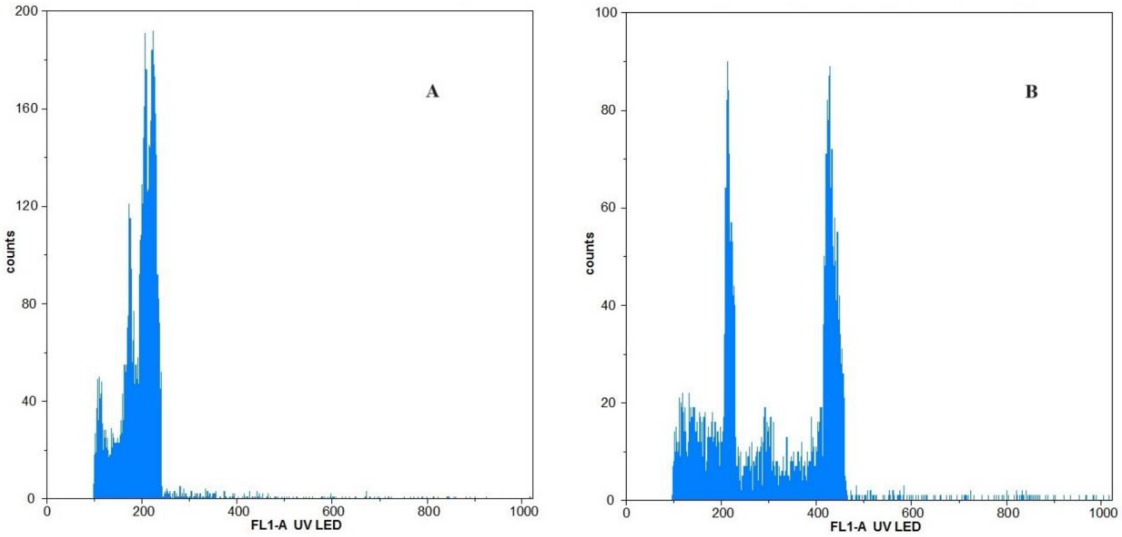

Figure 3: specimen mixoploid, FCM Histogram, 2. measurement, *Hedera helix* L.
(A) diploid, (B) mixoploid

Source: Author

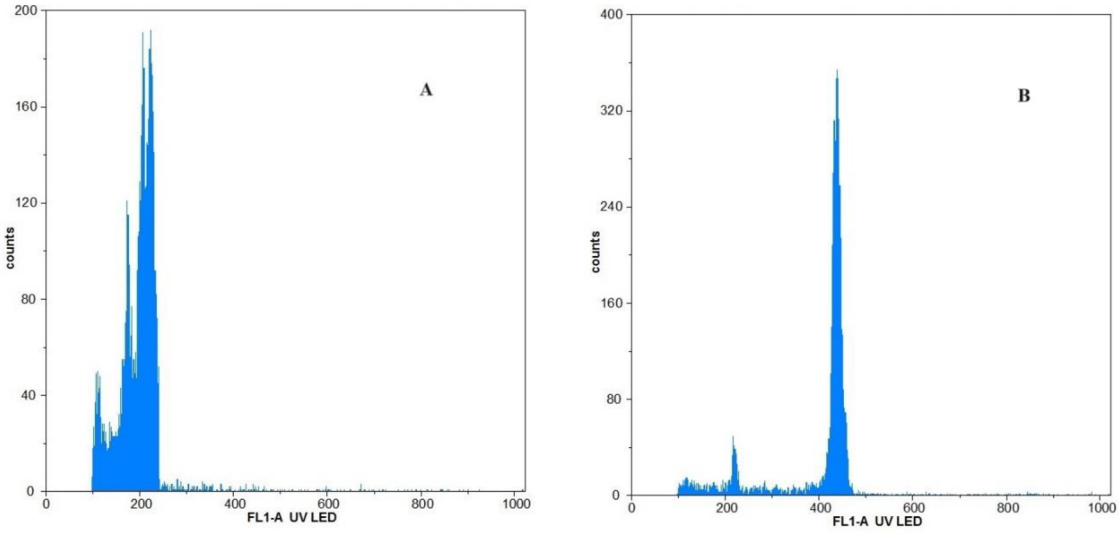

Figure 4: FCM Histogram of autotetraploid specimen, 2. measurement, *Hedera helix* L.
(A) diploid, (B) tetraploid

Source: Author

5.3. Morphology

As only 2 autotetraploid plants and 1 mixoploid the sample size was too small to perform a statistical analysis. Therefore an optical comparison was conducted. In figure 3 plants were measured against a reference length of I cm. In figure 4 the same procedure was done with leaves. Control group on the left side, autotetraploid in the middle and mixoploid on the right.

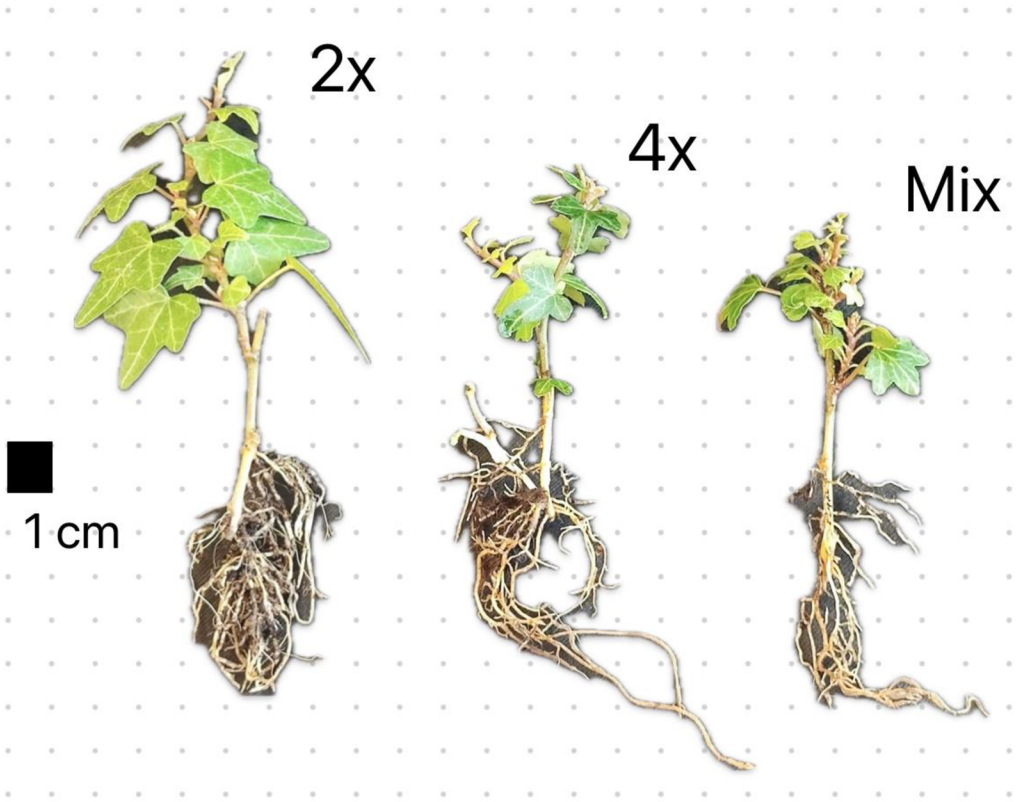

Figure 5: Comparison of whole plants with roots measured against 1 cm

Source: Author

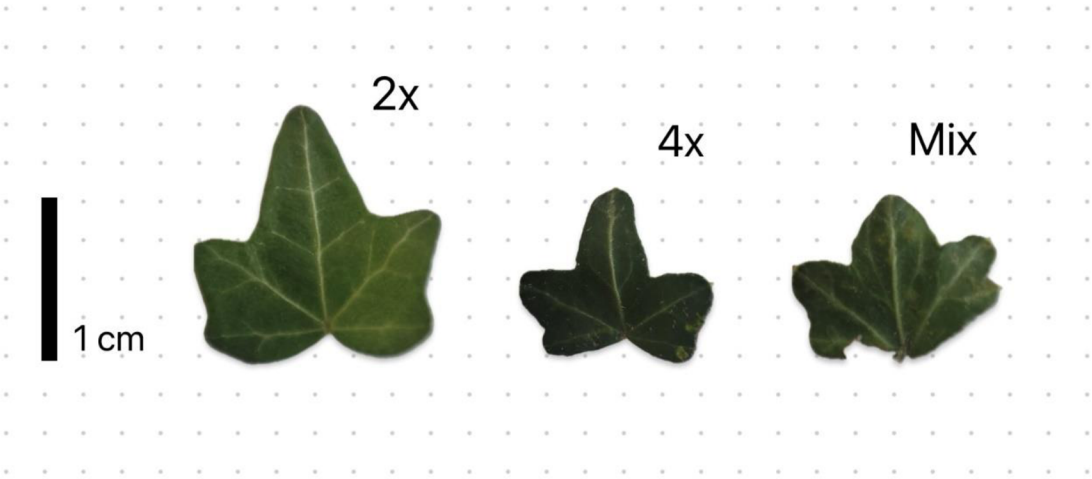

Figure 6: Leaves of control group, autotetraploid and mixoploid plants respectively

Source: Author

6. Discussion

The research conducted on the induction of *ex vitro* polyploidy in *Hedera helix* through the application of oryzalin presents a significant advancement in our understanding of possible avenues through which polyploidy can be induced. This investigation not only contributes to the sparse literature on non-crop species artificial polyploidy but also highlights the potential for broader applications in horticulture and ecological management. The successful induction of polyploidy, demonstrated by the creation of two autotetraploid plants and one mixoploid, underlines the effectiveness of oryzalin, a chemical noted for its antimitotic properties (Morejohn et al., 1987).

Additionally, the natural diversity in ploidy levels across the *Hedera* genus, as explored by Ackerfield & Wen (2002) and Valcárcel & Vargas (2010), provided a comparative framework for evaluating the induced changes in *Hedera helix*. These authors highlighted the morphological variability and speciation potential linked to natural polyploidy events within the genus, supporting the controlled induction's effectiveness compared to naturally occurring polyploidy.

Theoretically, this research enhances our understanding of polyploidy. From a practical perspective, it underscores the potential for creating polyploid plants in *ex vitro* conditions. This thesis presents a cost-effective way of creating polyploid plants. It succeeds where other studies that used more complex methods failed. Kara & Doğan (2022) used very advanced methods like NO2 exposure in combination with varying doses of oryzalin. Yet their study failed to produce polyploid plants. This might be due to the mechanism of function of oryzalin and the fact that it could not penetrate the thick plant tissue and produce antimitotic effects. However, the limited sample size of polyploids obtained in this study restricts the statistical analysis's breadth and the broader application of the findings. Future research could address this by increasing the experimental subjects and exploring the effects of varying oryzalin concentrations and exposure durations to refine the induction process. Notably decreasing the concentration of Oryzalin or exposure duration could yield favourable results. In this experiment, only 24h exposure duration yielded polyploid plants. The plants exposed for shorter duration were from a visual perspective, more lively and grew faster. Thou these findings are not

supported by statistical analysis, the visual clues could point a way toward the fact that lower concentration and duration are preferrable.

Additionally, examining the long-term growth of polyploid *Hedera helix* could provide comprehensive insights into their potential uses in environmental management and commercial exploitation. In summary, this thesis not only corroborates existing literature but expands upon it by applying polyploidy induction techniques to a species traditionally not focused on in genetic manipulation studies. The promising results pave the way for further investigations into optimizing polyploidy induction and exploring its practical applications. Further studies could investigate the content of secondary metabolites as some bioactive compounds naturally found in hedera were studied and found to have antioxidant properties (Bezruk et. al. 2020). It is believed that induced polyploidy often increases the production of secondary metabolites (Gantait & Mukherjee 2021; Mohammadi et al. 2023; Bharati et al. 2023) It remains to be seen whether this will be true for autotetraploid specimens of *Hedera helix.* The absence of a correlation between morphological features and ploidy level in *Hedera* species might suggest that the production of secondary metabolites will not correlate with ploidy either.

7. Conclusion

This thesis succeeded at obtaining two autotetraploid plants 2n=4x=96 along with one mixoploid. It provides a new cost-effective method for polyploidy induction. It is the first study of this kind and pioneers a way for future research using oryzalin in *ex vitro* conditions on somatic plant cuttings. Though the pool of obtained polyploid plants wasn´t robust enough to conduct statistical readings on the morphological differences between diploid and autotetraploid plants, our research suggests that morphological differences could exist. The main hypothesis was confirmed to be valid: It is possible to obtain autotetraploid plants using the methods presented in this thesis. Future research is needed to fully investigate the potential of polyploid plants of *Hedera helix.* The results of this work could provide a valuable reference point for further genetical and morphological studies of the *Hedera* genus. Future research could also explore the biochemical properties of autopolyploid *H. helix* specimens.

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