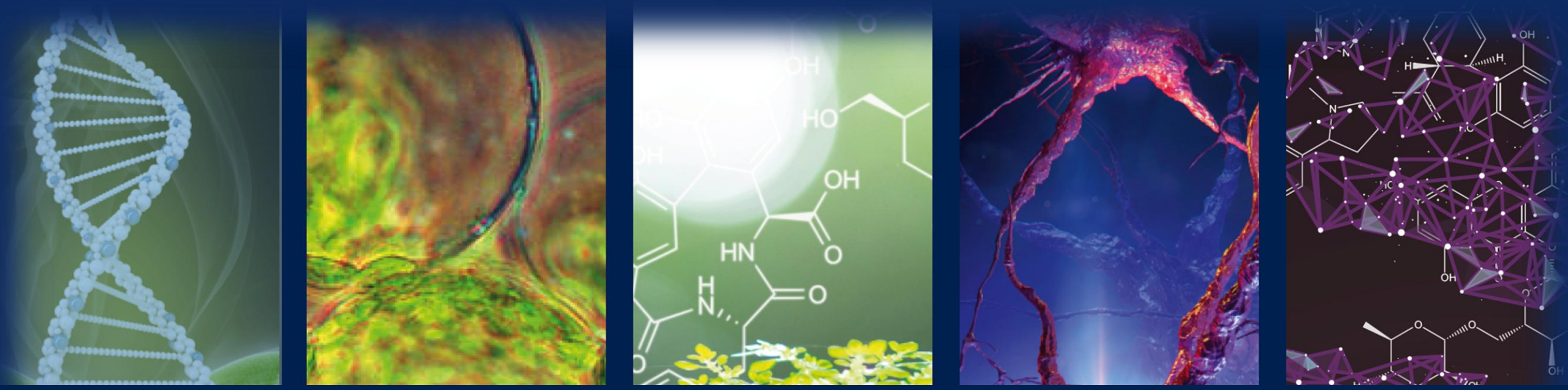

Unlocking the complex genome of *Nicotiana tabacum*

BAT
A BETTER TOMORROW

Dr Parameswari Paul

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AGENDA

01 Why Tobacco?

02 Current Statistics of Tobacco Genome

03 What We Lack?

04 Our Aim

05 NitabV6 genome

06 Conclusion

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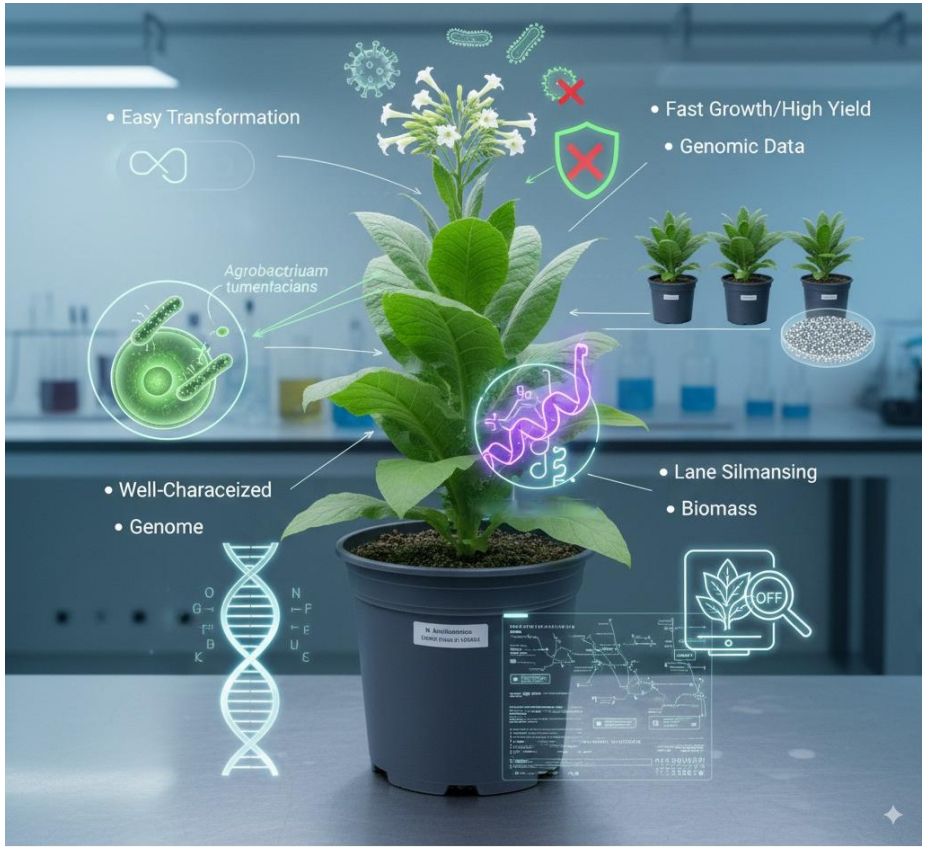
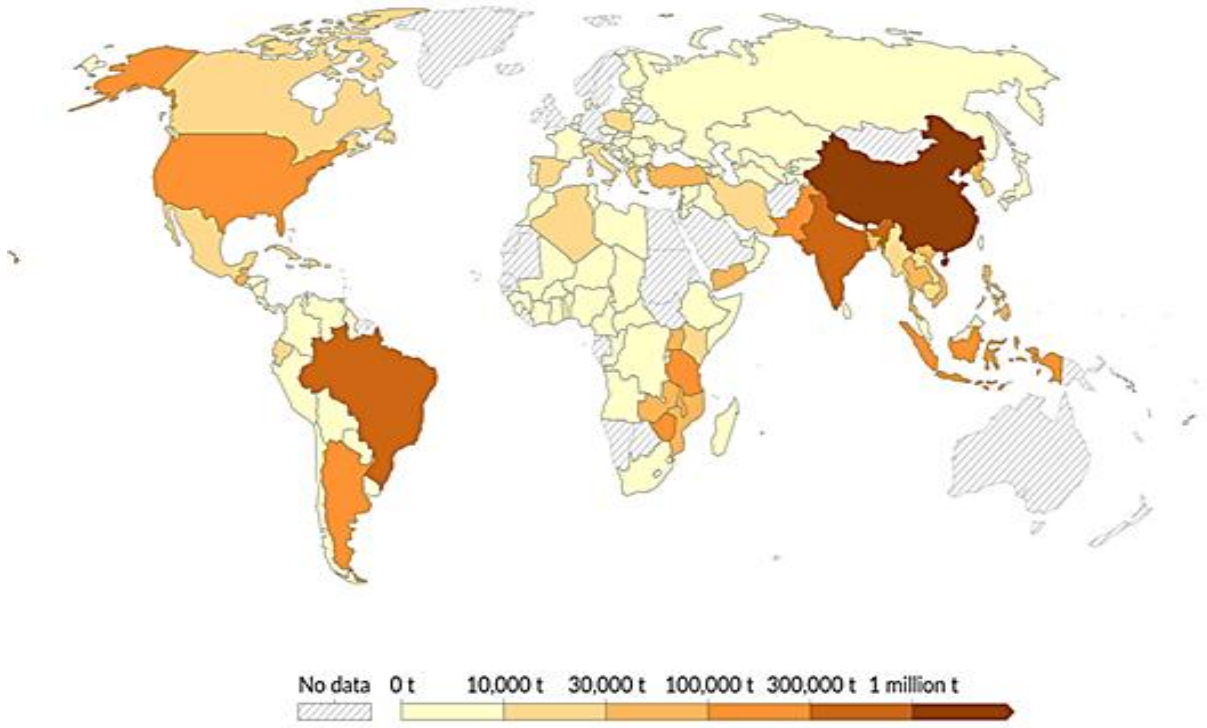
Why Tobacco?

Nicotiana tabacum as a Model Plant



Dependence Worldwide

Tobacco production, 2023



Production measured in tonnes, Source Food and Agriculture Organisation of United Stated 2023.

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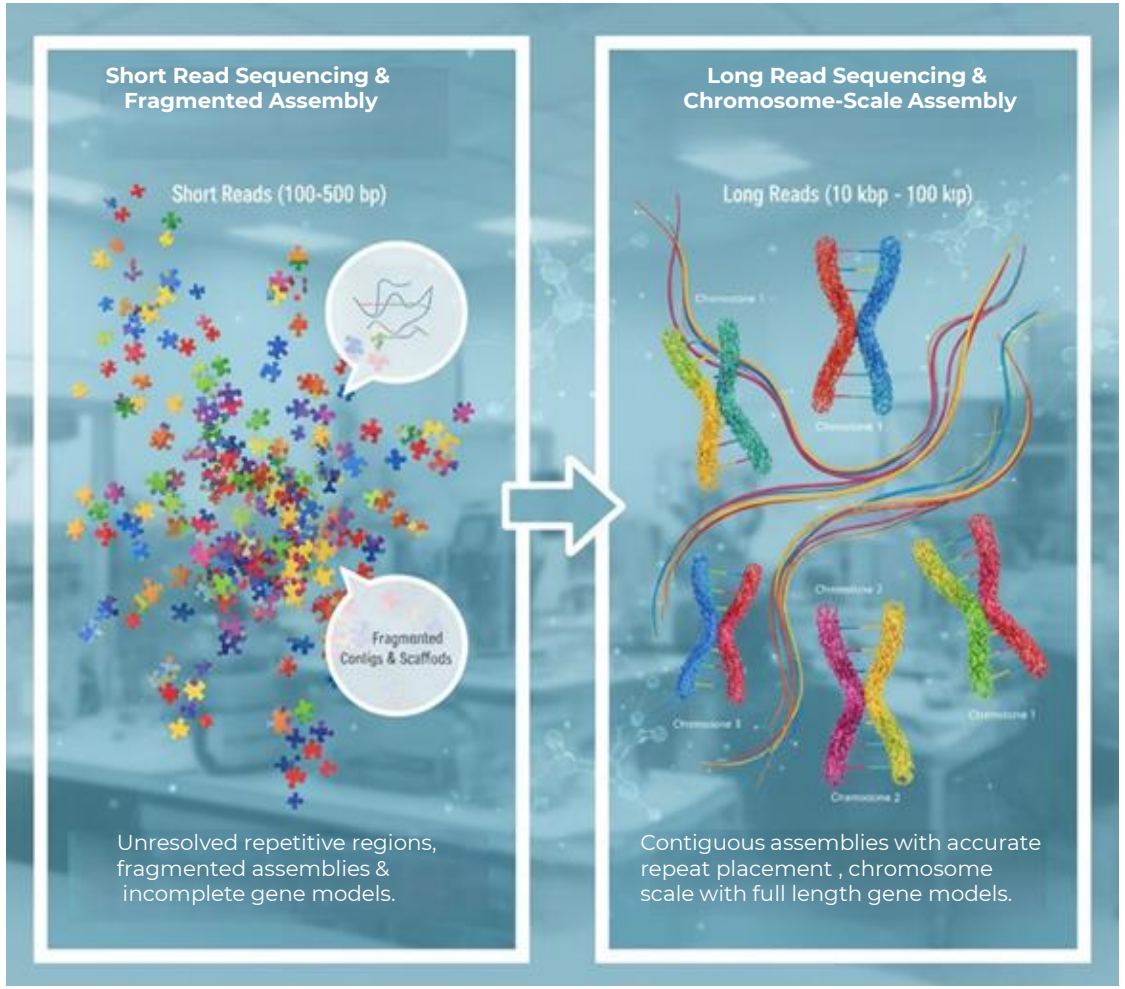
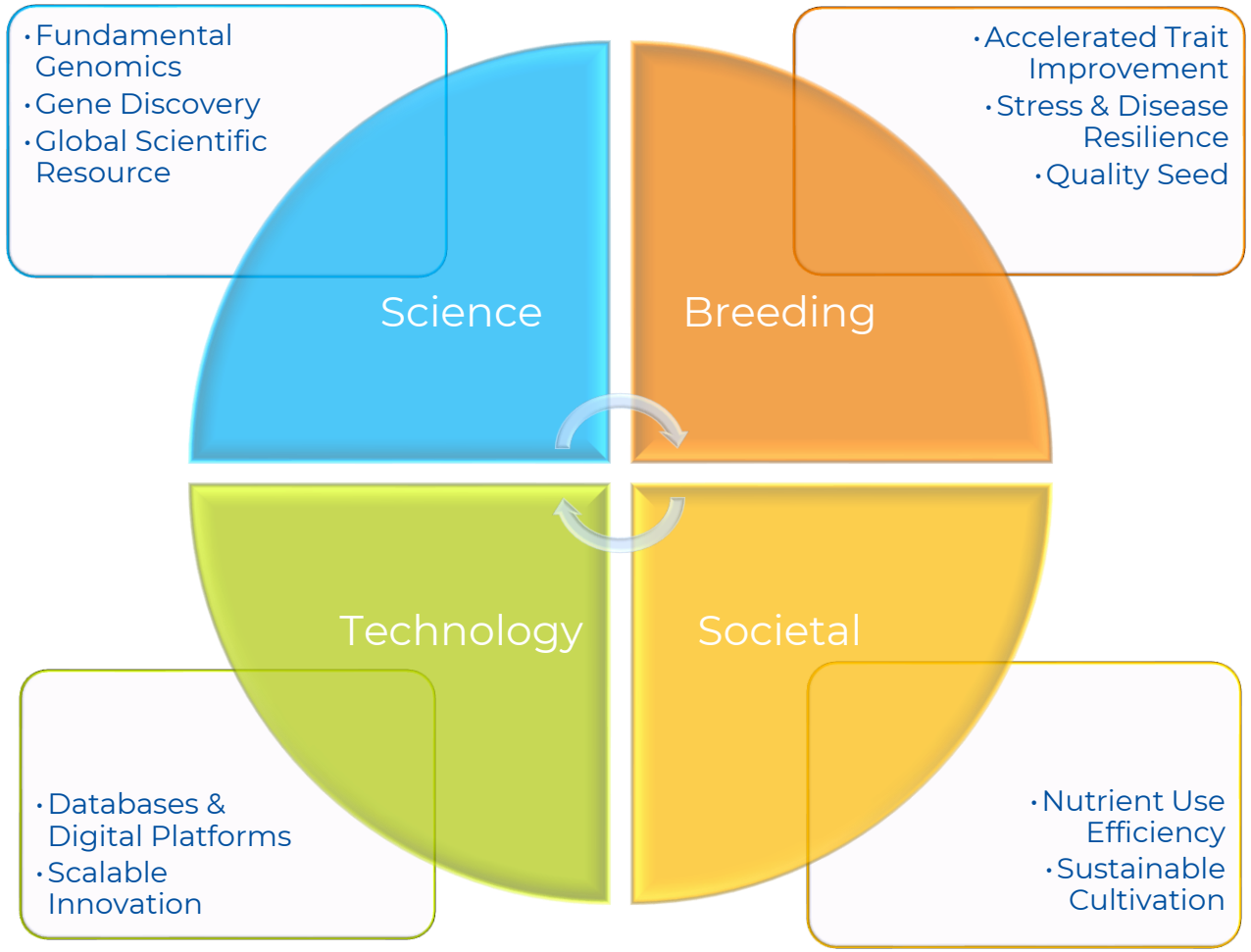
Why Now?



The Impact To Community



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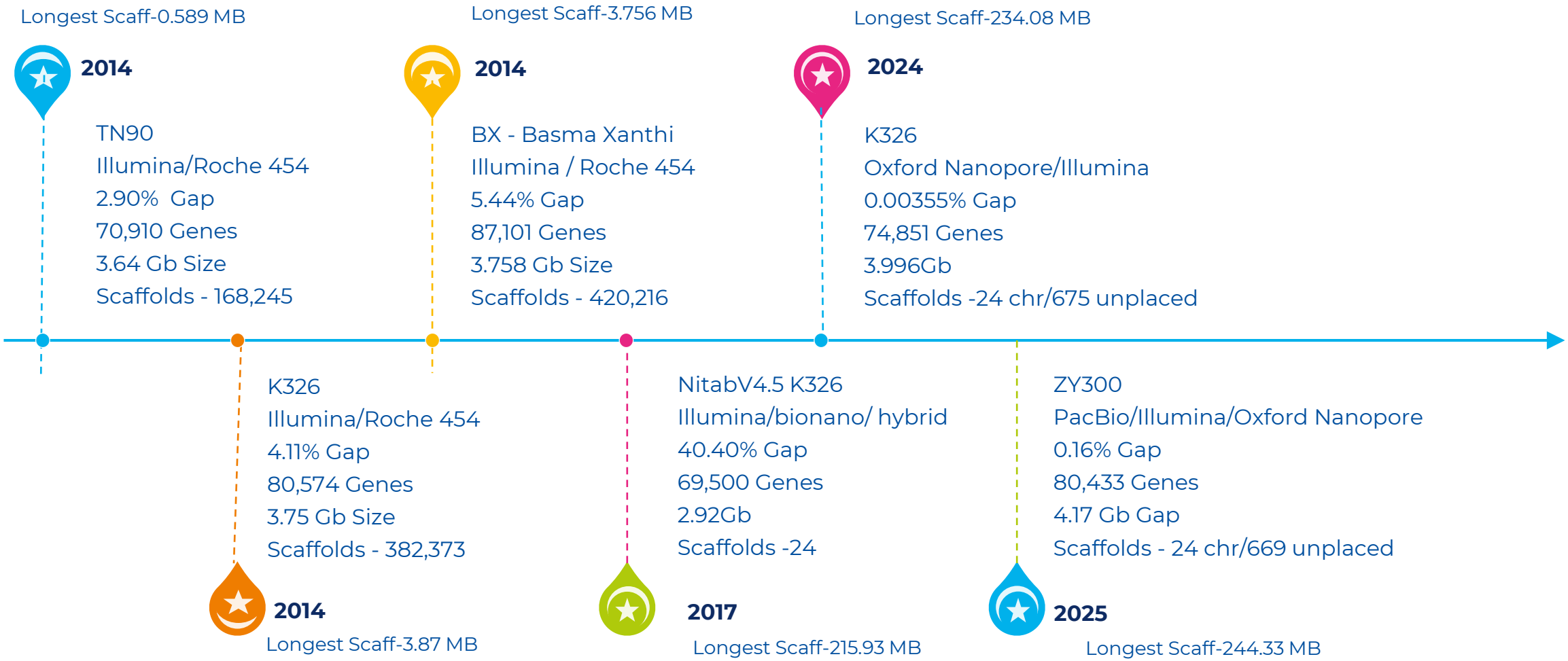
Current Statistics of Tobacco Genome



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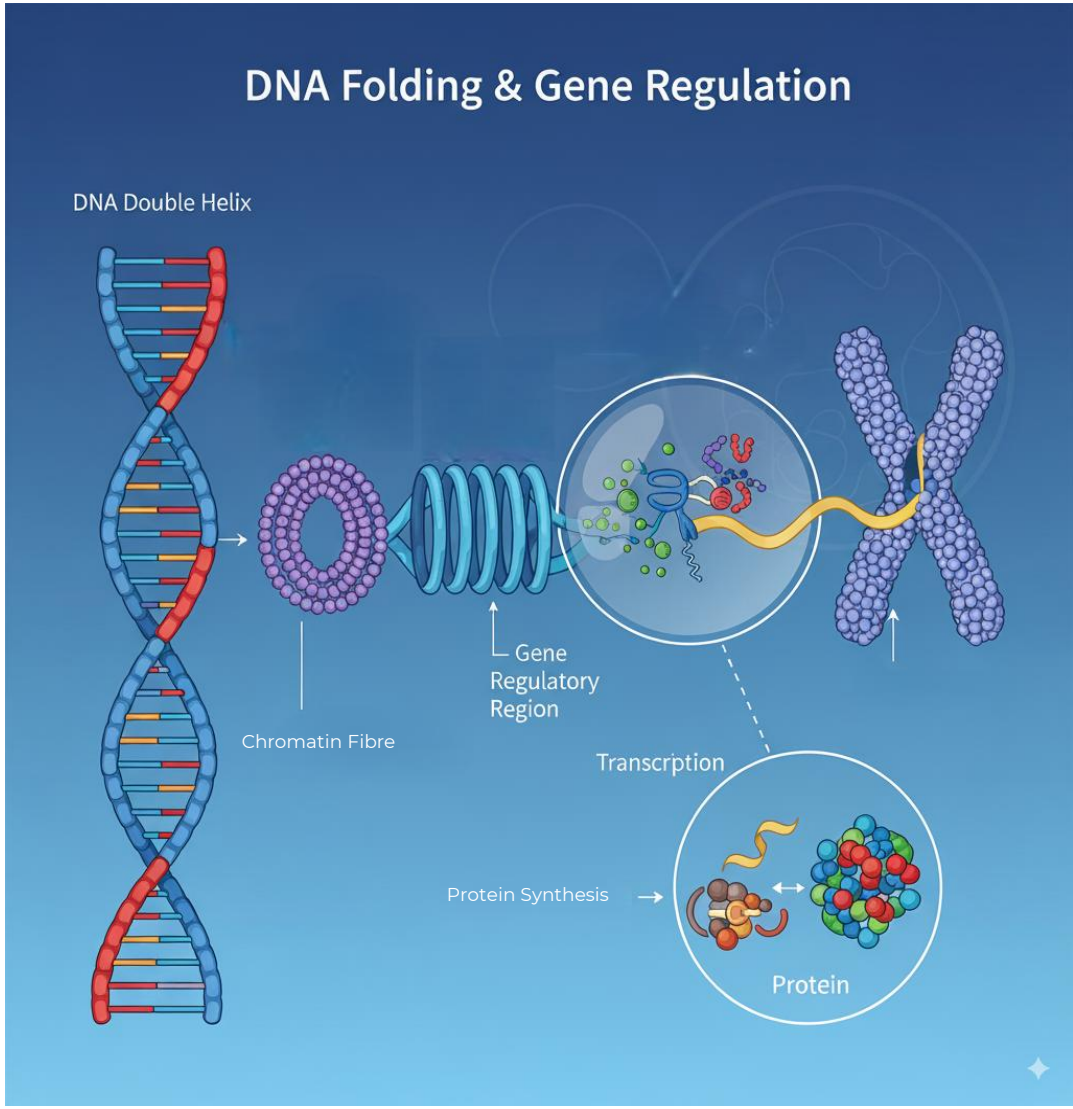
What is missing?

- Despite decades of research, tobacco lacked a **highly contiguous** reference genome due to its polyploidy and repetitive content.
- Understanding the **3D genome** structure is crucial for deciphering gene regulation and advancing precision breeding in response to climate change.
- A **Haplotype resolved** assembly genome assembly separates maternal and paternal genomes, enabling precise study of traits, plant disease, and inheritance.



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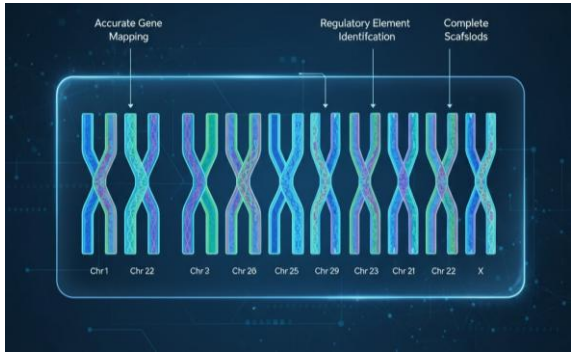
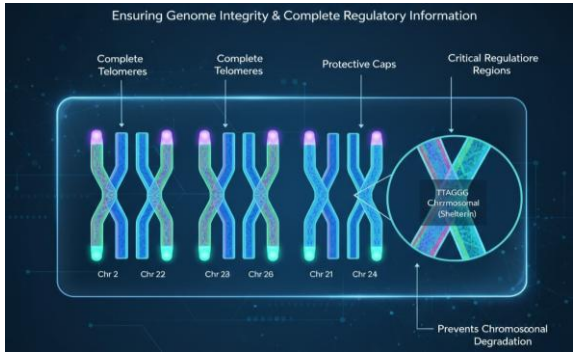
Our Aim

- Generate a near-complete, chromosome-scale genome assembly, resolve haplotypes, and explore 3D genome structure to understand gene regulation.

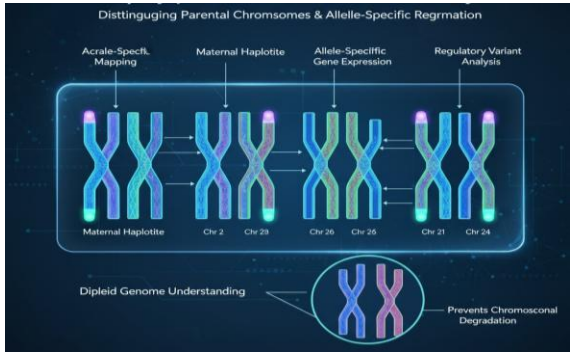
Chromosome Scale



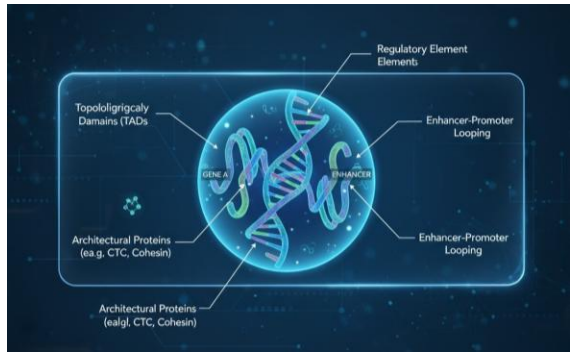
Telomere to Telomere



Haplotype resolved



3D genome



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The new *N. tabacum* NitabV6

Our Pipeline

HiFi Sequencing

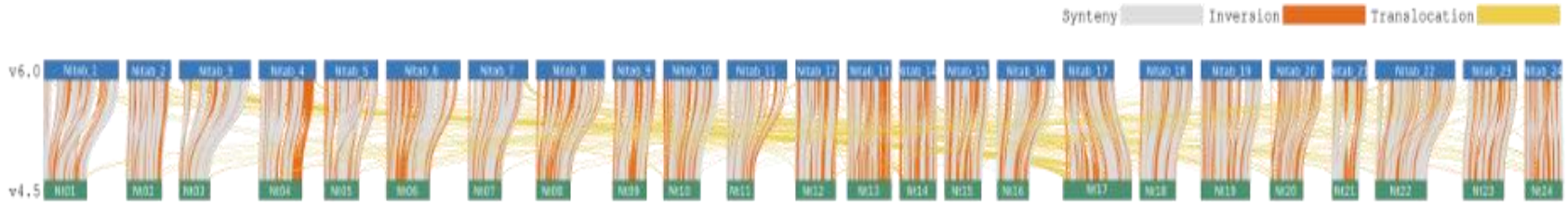
Omni-C, Deep Hi-C Scaffolding

Purge Duplication

Correction using Genetic map

Genome Statistics

- Chromosome scale genome assembly of NitabV6 with 4.1Gb size.
- Nearly gap free (0.0017%).
- Telomere to Telomere completeness.
- Less fragmented (24 chromosome scale + 35 unplaced).
- Haplotype resolved.
- Longest scaffold 251.65 MB with all the scaffolds >50kb.
- First 3D genome explored.



Comparison of our previous V4.5 genome version (2017) to the current NitabV6 (2025)

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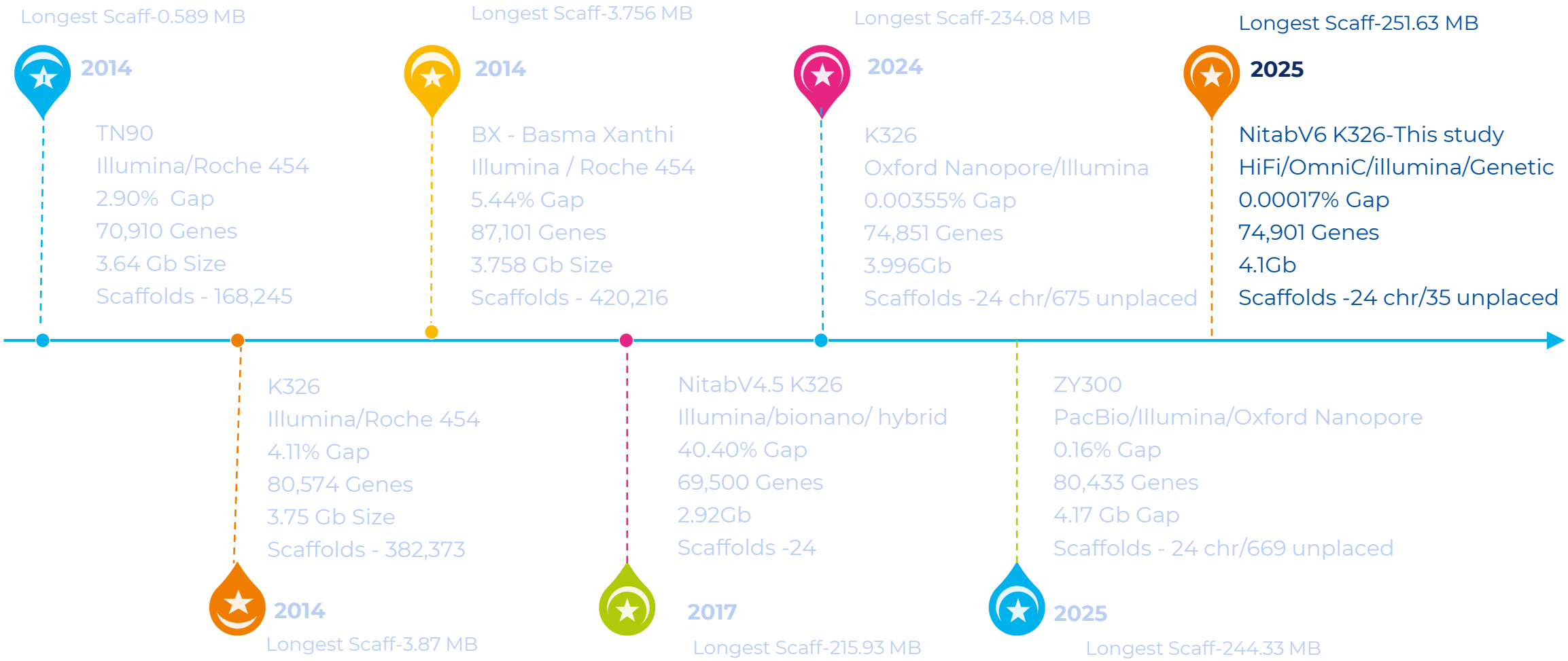
Current Statistics of Tobacco Genome



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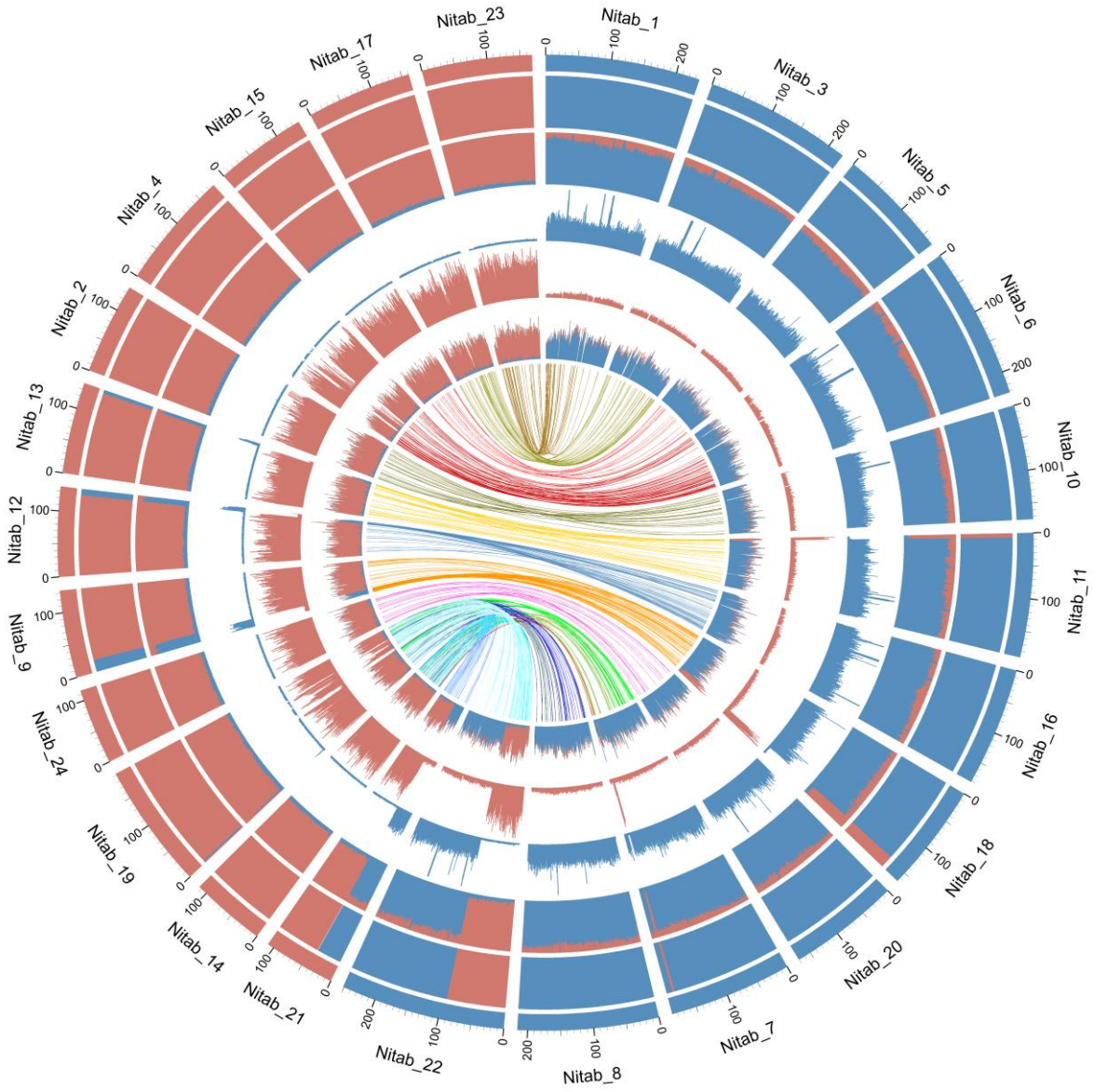
Deep Dive into NitabV6



- Circus plot showing NitabV6 assignments with S-subgenome and T-subgenome.
- Approximately 57.4% and 40.9% of *N. tabacum*'s genome originated from *N. sylvestris* and *N. tomentosiformis* respectively.
- stretch of T-subgenome at the tail of Chr11's left arm and Chr07 and Chr18's right arm
- S-subgenome at the tail of Chr12's, Chr13's right arm and Chr19's left arm, which were previously unidentified

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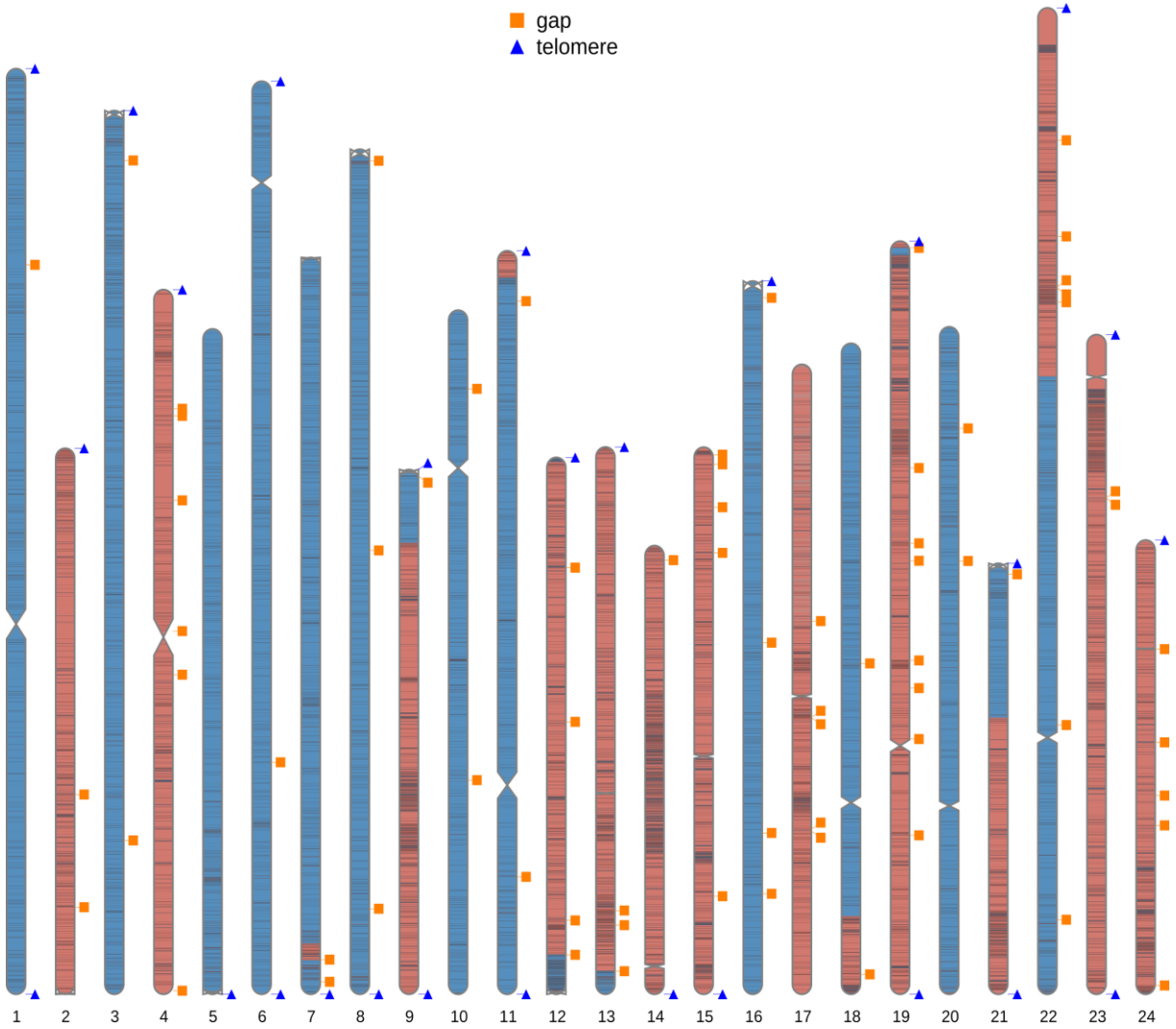
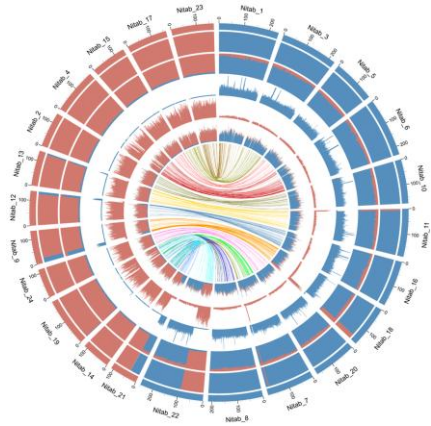
Deep Dive into NitabV6





- 12chr with telomeres in one end.
- 4 chr with no telomeric regions.
- 15 candidate centromeres were identified.
- 5 intact chromosomes having telomere ends and centromere candidates.

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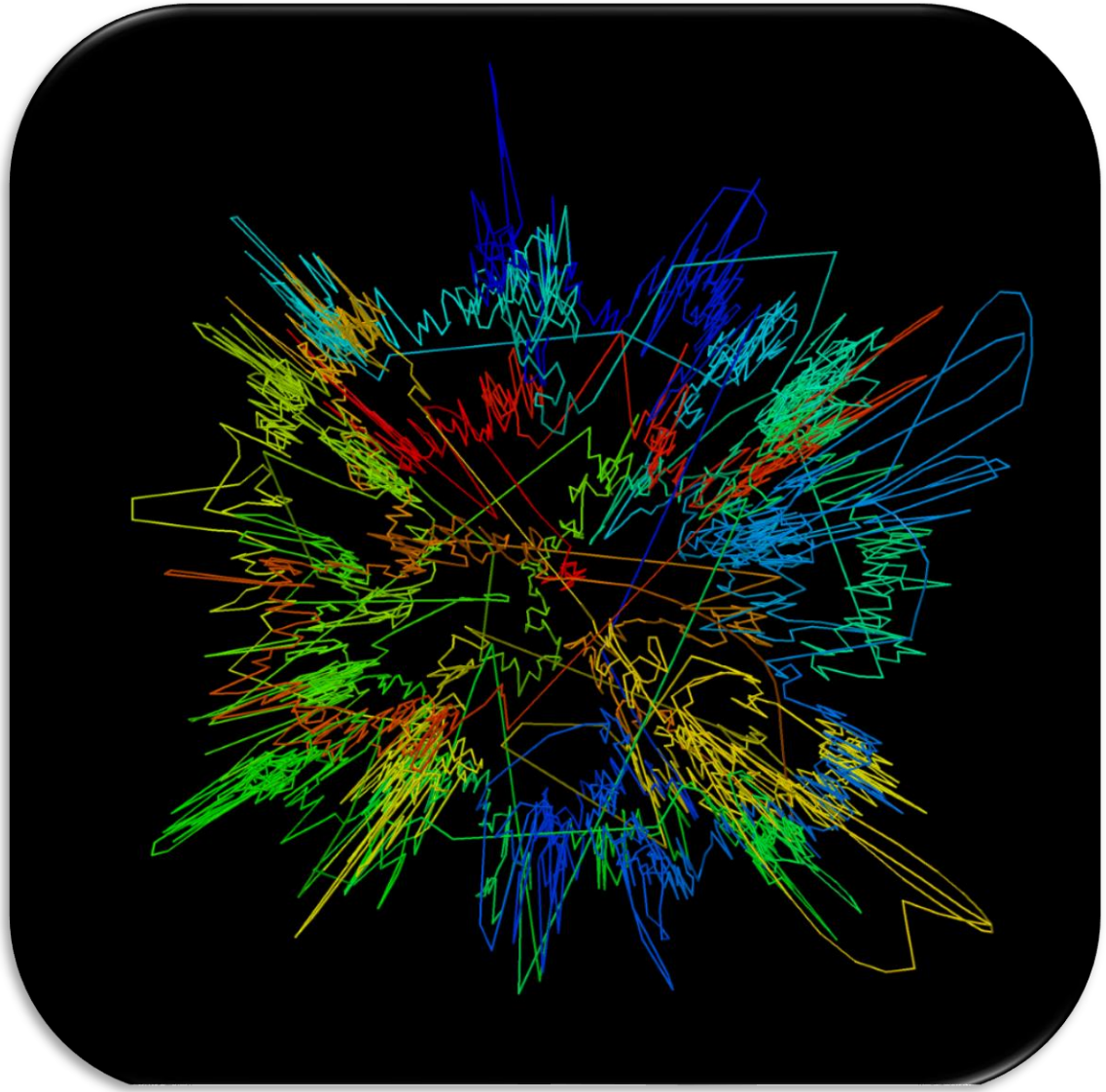
3D genome of NitabV6



- Deep sequencing (120x) carried out to explore the 3D architecture of NitabV6.
- Genome-wide and chromosome reconstruction.
- Representation of 3D organization of NitabV6.
- Each color represents the chromosomes in the genome.
- Reveals how DNA folding brings genes and regulatory elements into contact.

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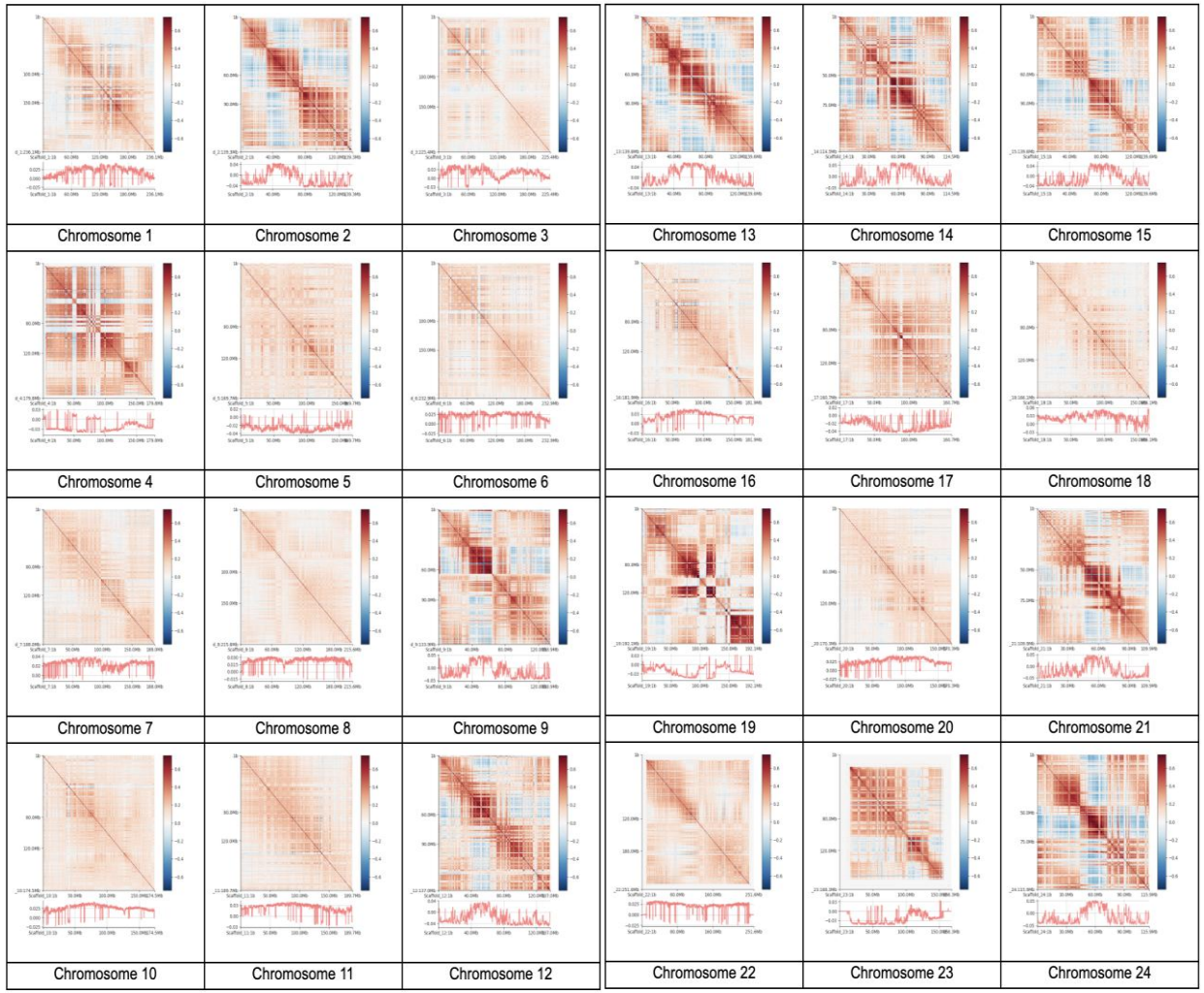
Deep Dive into NitabV6...continued



- Pearson's correlation matrix and the eigenvector's decomposition was calculated for the deep Hi-C data.
- A/B compartment studies were carried to show how the genome folds into active (A) and inactive (B) chromatin regions.
- Chromosomes 5, 6, 8, 10 and 11 were predominantly identified with active regions.
- Chromosomes 2, 4, 9, 12, 13, 14, 15, 19, 21, 23, and 24 displayed dominant regions with active (A) and inactive (B) compartments,

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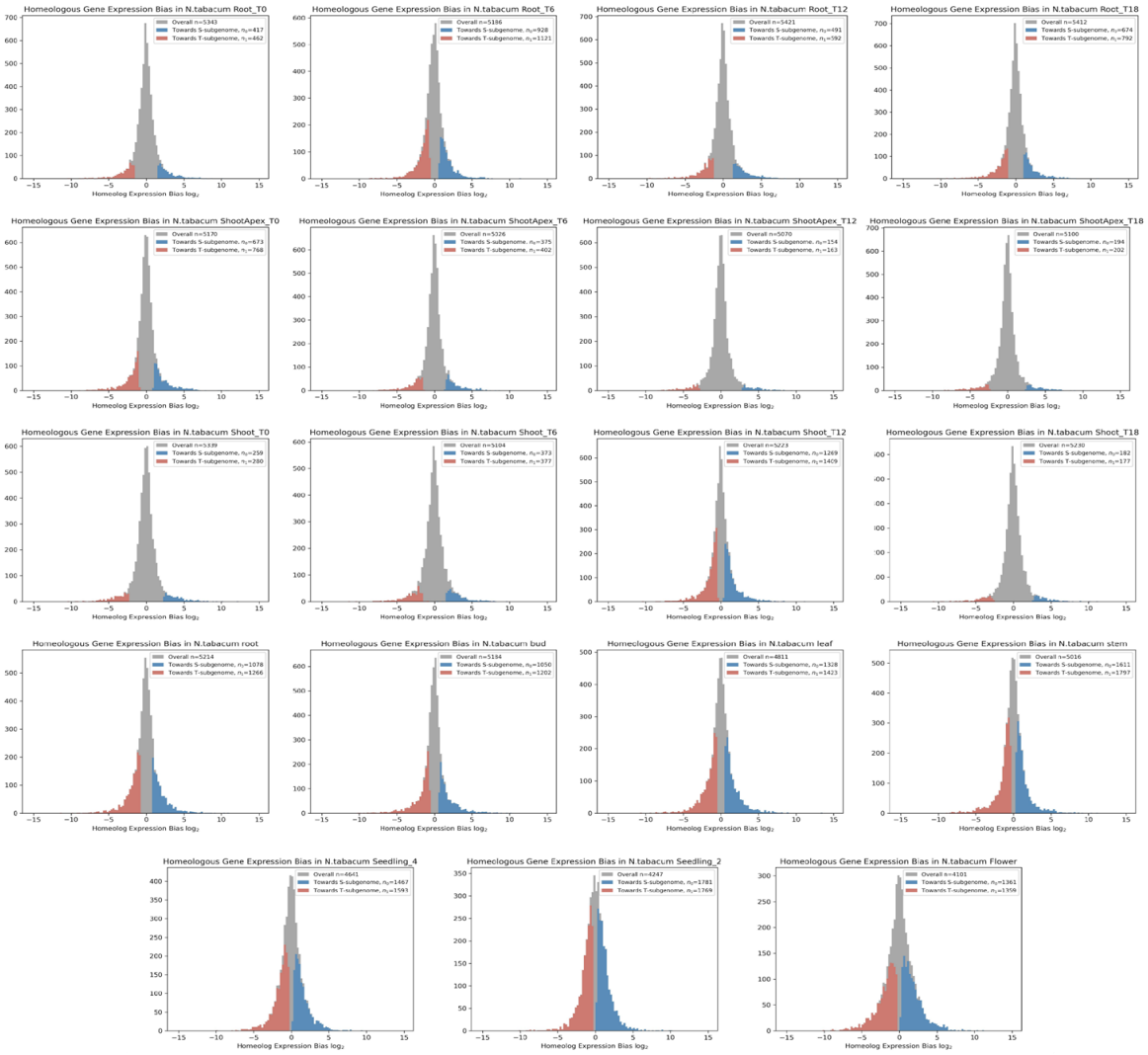
Deep Dive into NitabV6...continued



- 8,042 homoeologous gene pairs were identified and their expression profiles were assessed across 19 tissues.
- Tissues in different developmental stages, including shoot apex, shoot, root, bud, leaf, stem, seedling, and flower were utilized in the study.
- Expression bias between the S and T subgenomes was quantified using the homoeologous expression bias (HEB) framework (FDR 0.05).
- A strong T-subgenome bias was observed in 16 of 19 tissues, most notably in root, shoot apex, bud, leaf, stem.
- In contrast, three tissues flower, 2-week-old seedlings, and shoot a weaker S-subgenome bias was observed.
- S-subgenome dominates genome, T-subgenome dominates transcriptionally.
- Suggesting that **subgenome dominance** may **emerge from higher-order nuclear organization** rather than solely genetic or evolutionary factors.

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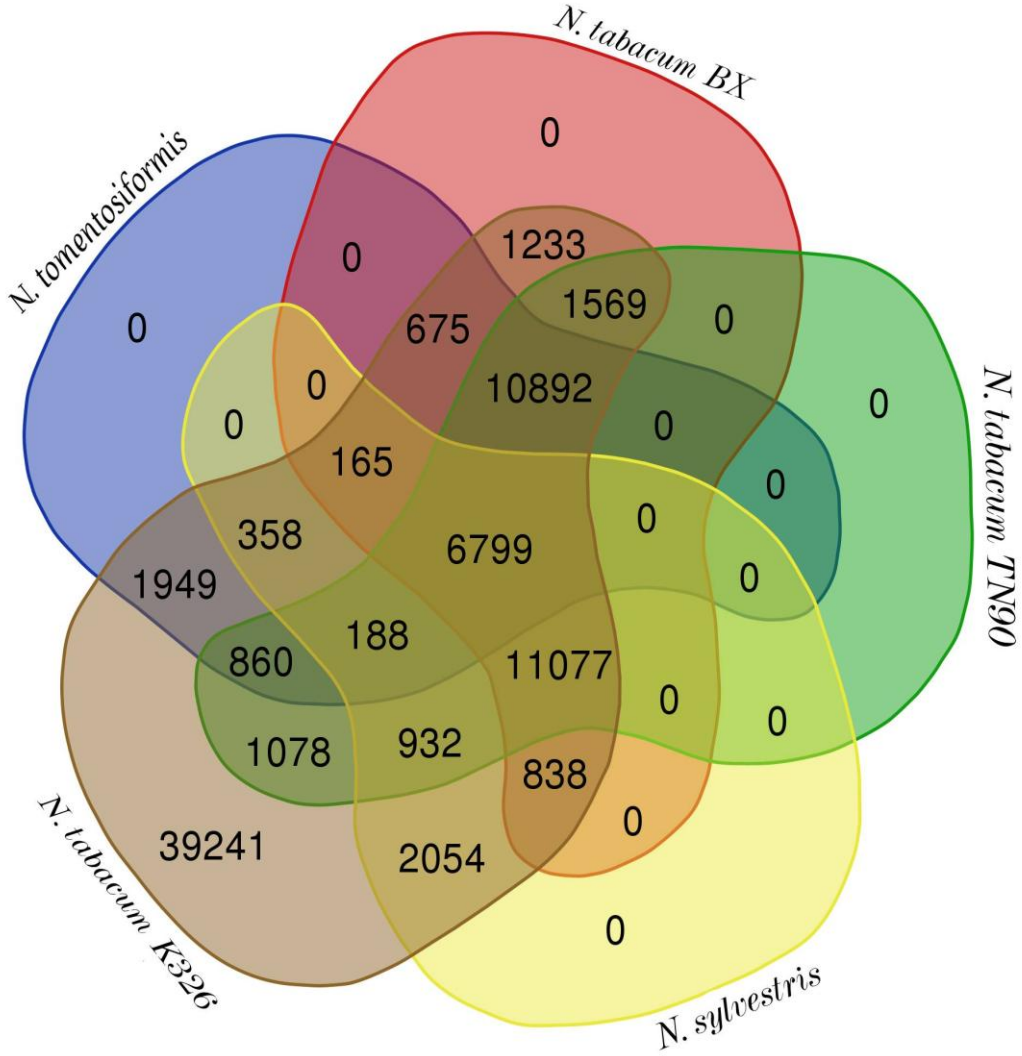
Deep Dive into NitabV6...continued



- NitabV6, *N. sylvestris, N. tomentiformis, N. tabacum* TN90 and *N. tabacum* Bx.
- The results highlighted that 93.2% of the genes were orthologous, while 6.8% remained unassigned to the any of the orthogroups.
- We observed 888 gene families to be expanded in *N. tabacum*.
- 18 gene families were found to be contracted.
- The genome shares 6,799 core genes with the other genomes (TN90, Bx, *N. tomentiformis and N. sylvestris*) with 39,241 genes unique to the plant.

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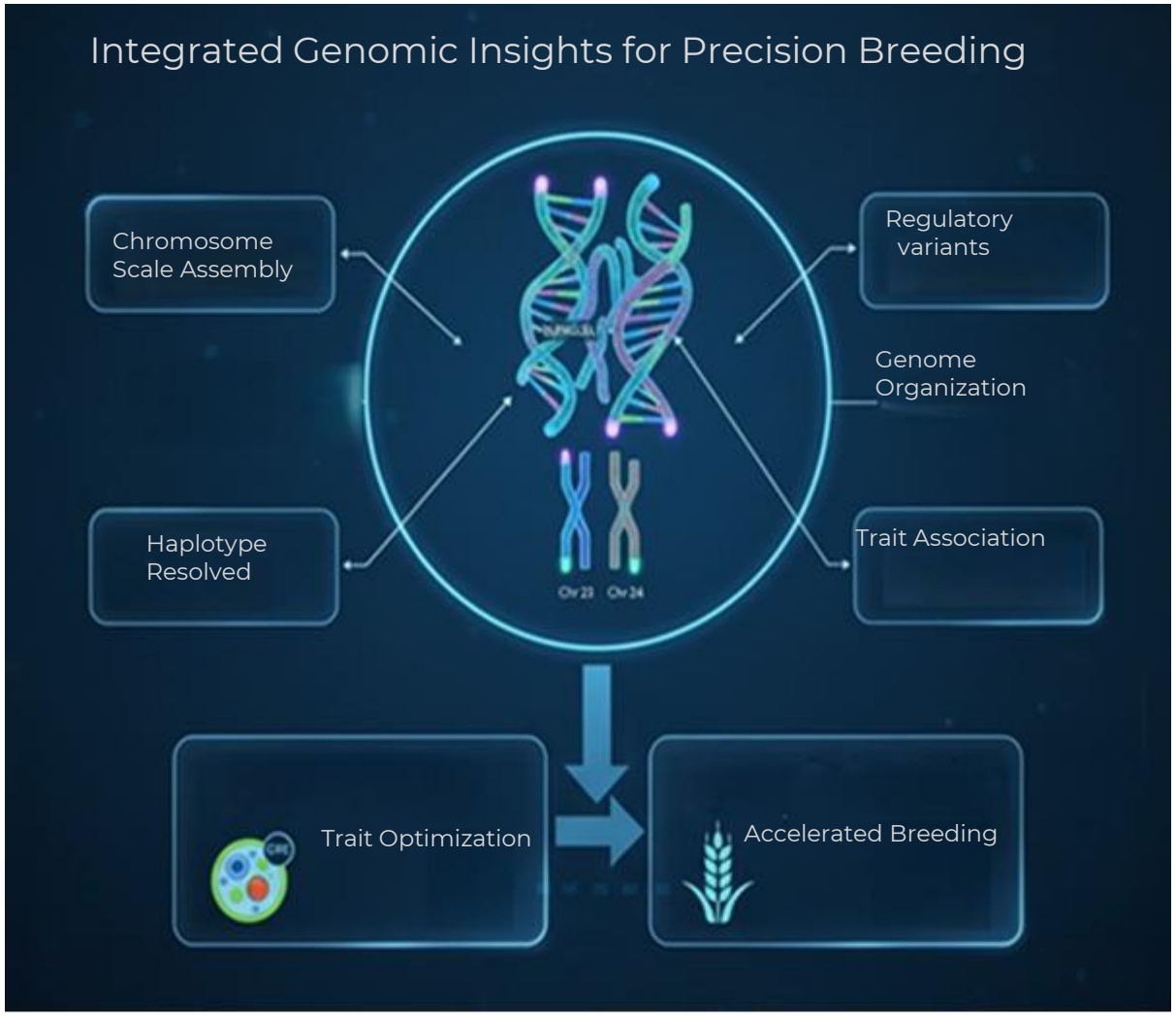
Conclusion



The chromosome-scale tobacco genome can accelerate plant breeding by powering marker-assisted pipelines and predictive trait selection, directly addressing industry demands for quality, climate resilience, and sustainability.

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The Team

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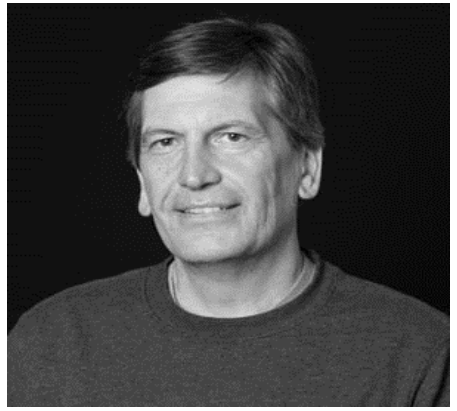

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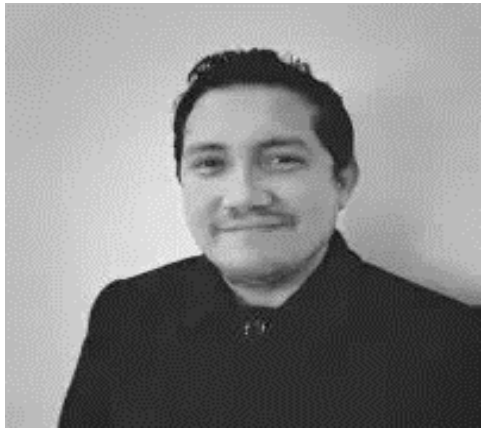

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