Asian Journal of

Poultry Science

ISSN 1819-3609

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS
Asian Journal of Plant Sciences
ISSN 1682-3974
DOI: 10.3923/ajps.2025.134.142

Research Article
Evaluating the Agronomic Impact of Induced Polyploidy in *Physalis ixocarpa*

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Abstract
Background and Objective: Husk tomato (*Physalis ixocarpa*), native to Mexico, is widely consumed in dishes and sauces. Despite its adaptation to adverse climates, there is a need for higher-yielding varieties. Polyploidy has enabled the development of larger fruits and increased yields in crops such as watermelon and potato. This study evaluated the effects of chemically induced polyploidy on the morphology, seed characteristics and fruit yield of *Physalis ixocarpa*. **Materials and Methods:** This study was conducted in two stages. In the first stage, colchicine and oryzalin were applied at four concentrations (0.08, 0.12, 0.16 and 0.20%) to induce polyploidy in germinated seeds. Ploidy level was verified through cytological analysis and seedling morphological characteristics were evaluated. In the second stage, yield and fruit quality were analyzed in both diploid and polyploid plants, highlighting the positive effects of polyploidy on crop performance. The experiment was arranged in a Randomized Complete Block Design (RCBD) with a 2×6 factorial structure and mean comparisons were performed using Tukey's test at a significance level of $p<0.05$. **Results:** Higher seedling survival was observed with 0.20% colchicine and 0.08% oryzalin. Polyploid seedlings exhibited smaller stomata, a lower stomatal index and larger seeds, with a 9.6% reduction in seed coat thickness, smaller embryo size and a different embryo position. Germination was higher in polyploids (83.33%). Polyploid plants achieved greater fruit weight per plant (1.277 kg), number of fruits per plant (35) and yield per hectare (56 t). Their fruits were larger, had higher soluble solids content (4.97%) and lower firmness (2.92 kg m^{-2}) compared to diploids (4.5 kg m^{-2}). **Conclusion:** The induced polyploidy improves fruit yield and quality, although it reduces firmness. The application of colchicine and oryzalin proved effective for inducing polyploidy in *Physalis ixocarpa*.

Key words: Husk tomato, polyploidy, colchicine oryzalin, morphology, stomata, seed, yield

Citation: Santiago-M., B.L., A. González-Cortés, R. Mendoza-Villarreal, A. Hernández-Pérez and V. Robledo-Torres, 2025. Evaluating the agronomic impact of induced polyploidy in *Physalis ixocarpa*. Asian J. Plant Sci., 24: 134-142.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Physalis ixocarpa is a species native to Mexico, commonly known as "tomate verde" or tomatillo. It grows in almost all types of soil and climates, with frost being the main limitation to its development. The fruit is manually harvested once the husk falls off. It contains proteins, fats, carbohydrates and iron, among other nutrients. Due to its medicinal properties, it is used in the treatment of respiratory ailments, ear infections, gastric inflammation and hypertension. The main use of tomatillo is in various dishes and sauces[1].

In 2023, approximately 40,176.37 hectares of tomatillo (*Physalis ixocarpa*) were harvested in Mexico, with a production value of 5,115.07 million pesos. The main producing states were Sinaloa (6,954.05 ha), Jalisco (4,988.15 ha), Zacatecas (2,962.96 ha), Michoacán (2,591.82 ha) and Puebla (4,330.01 ha), with a national average yield of 18.36 t/ha[2].

Although tomatillo is a crop that adapts well to adverse climates, there is still a need for varieties with improved adaptability and yield. Through genetic improvement, humans have unintentionally selected polyploid specimens, which have provided several advantages, such as larger fruits and increased yield.

Polyploidy is one of the major evolutionary processes in plants. It has occurred naturally, often through multiple independent events. As a dynamic and recurrent phenomenon, polyploidy leads to both genetic and epigenetic changes[3].

Genetic improvement via the induction of tetraploids in species such as watermelon, potato and husk tomato has opened a promising avenue for the development of new high-yielding and high-quality varieties or hybrids[4]. Efforts have been made to induce this polyploid condition in major crops of interest by applying chemical treatments such as colchicine and oryzalin[5]. These treatments have been successful; however, the growth and development of polyploid plants differ from those of diploid specimens.

Therefore, the objective of this research was to induce polyploidy in *Physalis ixocarpa* and to characterize the morphology of polyploid plants and seeds, as well as their fruit yield and quality.

MATERIALS AND METHODS

Study area: Colchicine (BioReagent, Sigma Life Science) and oryzalin (Pestanal, Sigma-Aldrich) were applied to diploid husk tomato (*Physalis ixocarpa*) seeds of the HM-UAAAN genotype to induce polyploidy. The seeds were obtained from the germplasm collection of the Department of Horticulture at the Universidad Autónoma Agraria Antonio Narro (UAAAN). The study was conducted between August and December, 2023 in the Tissue Culture and Mineral Analysis Laboratory of the same department.

Induction of polyploidy: Stock solutions (1%) of each mutagenic agent were prepared and stored in amber glass bottles wrapped in aluminum foil to protect from light, then refrigerated. Working solutions were subsequently prepared at four concentrations: 0.08, 0.12, 0.16 and 0.20%.

Eight treatments (four per agent) were established with two replicates each, using 20 seeds per replicate (Table 1). Seeds were germinated and once the radicle reached <0.5 cm, they were treated with the solutions for 24 hrs in darkness. Afterwards, seeds were washed thoroughly and 15 per treatment were sown in 200-cell polystyrene trays. Five seedlings per treatment were selected to standardize the squash technique, a cytogenetic method used to visualize chromosomes in somatic cells and assess mitotic division.

Surviving plants were analyzed 30 days after sowing for stomatal index, stomatal density, stomatal length and stomatal width. Plants were then transplanted into pots for seed production.

Procedure for evaluating plant survival: Twenty days after sowing, the initial survival percentage (ISP) was recorded based on the number of seedlings developed. Final survival percentage (FSP) was recorded before harvest.

Cytological variables: To confirm the ploidy level, mitotic analysis was performed on somatic cells from root apices collected between 8:00 and 10:00 a.m., when mitotic activity is highest. Chromosomes were visualized using the squash technique[6]. Additionally, a meiotic analysis was conducted on germ cells obtained from immature floral buds, which were collected and preserved in Farmer’s fixative for later observation.

Method for stomatal observation: Ten diploid and ten polyploid seedlings were sampled before transplanting. Epidermal impressions were taken from both adaxial and abaxial leaf surfaces using clear nail polish (COSMETICLAB). After drying, the film was removed with transparent adhesive tape and mounted on microscope slides. Three random fields at 40× magnification were observed per impression, for a total of 60 fields per population. In each field, stomata and epidermal cells were counted and three stomata per field were measured for width and length (μm)[7].

Table 1: Treatments with colchicine and oryzalin at four concentrations applied to *Physalis ixocarpa* seeds

Colchicine		Oryzalin	
T1	0.08%	T5	0.08%
T2	0.12%	T6	0.12%
T3	0.16%	T7	0.16%
T4	0.20%	T8	0.20%

Estimation of morphological variables in polyploid plants: At 45 days after transplanting, the following traits were measured: height to first bifurcation (HB) and total plant height (PH) in cm (measured with a tape, Truper, Mexico, CDMX, Mexico), stem diameter (SD) and average flower diameter (AFD) in mm (measured with digital caliper, model HER-411, Steren, Mexico City, CDMX, Mexico) and number of flowers (No. F), leaves (No. L) and fruits (No. Fr) per plant.

Methodology for seed morphological analysis in diploid and polyploid plants: Seeds from diploid and polyploid plants were abraded symmetrically using 180-grit waterproof sandpaper to expose the embryo and endosperm. Each seed was cleaned with a brush and stained with IKI solution (0.5% potassium iodide+elemental iodine to saturation) to visualize starch and distinguish embryo, perisperm and seed coat[8]. After 15 min, seeds were individually photographed. Internal traits were evaluated: seed coat thickness (SCT, mm) and embryo length (EL, mm). Images were captured with a Dino-Lite digital microscope (model AM4113T, Dino-Lite, Torrance, CA, USA) and analyzed using DinoCapture 2.0 software (Dino-Lite, Torrance, CA, USA).

Evaluation of germination percentage: Germination rates were evaluated for diploid and polyploid seeds. Seeds were sown in 200-cell polystyrene trays filled with a 70/30 peat moss/perlite mixture. During the first three days, trays were covered with black polyethylene bags to promote germination. After one week, germinated seeds were counted in three replicates of 100 seeds per treatment.

Agronomic evaluation: The experiment was established under a macrotunnel and shade mesh. Diploid and polyploid genotypes obtained from stage 1 were evaluated for fruit yield and quality.

Diploid and polyploid seeds were sown in 200-cell polystyrene trays. Ten days after germination, fertigation was initiated with FertiDrip at 1 g/L per week. Transplanting occurred 30 days after germination. Raised beds were spaced 1.5 m apart and plants were arranged in double rows with 30 cm spacing between plants.

The nutrient solution applied contained: 150 ppm N, 50 ppm P, 200 ppm K, 150 ppm Ca, 50 ppm Mg, 96 ppm S, 1.5 ppm Fe, 0.74 ppm Mn, 0.14 ppm B, 0.12 ppm Zn, 0.06 ppm Cu and 0.04 ppm Mo. The first harvest was used to estimate yield and identify promising genotypes.

Yield variables considered: Evaluated traits included: Fruit weight per plant (FWP, g), number of fruits per plant (NFP), average fruit weight (AFW, g) and yield (YLD, t/ha).

Evaluation of fruit quality parameters: The following traits were measured: Equatorial fruit diameter (EFD, mm), polar fruit diameter (PFD, mm), using a digital caliper (model HER-411, Steren, Mexico City, CDMX, Mexico); soluble solids content (SSC) using a digital refractometer (model RHB-55ATC, Aoqi, Shenzhen, Guangdong, China) and firmness (F, kg m^{-2}), measured with a handheld fruit pentrometer (model GY-4, Lichen, Zhejiang, China).

A Randomized Complete Block Design (RCBD) with a 2×6 factorial arrangement was used. Data were analyzed by ANOVA and mean comparisons were conducted using Tukey's test at a significance level of $p \leq 0.05$, using InfoStat software[9].

RESULTS

Survival percentage of plants: At the seedling stage, the highest survival rates were recorded in the treatments with 0.16 and 0.20% colchicine, with 36.6 and 33.3%, respectively. In the case of oryzalin, the 0.08% and 0.12% treatments showed a survival rate of 26.6%.

Before harvest, the highest survival rates were observed in the 0.20% colchicine treatment (26.6%) and the 0.08% oryzalin treatment (16.6%).

Ploidy level: Polyploid plants with a total of 48 chromosomes were obtained through chromosome counts (mitosis and meiosis) at the following concentrations: 0.08, 0.12, 0.16 and 0.20% colchicine and 0.12 and 0.16% oryzalin (Fig. 1(a-c) and 2(a-c)).

Stomatal characteristics observed: Significant differences were found between ploidy levels in stomatal length ($p = 0.039$) and adaxial stomatal index ($p = 0.0010$). Tetraploid seedlings exhibited shorter stomata (8.15 µm) and a lower stomatal index (16.55%) compared to diploid plants (8.60 µm and 19.08%, respectively, on the adaxial leaf surface) (Fig. 3 and 4). No significant differences were observed in the number of epidermal cells, the number of

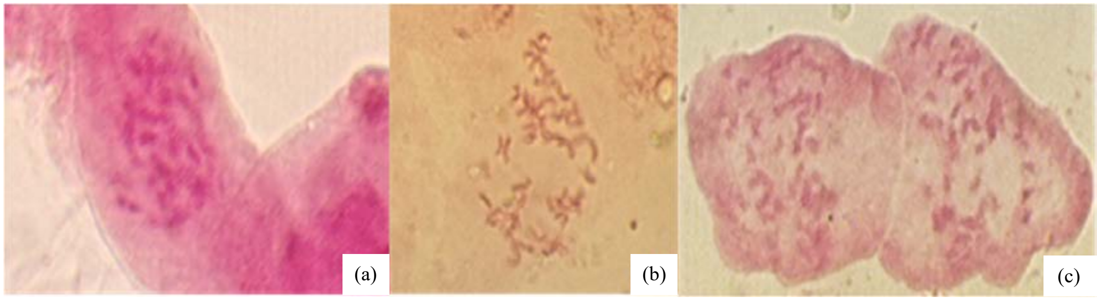

Fig. 1(a-c): Mitotic chromosomes in polyploids (2n = 4× = 48) in dividing cells from root apices of *Physalis ixocarpa*, (a) Prophase, (b) Metaphase and (c) Telophase

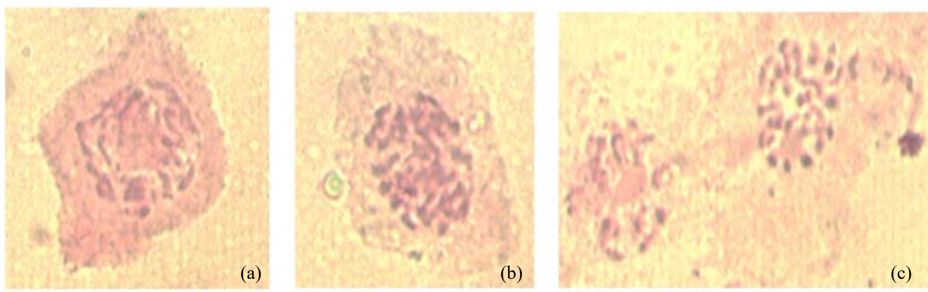

Fig. 2(a-c): Chromosomes during meiosis in the polyploid 2n = 4× = 48 in dividing cells from flower buds of *Physalis ixocarpa*, (a) Zigotene, (b) Pachyteney and (c) Metaphase I

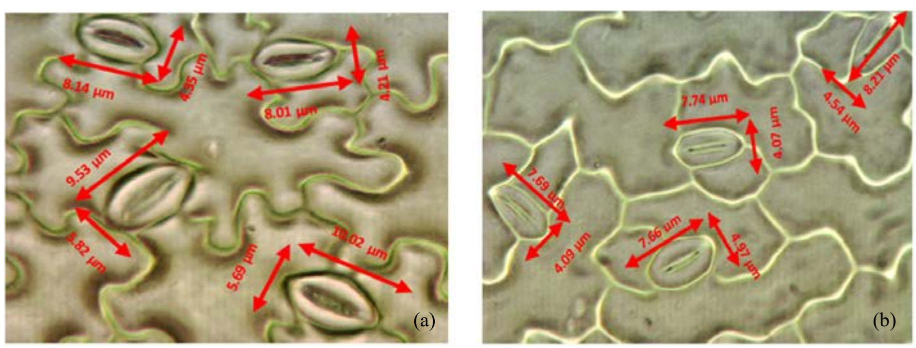

Fig. 3(a-b): Stomata on the (a) Abaxial and (b) Adaxial surfaces of diploid seedling leaves

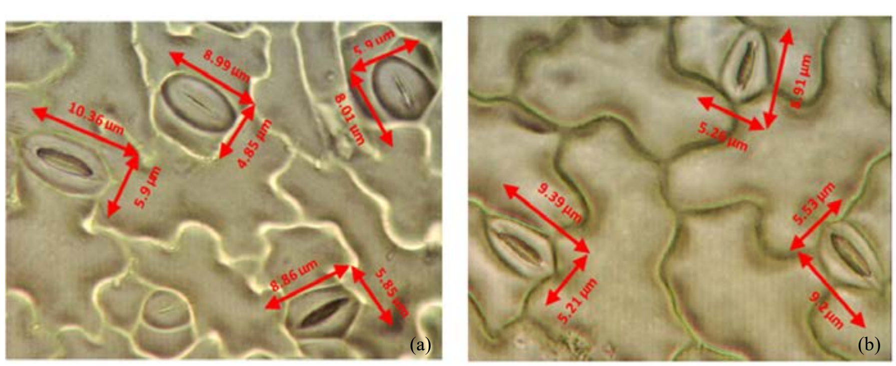

Fig. 4(a-b): Stomata on the (a) Abaxial and (b) Adaxial surfaces of polyploid seedling leaves

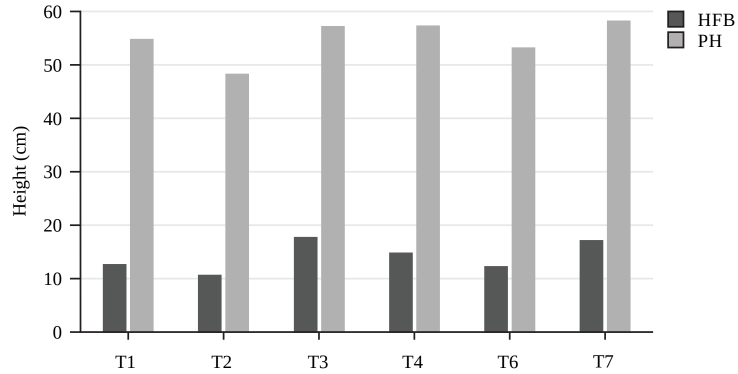

Fig. 5: Height to the first bifurcation (HFB) and total plant height (PH) in polyploid *Physalis ixocarpa* plants

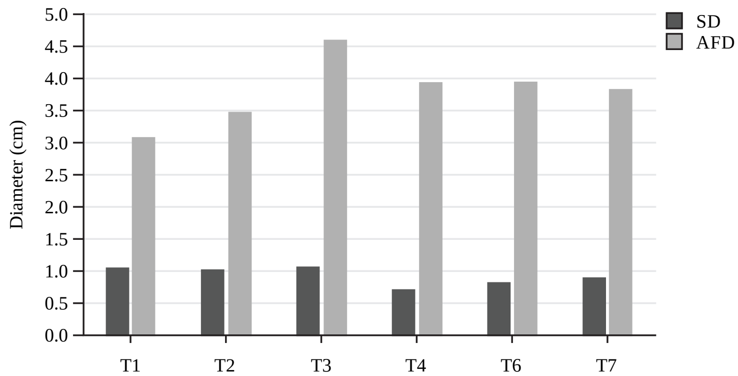

Fig. 6: Stem diameter (SD) and average flower diameter (AFD) in polyploid *Physalis ixocarpa*

stomata or stomatal density. On the abaxial side of the leaf, stomatal width was significantly lower in tetraploid plants (5.42 µm) than in diploid plants (5.83 µm) (p = 0.0063). In the early seedling stage, diploid and tetraploid plants exhibited noticeable morphological differences. Diploid seedlings showed a more elongated and vigorous growth pattern, with thinner stems and narrower leaves. In contrast, tetraploid seedlings appear more compact, with thicker stems and broader cotyledons. These differences were evident during the establishment phase and may reflect early physiological alterations caused by chromosome duplication.

Morphological variables observed in polyploid plants: Polyploid plants exhibited the following average values: Plant height, 54.58 cm; height to the first bifurcation (Fig. 5), 14 cm, which shows the measurement of the height from the base of the stem to the first bifurcation point. This value is consistently lower in polyploid plants, indicating a more compact growth habit. Figure 6 shows that stem diameter (SD) ranged from approximately 0.7 cm (T3) to 0.9 (T6 y T7), while average flower diameter (AFD) reached up to 4.6 cm (T3), indicating greater structural development in polyploid plants. The number of reproductive and vegetative structures in polyploid *Physalis ixocarpa* plants is summarized in Fig. 7. On average, each plant produced 12.3 fruits, 15 flowers and 126.16 leaves. These values reflect a favorable balance between vegetative growth and reproductive development in polyploid individuals.

Seed morphological traits in polyploid and diploid plants: Only the polyploid plants from the 0.12 and 0.16% oryzalin treatments produced a higher number of viable seeds. Significant differences were found between ploidy levels in the variables of equatorial diameter (p = 0.0055) and polar diameter (p = 0.01) of the seed, with tetraploid seeds being larger (2.70 mm and 2.20 mm) compared to diploid seeds (2.54 mm and 2.03 mm), respectively. Seed coat thickness was reduced by 9.6%. Embryo size was affected by the increase in chromosome number (p<0.0001), with tetraploid embryos measuring 7.35 mm compared to 9.35 mm in diploid embryos. Embryos in diploid seeds displayed a more coiled position, whereas embryos in polyploid seeds adopted a more extended position (Fig. 8(a-b)).

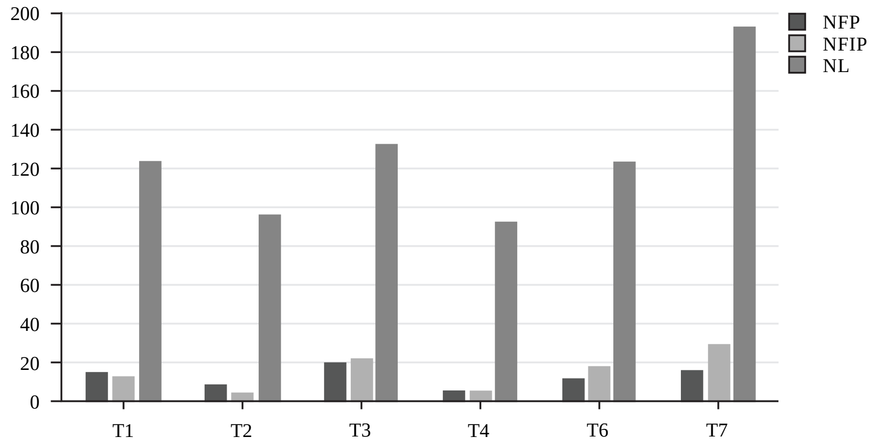

Fig. 7: Number of fruits per plant (NFP), number of flowers per plant (NFlP) and number of leaves (NL) in polyploid *Physalis ixocarpa* plants

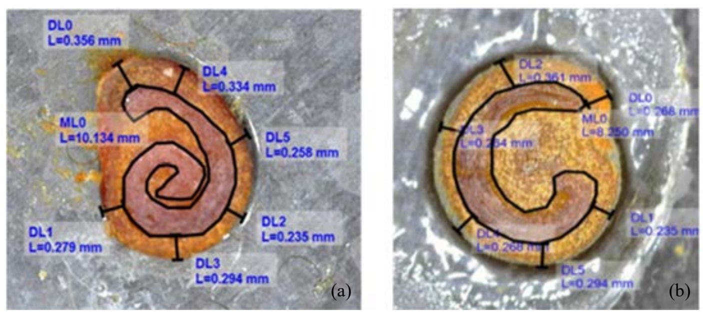

Fig. 8(a-b): Embryo position in *Physalis ixocarpa* seeds, (a) Diploid seed, (b) Polyploid seed
DL: Diploid leaves and ML: Mutated (or Modified) leaves

Germination percentage of diploid and polyploid seeds: Polyploid materials showed a significantly higher germination percentage (83.33%) compared to diploids (69.3%).

Yield variables in diploid and polyploid plants: Significant statistical differences ($p<0.05$) were observed between ploidy levels for all evaluated yield variables, according to the analysis of variance and Tukey's mean comparison test. Polyploid plants showed higher values for fruit weight per plant (1.277 kg/plant), number of fruits per plant (35), average fruit weight (36.90 g) and estimated yield per hectare (56 t/ha). The highest recorded yield in polyploid tomatillo was 61.27 t/ha; however, some individual plants in the field produced between 2.5 and 3.9 kg per plant. In contrast, diploid materials showed no significant differences among themselves ($p>0.05$), with average values of 0.21 kg of fruit weight per plant, 15 fruits per plant, an average fruit weight of 18.5 g and a yield of 12 t/ha.

Fruit quality results: Statistically significant differences ($p<0.05$) were observed between ploidy levels in all fruit quality variables. Polyploid fruits had greater equatorial and polar diameters (4.65 and 3.82 cm, respectively) compared to diploid fruits (3.71 and 3.13 cm, respectively). Polyploid fruits also exhibited a higher soluble solids content (4.97%) than diploid fruits (4.71%) ($p<0.05$). For fruit firmness, highly significant differences were found ($p<0.0001$), with diploid fruits showing greater firmness (4.5 kg m^{-2}) compared to polyploid fruits (2.92 kg m^{-2}).

DISCUSSION

The survival percentage observed in this study can be attributed to the toxic effects of colchicine treatment on plant cells and tissues, which inhibit development. For example, colchicine at 0.1% concentration was reported as toxic to *Citrus sinensis* explants, significantly reducing the average number of adventitious shoots and causing 90% of shoots to fail to develop or show morphogenic response. However, in *Agave marmorata*, induction of polyploidy with 0.15% colchicine resulted in the best sprouting parameters per explant and a higher survival percentage. These contrasting effects highlight the importance of optimizing colchicine concentration depending on species and explant type to maximize polyploid induction while minimizing toxicity[10,11]. Unlike colchicine oryzalin is used at very low concentrations (1×10 M) to induce chromosome duplication. It has been shown that at concentrations of 0.06 and 0.08%, it results in lower survival rates and effectiveness in *Physalis peruviana*[12,13]. When colchicine and oryzalin come into contact with cells, they inhibit microtubule polymerization by binding to tubulin, causing an unequal distribution of chromosomes, which leads to a change in the ploidy of the daughter cells[10].

In polyploid plants, the cell cycle has a longer duration, which affects the rate of cell division and consequently influences growth and differentiation dynamics[14,15]. This impacts the overall development time of the crop and may explain the differences observed in stomatal size in seedlings, especially considering that both measurements were taken from seedlings of the same age. Additionally, environmental abiotic factors also contribute to these variations[7,16]. Polyploid plants exhibited a reduction in stomatal density compared to diploid plants, a condition attributed to the fact that stomatal density is inversely proportional to ploidy level[17].

Ramirez-Godina *et al.*[18] reported the following average values in tetraploid populations: flower diameter ranged from 2.47 to 2.68 cm, stem diameter averaged 2.53 cm and plant height varied between 58.60 and 67.13 cm. Chromosome duplication increases the amount of genetic material, enables the functional divergence of genes and promotes evolutionary diversification[5], moreover, polyploidization induces both structural and functional changes, leading to chromosomal rearrangements, gene loss, epigenetic modifications and RNA alterations that ultimately affect gene expression[19,20]. These outcomes result from the increased number of gene alleles in polyploids. As a consequence, the offspring exhibit different behavior from the parental species; gene copies may evolve to acquire new or modified functions, allowing ecological niche expansion or greater adaptability to environmental changes. This often leads to larger leaves, stems and flowers[10], Several studies have shown that colchicine-induced polyploidy in *Aloe vera* and *Kalanchoe daigremontiana* increased plant height, number of leaves and leaf volume, thereby promoting growth and biomass accumulation[21,22], however, inducing polyploidy also prolongs the cell cycle and plant size does not always increase. In one study, higher concentrations of colchicine applied to *Colocasia esculenta* resulted in smaller plants with fewer leaves[15,23].

The viability of seeds in species treated with colchicine may be compromised due to side effects such as sterility or abnormal growth. Colchicine can also induce the formation of chimeric plants as a result of asynchronous cell divisions[24]. In polyploid fruits, seed production is often reduced due to meiotic irregularities, including chromosome loss during anaphase I and II, which leads to decreased fertility[17], additionally, the effectiveness of an antimitotic agent depends on several factors, such as the type of tissue selected, plant genotype, method of application and growth conditions[25], studies have indicated that one of the main barriers to polyploid rice breeding is the low seed production rate[26,27].

Seed size and seed coat cell size increase with higher ploidy levels[7], it is suggested that all polyploid seeds result from double fertilization, which contributes to their increased size[28]. The reduction in seed coat thickness may be a consequence of polyploidization, as it alters cellular structure, reduces lignification and affects the expression of genes involved in seed coat formation and development[29]. Embryo growth in polyploid seeds promotes early mitosis and a greater number of dividing cells, supporting root development and nutrient reserve distribution[30]. In polyploids, the higher germination rate is positively correlated with seed size and mass, which is attributed to more efficient nutrient distribution[31].

Polyploid materials exhibit genomic redundancy and polysomic inheritance, which increase genome size and flexibility. These features support adaptive divergence, enhancing plant vigor and persistence under adverse environmental conditions[32], In Mexico, the average tomatillo yield in 2024 was 18.3 t/ha; however, polyploid materials may triple this value. Selected genotypes have shown potential yields ranging from 100 to 170 t/ha. Yield in polyploid materials was three times higher than that of diploids, despite receiving the same fertilization and irrigation frequency. This increase is attributed to larger cell size and more active metabolism in polyploid plants, which leads to the development of thick, suberized root cortices that reduce conductivity and limit water absorption. Lower stomatal densities are associated with reduced transpiration rates, allowing polyploid plants to maintain turgor under water deficit conditions, thereby optimizing water and input use efficiency[33].

The increase in fruit size and weight is determined by enhanced cell proliferation and expansion, which modifies fruit shape, resulting in larger diameters attributed to increased ploidy level. Polyploid fruits exceed the sizes reported for the varieties Diamante, San Martín and Rendidora, which range from 3.9 to 4.6 cm in equatorial diameter[18]. The higher soluble solids content in polyploid fruits is attributed to increased photosynthetic activity, which alters both primary and secondary carbon metabolism and its metabolic flux . During fruit ripening, starch is hydrolyzed and in *Physalis* species, soluble solids contents of around 5 to 6.5 have been reported. The values obtained in this study make them suitable for consumption as a vegetab[19].

CONCLUSION

Oryzalin at concentrations of 0.12% and 0.16% proved to be more effective for inducing polyploidy in germinated seeds of *Physalis ixocarpa*, resulting in high percentages of viable polyploid seeds. The highest survival rate was observed with 0.12% oryzalin. Chromosome duplication slows down the cell cycle and extends the crop duration. It also reduces stomatal density, minimizing water loss and enhancing plant resilience under adverse environmental conditions. The traits observed in polyploid plants are attributed to the genetic variability resulting from polyploidization.

The polyploid *Physalis ixocarpa* lines developed in this study tripled the yield of their diploid counterparts, while using the same amount of production inputs and also improved fruit quality, supporting a more sustainable production system.

SIGNIFICANCE STATEMENT

This study discovered the potential of induced polyploidy using colchicine and oryzalin as an effective method for enhancing yield and fruit quality in husk tomato (*Physalis ixocarpa*), which can be beneficial for plant breeders and agricultural producers seeking to improve crop performance under diverse climatic conditions. The induced polyploid plants exhibited larger fruit and seed sizes, increased soluble solids and higher overall yield, despite a slight reduction in fruit firmness. This approach demonstrates a promising strategy for crop improvement in underutilized but economically significant species. This study will help researchers to uncover the critical areas of polyploidy-induced physiological and genetic changes that many researchers were not able to explore. Thus, a new theory on polyploidy-based horticultural enhancement may be arrived at.

ACKNOWLEDGMENTS

The authors thank the Universidad Autónoma Agraria Antonio Narro for providing the necessary facilities that made this work possible, as well as the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the support provided through the scholarship granted for the development of this study. We also acknowledge the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (Secretariat of Science, Humanities, Technology and Innovation) for the logistical and institutional support that facilitated the progress of this research.

REFERENCES

1. González-Pérez, J.E. and J.Á. Guerrero-Beltrán, 2021. Tomatillo or husk tomato (*Physalis philadelphica* and *Physalis ixocarpa*): A review. Sci. Hortic., Vol. 288. 10.1016/j.scienta.2021.110306.
2. dos Santos Barroso, N., M.O. de Souza, L.C. da Silva Rodrigues and C.R. Pelacani, 2017. Maturation stages of fruits and physiological seed quality of *Physalis ixocarpa* Brot. Ex Hormen. Rev. Bras. Fruticultura, Vol. 39. 10.1590/0100-29452017151.
3. Anatskaya, O.V. and A.E. Vinogradov, 2022. Polyploidy as a fundamental phenomenon in evolution, development, adaptation and diseases. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms23073542.
4. Kaseb, M.O., M.J. Umer, X. Lu, N. He and M. Anees *et al*., 2023. Comparative physiological and biochemical mechanisms in diploid, triploid, and tetraploid watermelon (*Citrullus lanatus* L.) grafted by branches. Sci. Rep., Vol. 13. 10.1038/s41598-023-32225-z.
5. Miri, S.M., 2020. Artificial polyploidy in the improvement of horticultural crops. J. Plant Physiol. Breed., 10: 1-28.
6. Robledo-Torres, V., F. Ramirez-Godina, R. Foroughbakhch-Pournavab, A. Benavides-Mendoza, G. Hernandez-Guzman and M.H. Reyes-Valdes, 2011. Development of tomatillo (*Physalis ixocarpa* Brot.) autotetraploids and their chromosome and phenotypic characterization. Breed. Sci., 61: 288-293.
7. Godina, F.R., V.R. Torres, M.H.R. Valde's, L.E. Bocardo, M.A.T. Tapia and H.T.G. Osuna, 2015. Histological and morphological study of autotetraploid and diploid plants of tomatillo [In Spanish]. Mex. J. Agric. Sci., 6: 2291-2299.
8. Gong, Y., D. Zhang, J. Lv, P. Lv and X. Zhao *et al*., 2025. Breaking wild soybean seed physical dormancy: Optimal methods and seed coat structural changes. Genet. Resour. Crop Evol., 10.1007/s10722-025-02464-2.
9. Ashour, L., 2024. A review of user-friendly freely-available statistical analysis software for medical researchers and biostatisticians. Res. Stat., Vol. 2. 10.1080/27684520.2024.2322630.
10. Latado, R.R., M. Cristofani-Yaly, C.R. de Carvalho and M.A. Machado, 2007. Citrus autotetraploid plants obtained by *in vitro* treatment with colchicine. Pesqui. Agropecu. Bras., 42: 1429-1435.

11. Osorio-German, J.A., A.M. Arzate-Fernandez and T.H. Norman-Modragon, 2025. Induction of tetraploids in *Agave marmorata* Roezl, by using colchicicine as a breeding strategy. Trop. Subtrop. Agroecosyt., Vol. 28. 10.56369/tsaes.5803.
12. Wannakrairoj, S. and W. Tefera, 2013. *In vitro* chromosome doubling in korarima [*Aframomum corrorima* (Braun) P.C.M. Jansen] using colchicine and oryzalin. Agric. Nat. Resour., 47: 684-694.
13. Dhooghe, E., K. van Laere, T. Eeckhaut, L. Leus and J. van Huylenbroeck, 2011. Mitotic chromosome doubling of plant tissues *in vitro*. Plant Cell Tissue Organ Cult., 104: 359-373.
14. Corneillie, S., N. De Storme, R.V. Acker, J.U. Fangel and M. De Bruyne *et al*., 2019. Polyploidy affects plant growth and alters cell wall composition. Plant Physiol., 179: 74-87.
15. Doyle, J.J. and J.E. Coate, 2018. Polyploidy, the nucleotype, and novelty: The impact of genome doubling on the biology of the cell. Int. J. Plant Sci., 180: 1-52.
16. Ramírez-Godina, F., V. Robledo-Torres, R. Foroughbakhch-Pournavab, A. Benavides-Mendoza and M.A. Alvarado-Vázquez, 2013. Pollen viability, density, and stomatal size in autotetraploid and diploid *Physalis ixocarpa*. Bot. Sci., 91: 11-18.
17. Gonzalez-Cortés, A., F. Ramírez-Godina, M.H. Reyes-Valdés, V. Robledo-Torres, M.Á. Pérez-Rodríguez, J.A. Villarreal-Quintanilla and A. López-Benítez, 2019. Seed morphology of seven species from *Opuntia genus* (Cactaceae) collected at Coahuila's Southeast (Mexico) [In Spanish]. Bol. Soc. Argent. Bot., 54: 493-507.
18. Ramirez-Godina, F., V. Robledo-Torres, R.F. Pournabav, A. Benavides-Mendoza, J.L. Hernandez-Pinero, M.H. Reyes-Valdes and M.A. Alvarado-Vazquez, 2013. Yield and fruit quality evaluation in husk tomato autotetraploids (*Physalis ixocarpa*) and diploids. Aust. J. Crop Sci., 7: 933-940.
19. Ramos-López, B.I., Y.D. Ortiz-Hernández and I. Morales, 2018. Yield analysis of *Physalis ixocarpa* Brot. ex Hornem varieties under greenhouse and field conditions. Cienc. Rural, Vol. 48. 10.1590/0103-8478cr20180044.
20. Castañeda-Nava, J.J., F. Santacruz-Ruvalcaba, R. Barba-González , J. de Jesús Sánchez González and L. de la Cruz Larios, 2023. Evaluating the correlation of ploidy level, leaf size, stomata characteristics and tuber weight in *Dioscorea* spp. populations from Jalisco, México. Trop. Subtrop. Agroecosyt., Vol. 26.
21. Wu, J.H., A.R. Ferguson, B.G. Murray, Y. Jia, P.M. Datson and J. Zhang, 2012. Induced polyploidy dramatically increases the size and alters the shape of fruit in *Actinidia chinensis*. Ann. Bot., 109: 169-179.
22. Mercado, S.A.S., J.D.Q. Caleño and V.J.B. Urbano, 2021. Impact of colchicine on leaf morphology and stomatics of *Kalanchoe tubiflora* (Harv.) Raym.-Hamet (Crassulaceae). Botan. Pacifica, 10: 47-55.
23. Haring, F., M. Farid, S. Sudirman and M.F. Anshori, 2023. The morpho-somatic and chromosomal changes in colchicine polyploidy induction *Colocasia esculenta* var. Antiquorium. Plant Breed. Biotech., 11: 105-116.
24. Manzoor, A., T. Ahmad, M.A. Bashir, I.A. Hafiz and C. Silvestri, 2019. Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. Plants, Vol. 8. 10.3390/plants8070194.
25. Talebi, S.F., M.J. Saharkhiz, M.J. Kermani, Y. Sharafi and F.R. Fard, 2017. Effect of different antimitotic agents on polyploid induction of anise hyssop (*Agastache foeniculum* L.). Caryologia, 70: 184-193.
26. DeTian, C., C. JianGuo, C. DongLing, D. BingCheng and Z. Wei *et al*., 2007. The breeding of two polyploid rice lines with the characteristic of polyploid meiosis stability. Sci. China, Ser. C Life Sci., 50: 356-366.
27. Chen, R., Z. Feng, X. Zhang, Z. Song and D. Cai, 2021. A new way of rice breeding: Polyploid rice breeding. Plants, Vol. 10. 10.3390/plants10030422.
28. Bleckmann, A., S. Alter and T. Dresselhaus, 2014. The beginning of a seed: Regulatory mechanisms of double fertilization. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00452.
29. Stevens, A.V., A.B. Nicotra, R.C. Godfree and L.K. Guja, 2020. Polyploidy affects the seed, dormancy and seedling characteristics of a perennial grass, conferring an advantage in stressful climates. Plant Biol. J., 22: 500-513.
30. Dante, R.A., B.A. Larkins and P.A. Sabelli, 2014. Cell cycle control and seed development. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00493.
31. Hahn, M.A., T. Lanz, D. Fasel and H. Müller-Schärer, 2013. Increased seed survival and seedling emergence in a polyploid plant invader. Am. J. Bot., 100: 1555-1561.
32. von Well, E. and A. Fossey, 1998. A comparative investigation of seed germination, metabolism and seedling growth between two polyploid Triticum species. Euphytica, 101: 83-89.
33. Tossi, V.E., L.J.M. Tosar, L.E. Laino, J. Iannicelli and J.J. Regalado *et al*., 2022. Impact of polyploidy on plant tolerance to abiotic and biotic stresses. Front. Plant Sci., Vol. 13. 10.3389/fpls.2022.869423.