Qi, Dong; Rabara, R; Shen, Y; Della Vecchia, M Altria Client Services LLC, Richmond, VA 23219 Center for Research and Technology

CORESTA AP 2023

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Colchicine treated Tobacco – Potential to Change Biochemical **Composition of Leaves**











Colchicine and its effect on chromosomeduplication

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Source: (-)-Colchicine – AFG Scientific

Colchicine is a chemical that can induce abnormal cell division

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DIPLOID CELLS

MITOSIS WITH CHOLCHICINE



Souce: Colchicine results in doubling of chromosome number because of (toppr.com)



Proof of concept objectives:

and sensory profile.

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 Develop library of polyploid tobacco lines for trait development and discovery.

Screen developed tobacco lines for improved flavor









Polyploidy plants and line development

Ploidy manipulation: A traditional method to create new traits in plants

Polyploidy

Name	Number
Common wheat	6N = 42
Tobacco	4N = 48
Potato	4N = 48
Banana	3N = 27
Boysenberry	7N = 49
Strawberry	8N = 56

chromosome number up to 400N

Source: Bioninja-https://ib.bioninja.com.au/higher-level/topic-10-genetics-and-evolu/103-gene-pools-and-speciati/allopolyploidy.html

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Source: https://image.slidesharecdn.com/dnaextractionlab-120201174434-phpapp01/75/dna-extraction-lab-2-2048.jpg?cb=1668579615







Source: Errors in Cell Division - Course Hero

Material and method: How to induce polyploidy in Tobacco

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Line 1, Line 2, Line 3, Line 4 - tobacco seeds and seedlings

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Cellular level changes

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Method for inducing polyploidy in tobacco

Shaking in orbital shaker for better penetration

Adapted from Gantait, S., Mukherjee, E. Induced autopolyploidy—a promising approach for enhanced biosynthesis of plant secondary metabolites: an insight. J Genet Eng Biotechnol 19, 4 (2021).

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Science

Cellular level changes

Source: Gantait, S., Mukherjee, E. Induced autopolyploidy—a promising approach for enhanced biosynthesis of plant secondary metabolites: an insight. *J Genet Eng Biotechnol* **19**, 4 (2021).

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Polyploidy-potential changes in the plant

Product level changes

Source: Niazian, M., Nalousi, A.M. Artificial polyploidy induction for improvement of ornamental and medicinal plants. *Plant Cell Tiss Organ Cult* **142**, 447–469 (2020). https://doi.org/10.1007/s11240-020₋₀₁₈₈₈₋₁

Preliminary results

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Seed Source	Number of treated seeds	Number of treated seedlings	Doubling Confirmation (Flow Cytometry)	Seed Collection	Fertileoffspring
Line 1	100+	50+	9	9	
Line2	100+	50+	4	4	0
Line3	100+	50+	4	4	0
Line4	100+	50+	0	0	0

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Polyploid line development from colchicine treatment

✓ Treatments: 0.5 and 1% aqueous solution for 8 hours

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Colchicine effects - control versus colchicine treated

Control vs Treated

Control vs Treated

Other examples of colchicine's effects on plant materials

Inflorescence of poinsettia 'Dulce Rosa' and its colchicineinduced mutants. Bar = 5 cm. CK (control): 'Dulce Rosa', D-M1 to D-M7: mutant lines obtained by colchicine treatments.

Source: Pan, I., Lu, Y., Wen, P., & Chen, Y. (2019). Using Colchicine to Create Poinsettia (Euphorbia pulcherrima × Euphorbia cornastra) Mutants with Various Morphological Traits. *Hort Science horts*, 54(10), 1667-1672. Retrieved Oct 4, 2023, from <u>https://doi.org/10.21273/HORTSCI14143-19</u>).

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The inflorescence and pollen character and relative DNA content on poinsettias, 'Dulce Rosa', 'Princettia-Hot Pink', and their individual mutants. (A) Abnormal involucre of 'Dulce Rosa'. (B) Dehisced anther of 'Dulce Rosa' mutant D-M1. (C) Pollen germination of 'Dulce Rosa' mutant D-M1. (D) The relative DNA contents of CK (control): 'Dulce Rosa' and mutant D-M1. (E) The involucre of 'Princettia-Hot Pink'. (F) Anther dehisced 'Princettia-Hot Pink' mutant P-M13. (G) Non-pollen germination of 'Princettia-Hot Pink'. (H) Pollen tube elongation of 'Princettia-Hot Pink' mutant P-M13. (I) The relative DNA contents of CK (control): 'Princettia-Hot Pink' and mutant P-M13. Bar (A, B, E, F) = 0.5 cm; (C, G) = 200 μ m; (H) = 100 μ m

Comprisons of leaf morphology in chicory plants having different ploidy levels. Diploid (Panel A), an euploid (Panel B), mixoploid (20%) tetraploid +80%diploid;Panel C),mixoploid (83%tetraploid + 17%diploid; Panel D), and tetraploid (Panel E) leaves are shown. Scale bar $=5 \, \mathrm{cm}$.

Flowers and pollen grains from diploid (Panels A, C) and colchine-treated tetraploid (Panels B, D) chicory plants. Scale bars = 2 cm (Panels A, B); 50 μ m (Panels C, D).

Source: Ghotbi, Elnaz & Rezanejad, Farkhondeh & Zolala, Jafar & Dehghan, Ishmael. (2013). The effects of chromosome-doubling on selected morphological and phytochemical characteristics of Cichorium intybus L. Journal of Horticultural Science and Biotechnology. 88.701-709. 10.1080/14620316.2013.11513027.

Effect of chemical compound level on controls and treated plants: comparable, higher or lower levels.

Sensory attributes: warm, woody, smoky, nutty, roasted, bitter, peppery.

(Preliminary internal screening based on 03 participants. All panelists were adults aged 21+, who smoke conventional cigarettes)

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> Chemical classes of existing compounds: triterpenoid, diterpenoid, alkane, fatty acids, phenolic compound, aromatic compounds, aldehydes.

Aerosol Derived from Tobacco filler-Lines 1& 2 *Treated vs Control* - (one repetition)

Conclusions:

Developed and improved methods for creation of new polyploid tobacco lines via colchicine treatment ✓ Tobacco clones might better suited as final format of polyploid tobacco library ✓ Established polyploid tobacco line library for trait development

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Thank you!

Questions?

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