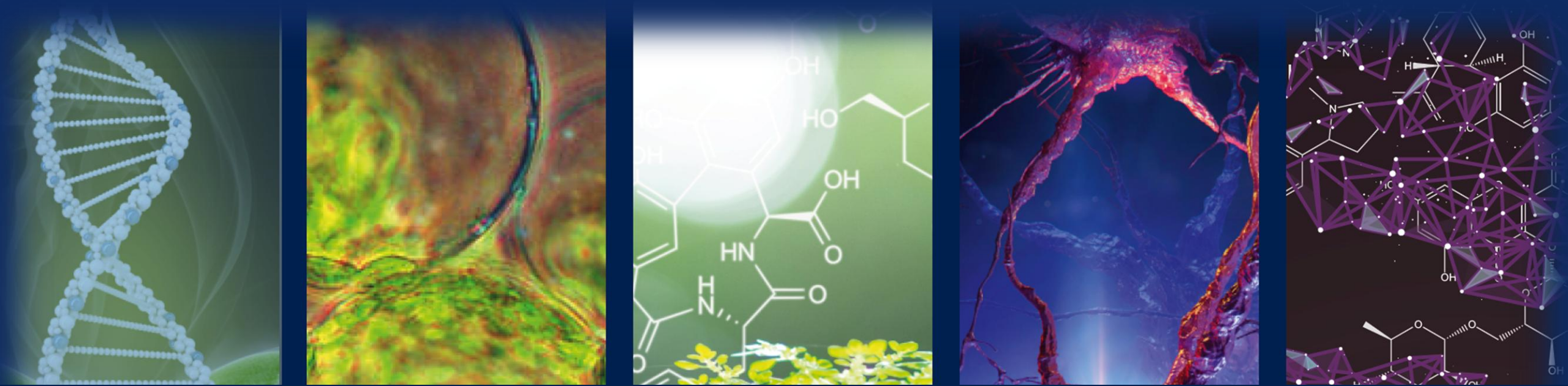




Unlocking the complex genome of *Nicotiana tabacum*



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AGENDA

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Why Tobacco?

02

Current Statistics of Tobacco Genome

03

What We Lack?

04

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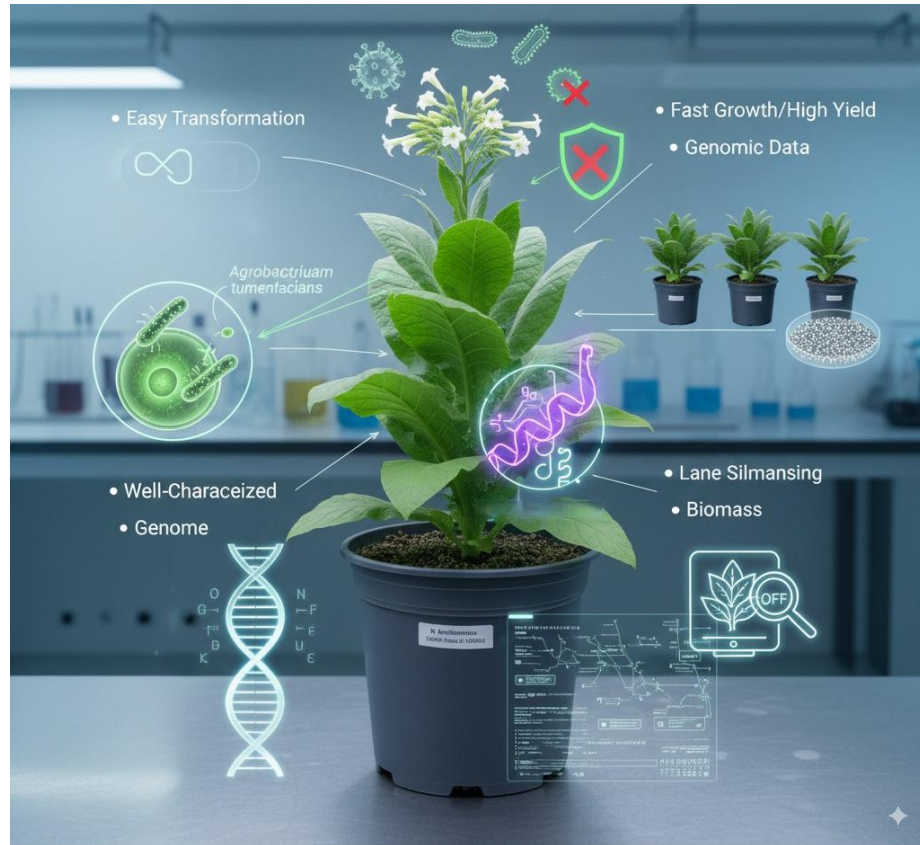
NitabV6 genome

06

Conclusion

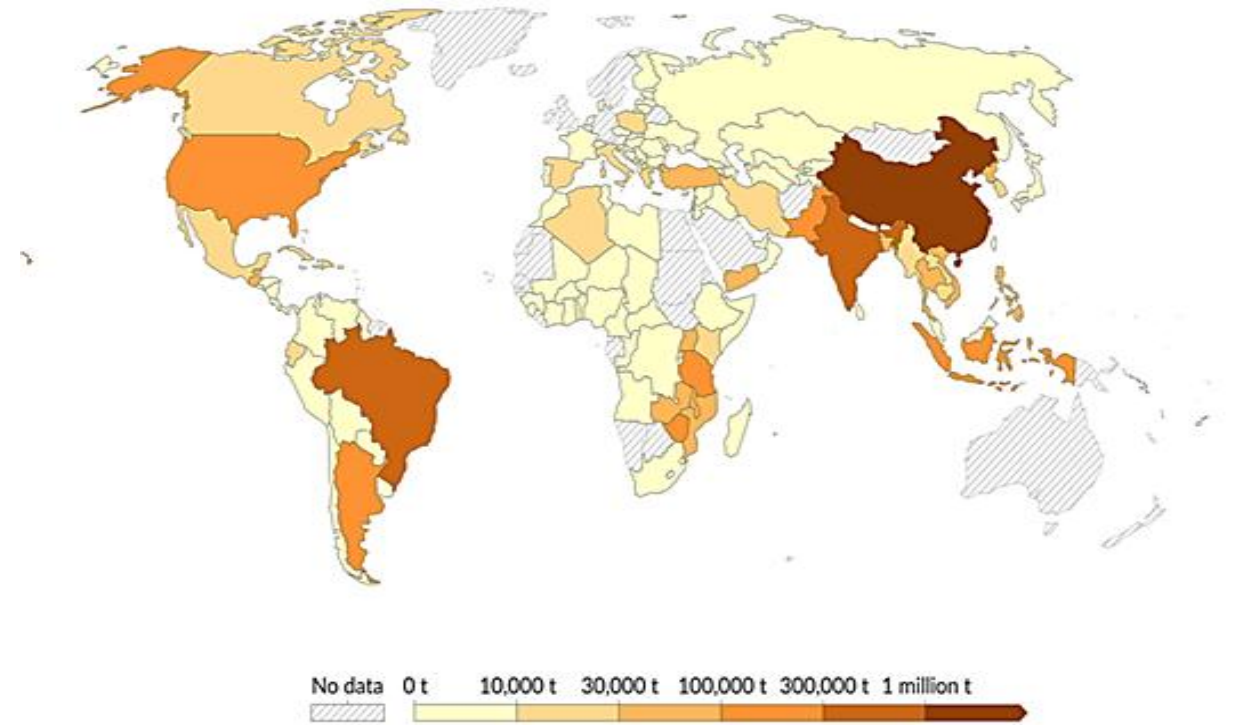
Why Tobacco?

Nicotiana tabacum as a Model Plant



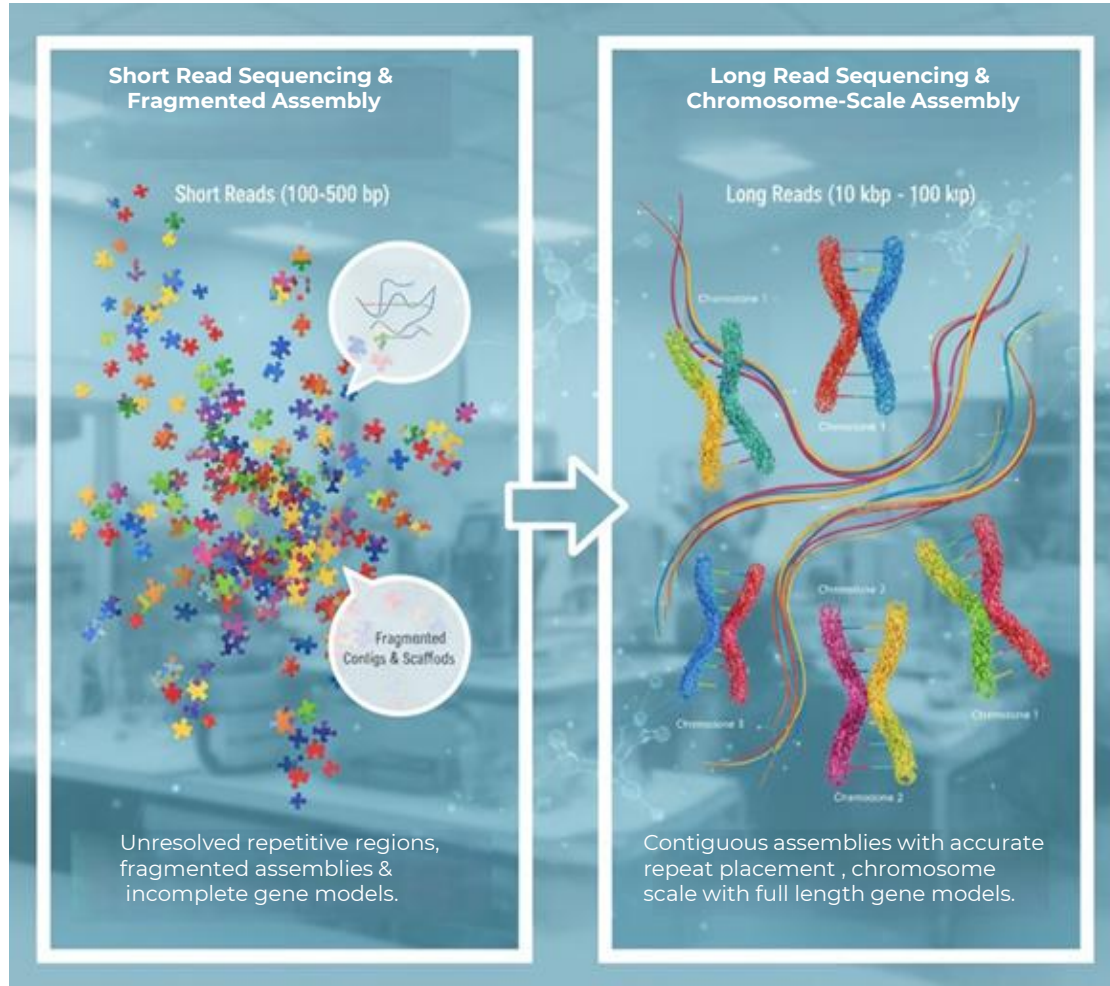
Dependence Worldwide

Tobacco production, 2023

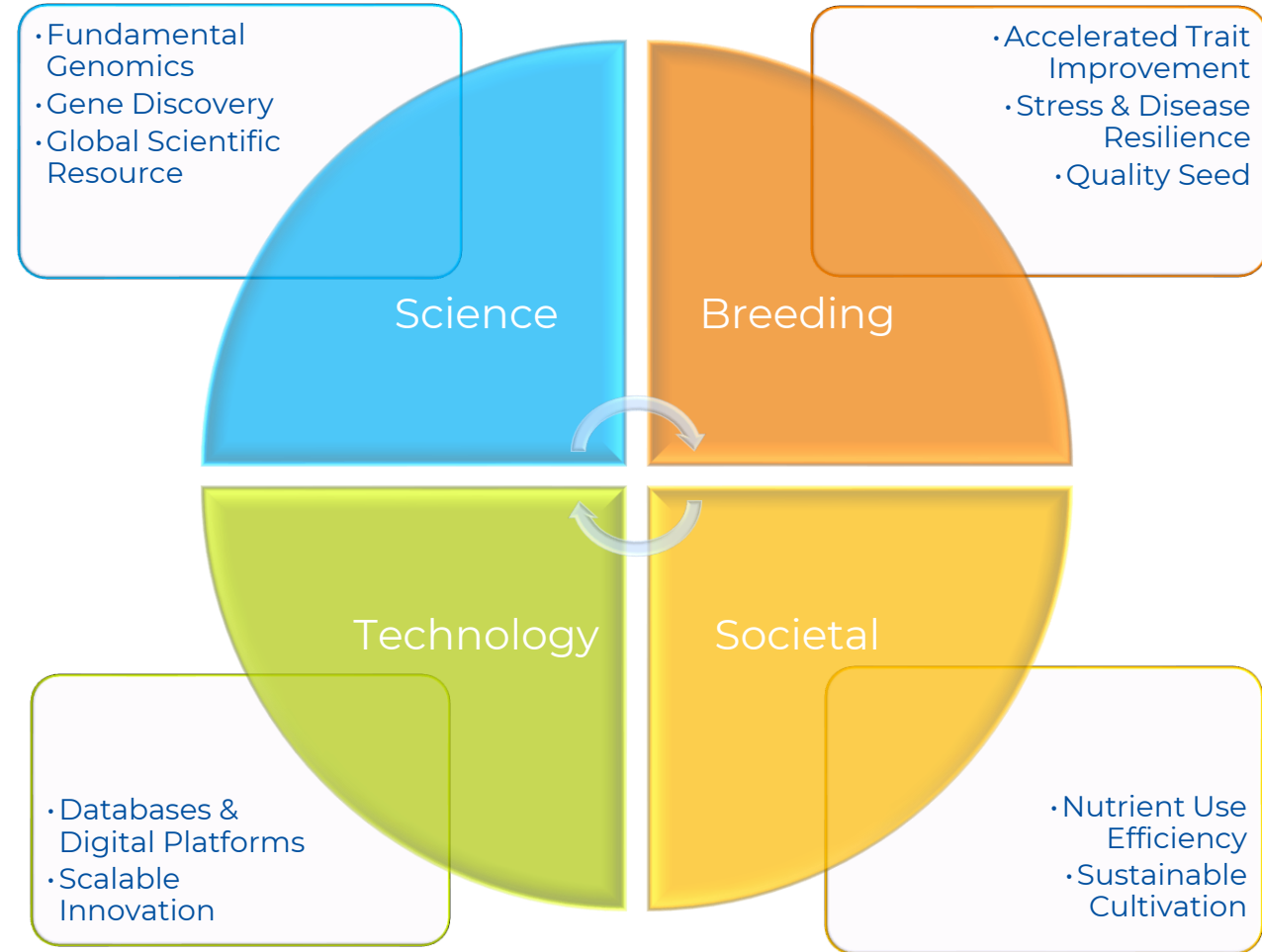


Production measured in tonnes, Source Food and Agriculture Organisation of United States 2023.

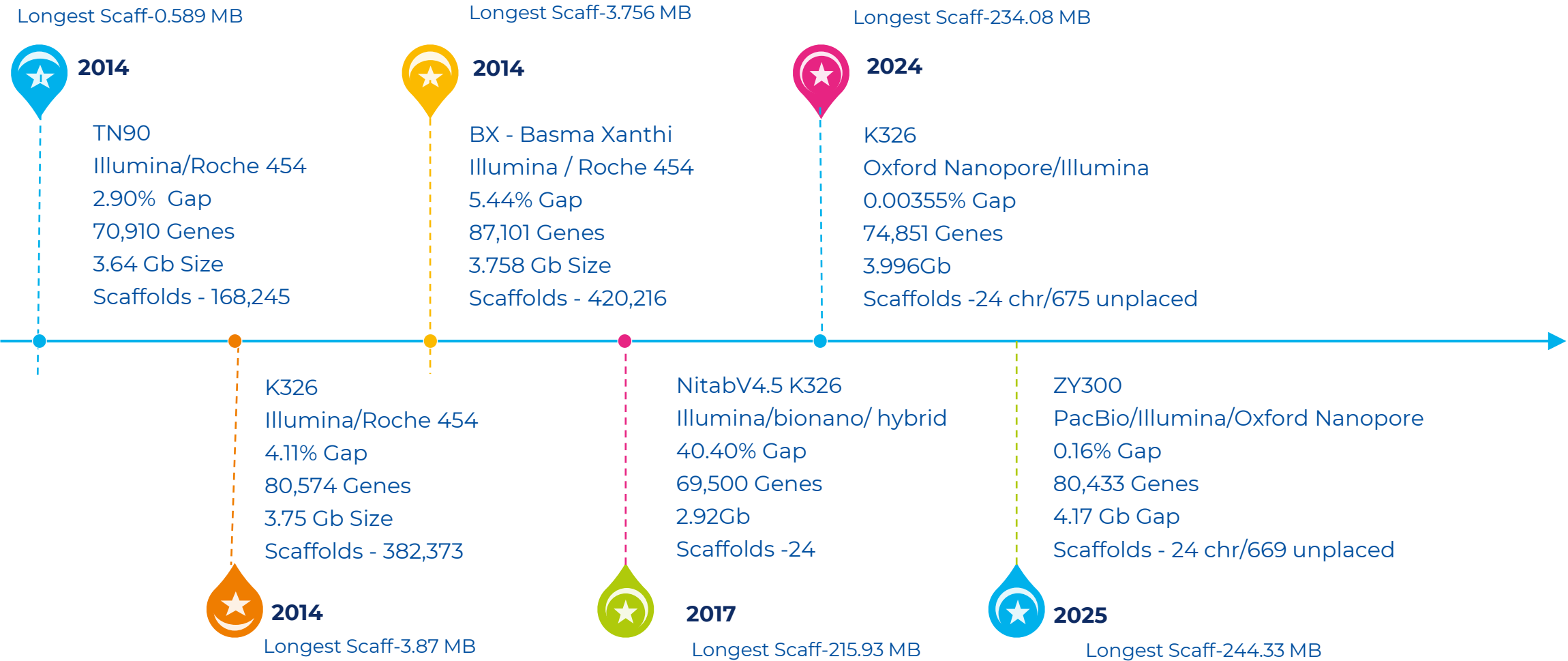
Why Now?



The Impact To Community

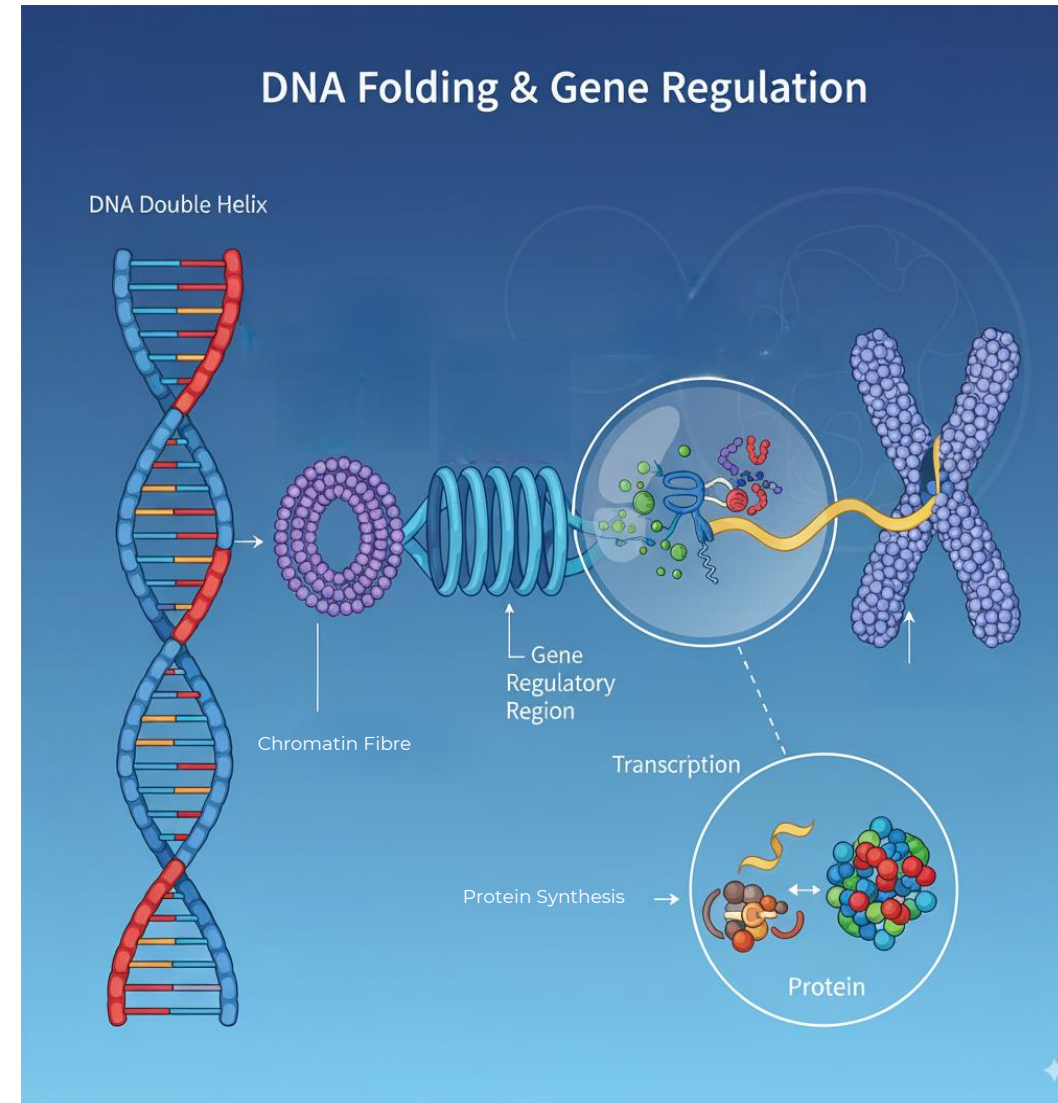


Current Statistics of Tobacco Genome



What is missing?

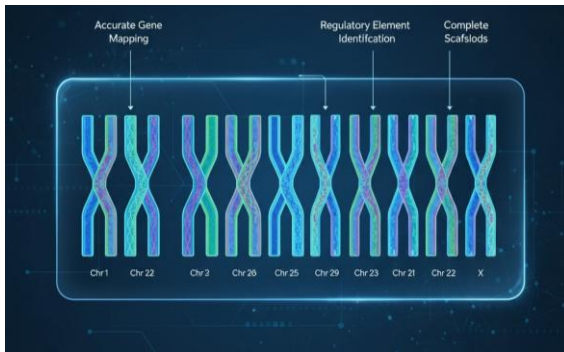
- Despite decades of research, tobacco lacked a **highly contiguous** reference genome due to its polyploidy and repetitive content.
- Understanding the **3D genome** structure is crucial for deciphering gene regulation and advancing precision breeding in response to climate change.
- A **Haplotype resolved**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



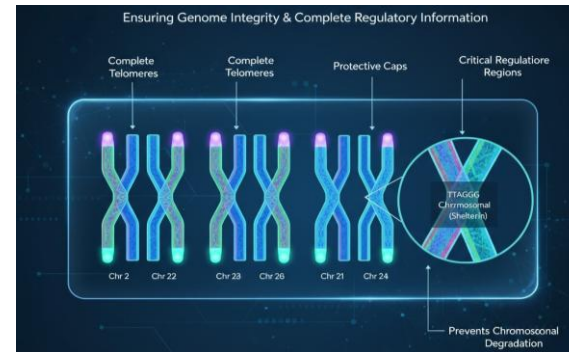
Our Aim

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

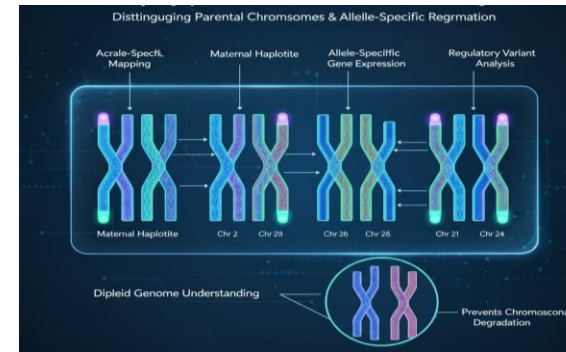
Chromosome Scale



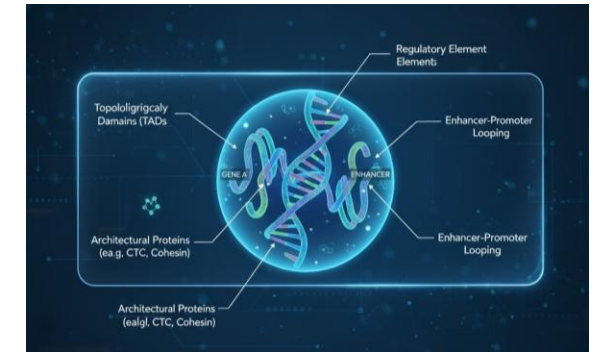
Telomere to Telomere



Haplotype resolved

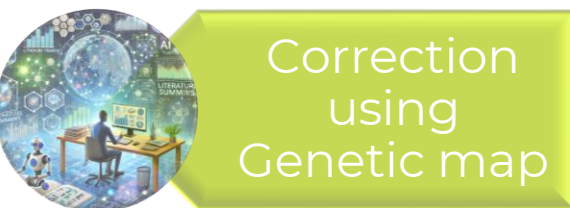
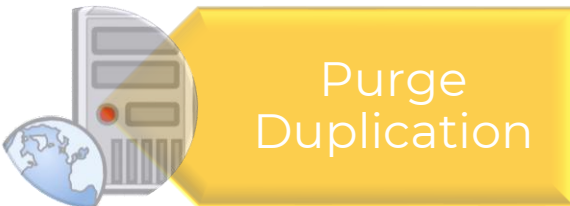


3D genome



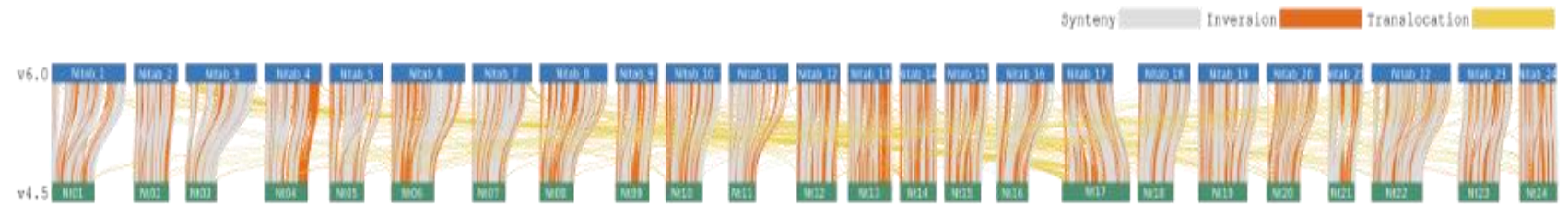
The new *N. tabacum* NitabV6

Our Pipeline



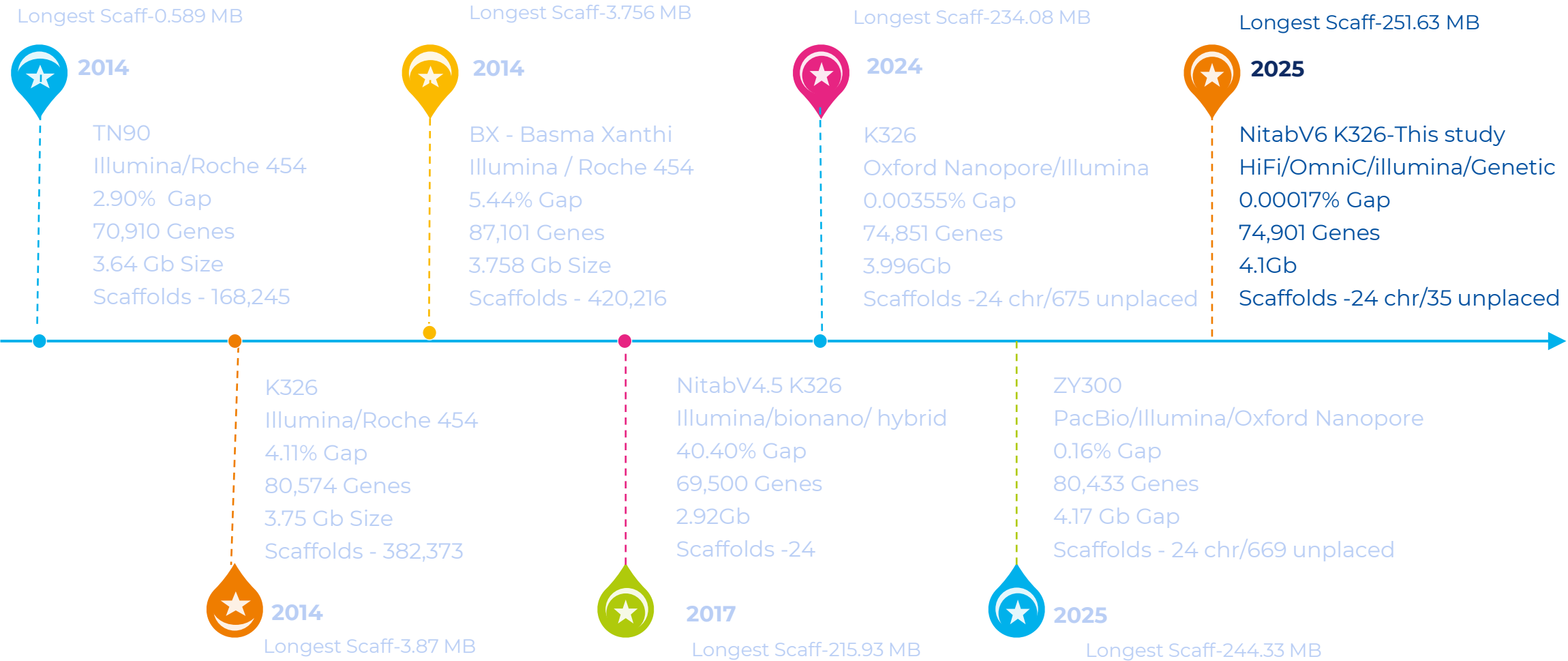
Genome Statistics

- 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.

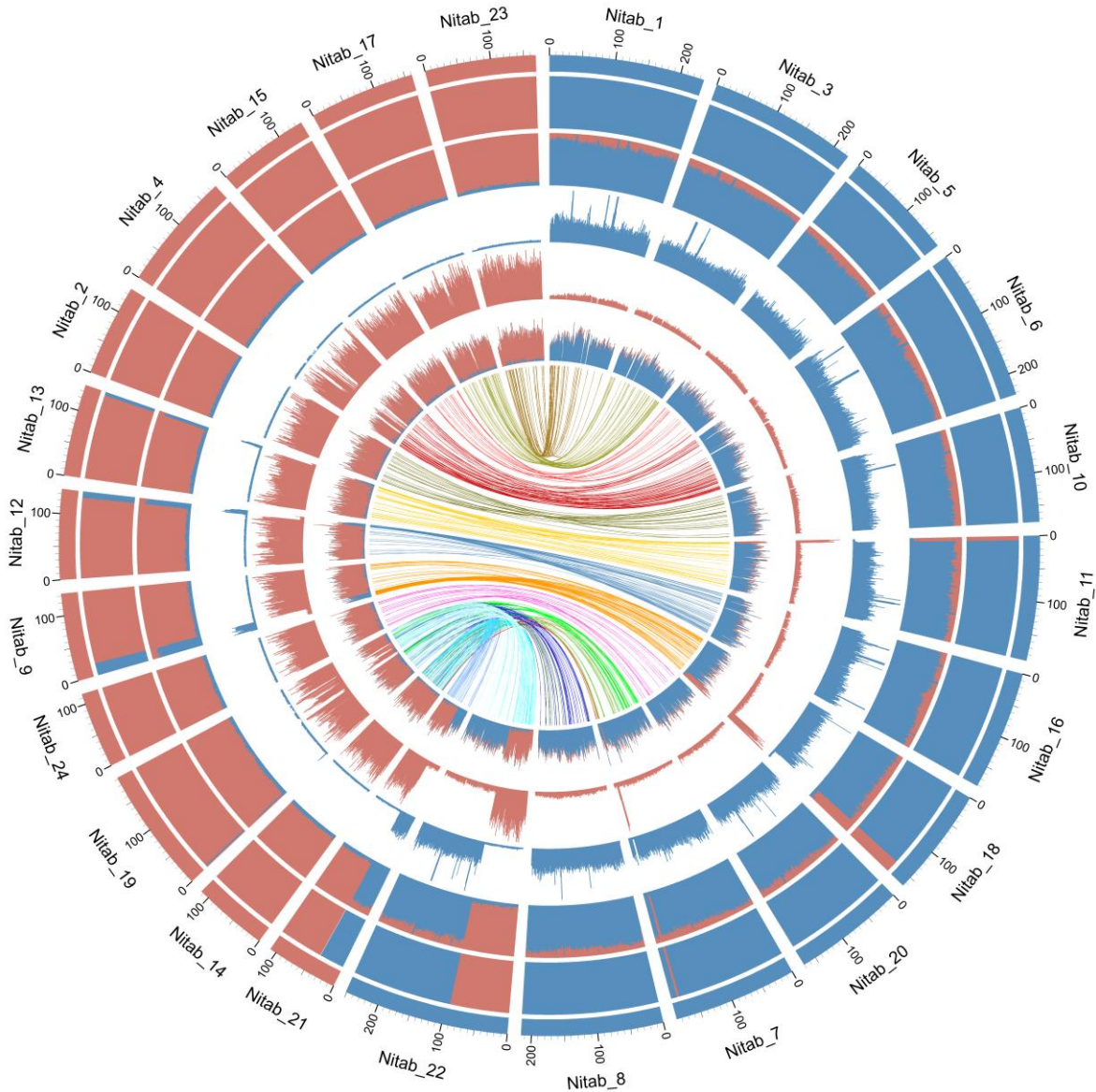


Comparison of our previous V4.5 genome version (2017) to the current NitabV6 (2025)

Current Statistics of Tobacco Genome

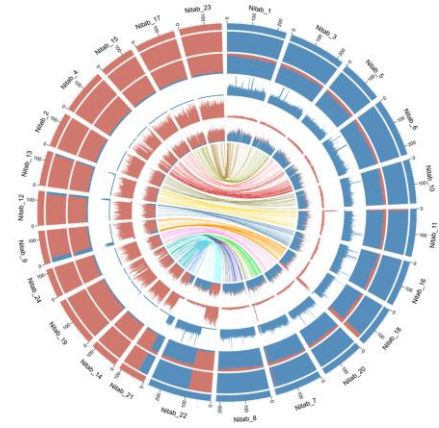
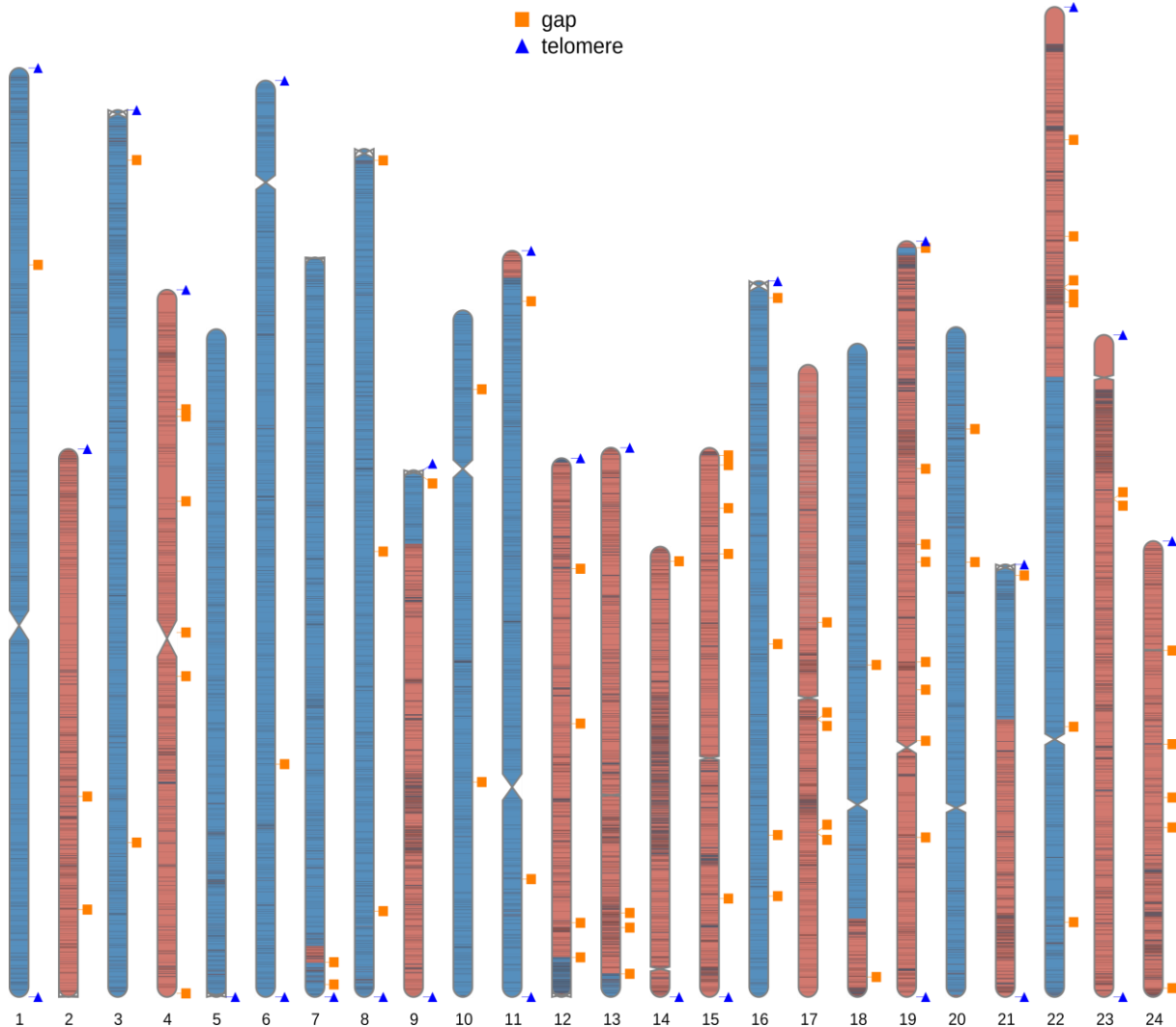


Deep Dive into NitabV6



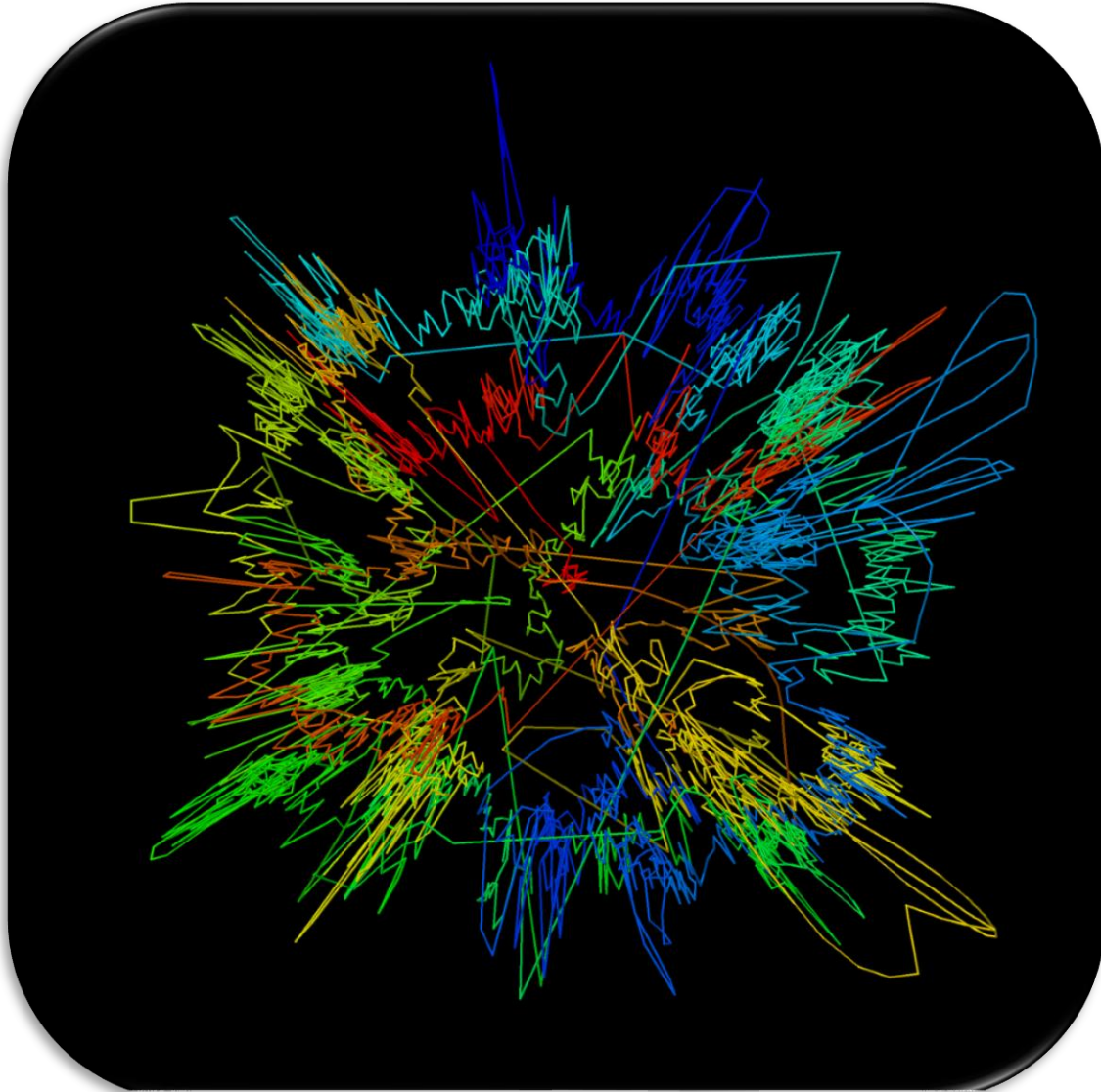
- Circus plot showing NitabV6 assignments with S-subgenome and T-subgenome.
- Approximately 57.4% and 40.9% of *N. tabacum*'s genome originated from *N. sylvestris* and *N. tomentosiformis* respectively.
- stretch of T-subgenome at the tail of Chr11's left arm and Chr07 and Chr18's right arm
- S-subgenome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

Deep Dive into NitabV6



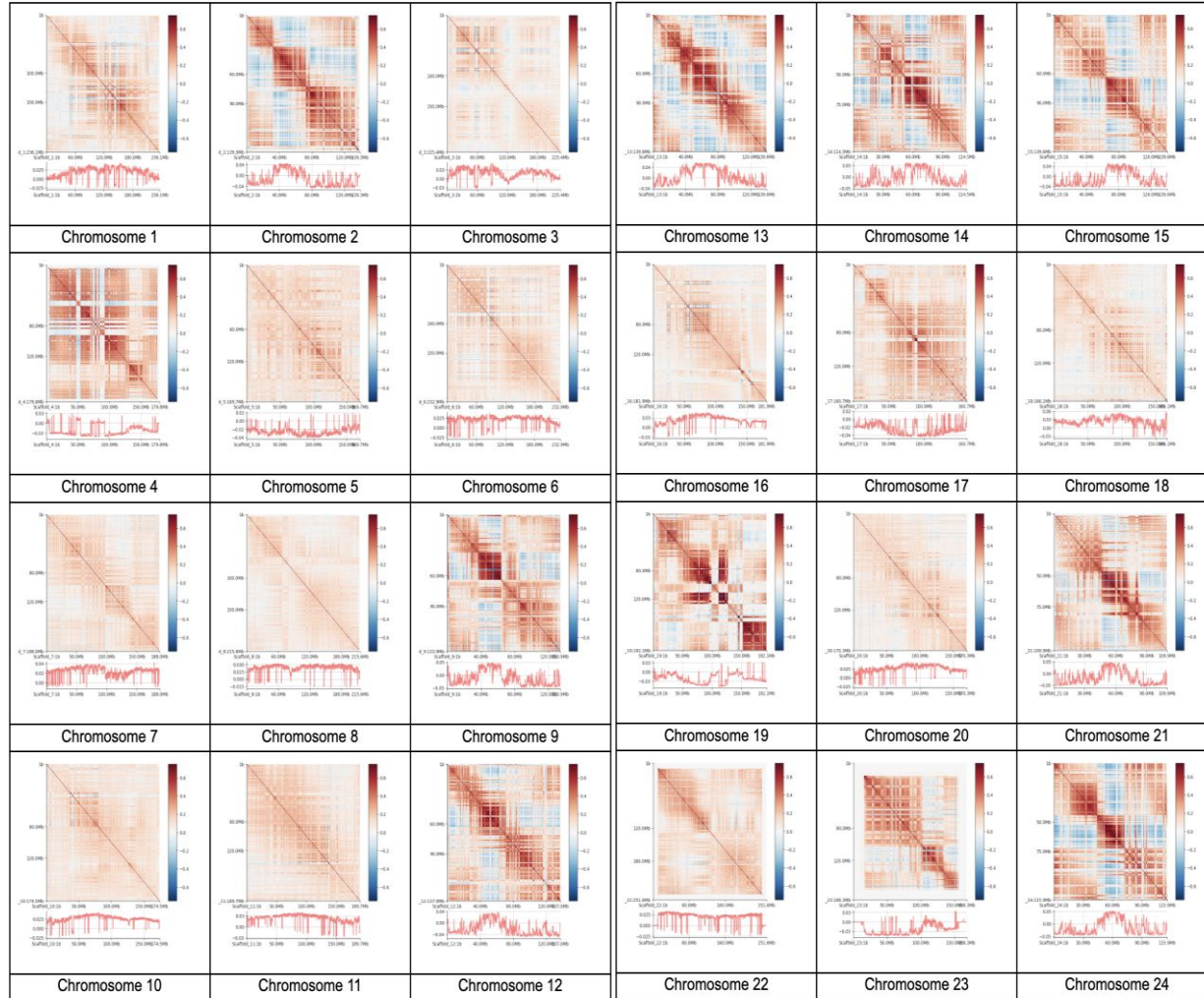
- 12chr with telomeres in one end.
- 4 chr with no telomeric regions.
- 15 candidate centromeres were identified.
- 5 intact chromosomes having telomere ends and centromere candidates.

3D genome of NitabV6



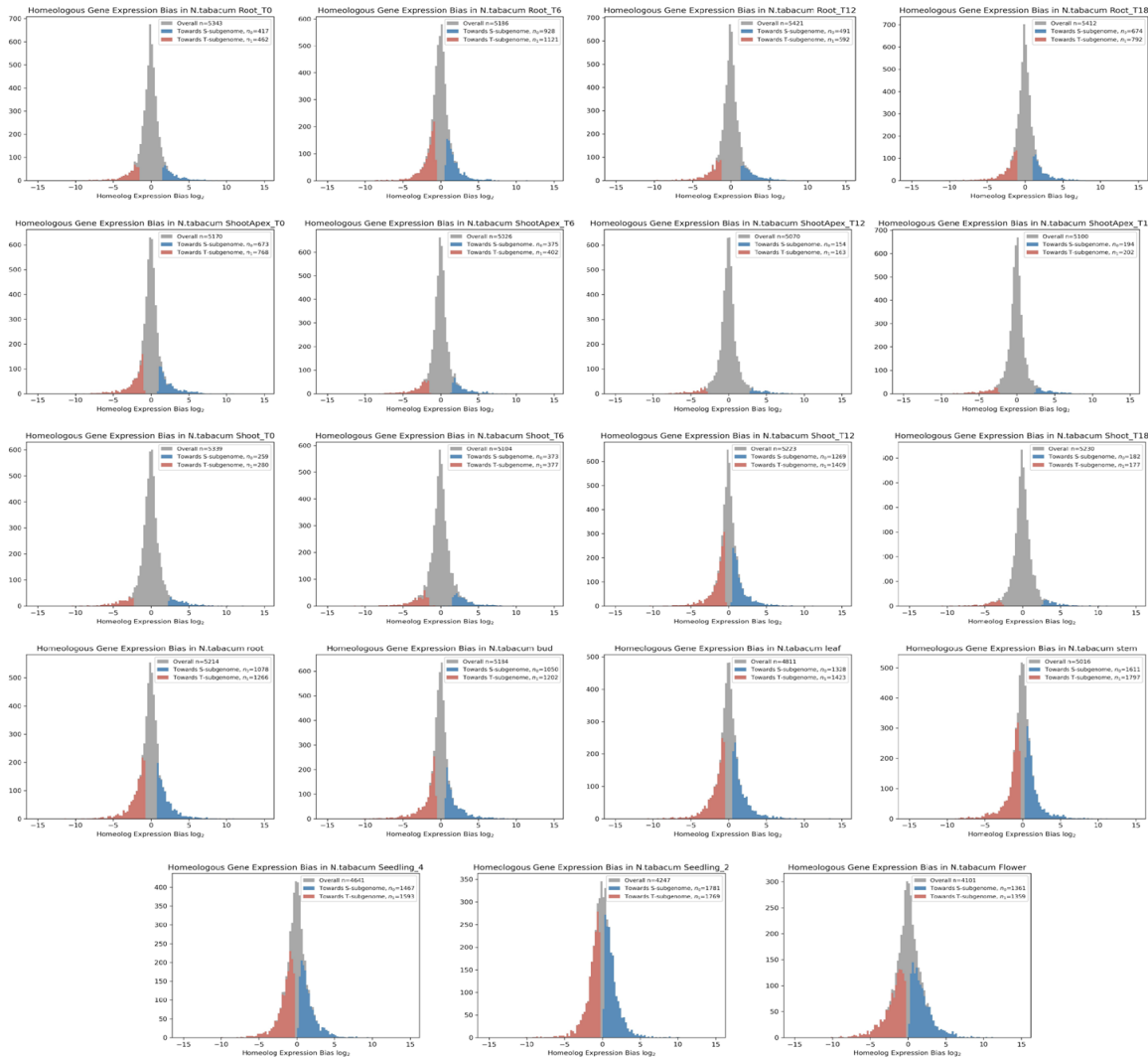
- Deep sequencing (120x) carried out to explore the 3D architecture of NitabV6.
- Genome-wide and chromosome reconstruction.
- Representation of 3D organization of NitabV6.
- Each color represents the chromosomes in the genome.
- Reveals how DNA folding brings genes and regulatory elements into contact.

Deep Dive into NitabV6...continued



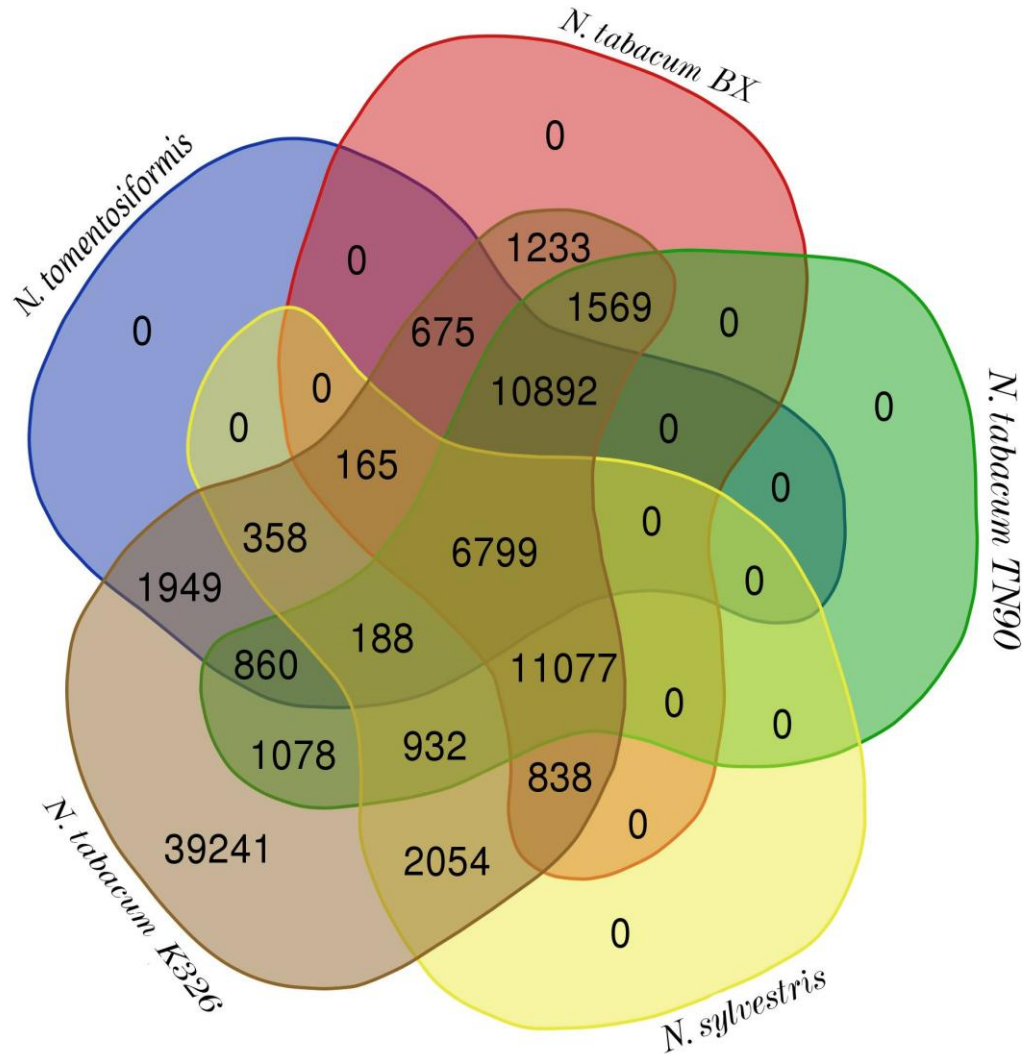
- Pearson's correlation matrix and the eigenvector's decomposition was calculated for the deep Hi-C data.
- A/B compartment studies were carried to show how the genome folds into active (A) and inactive (B) chromatin regions.
- Chromosomes 5, 6, 8, 10 and 11 were predominantly identified with active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

Deep Dive into NitabV6...continued



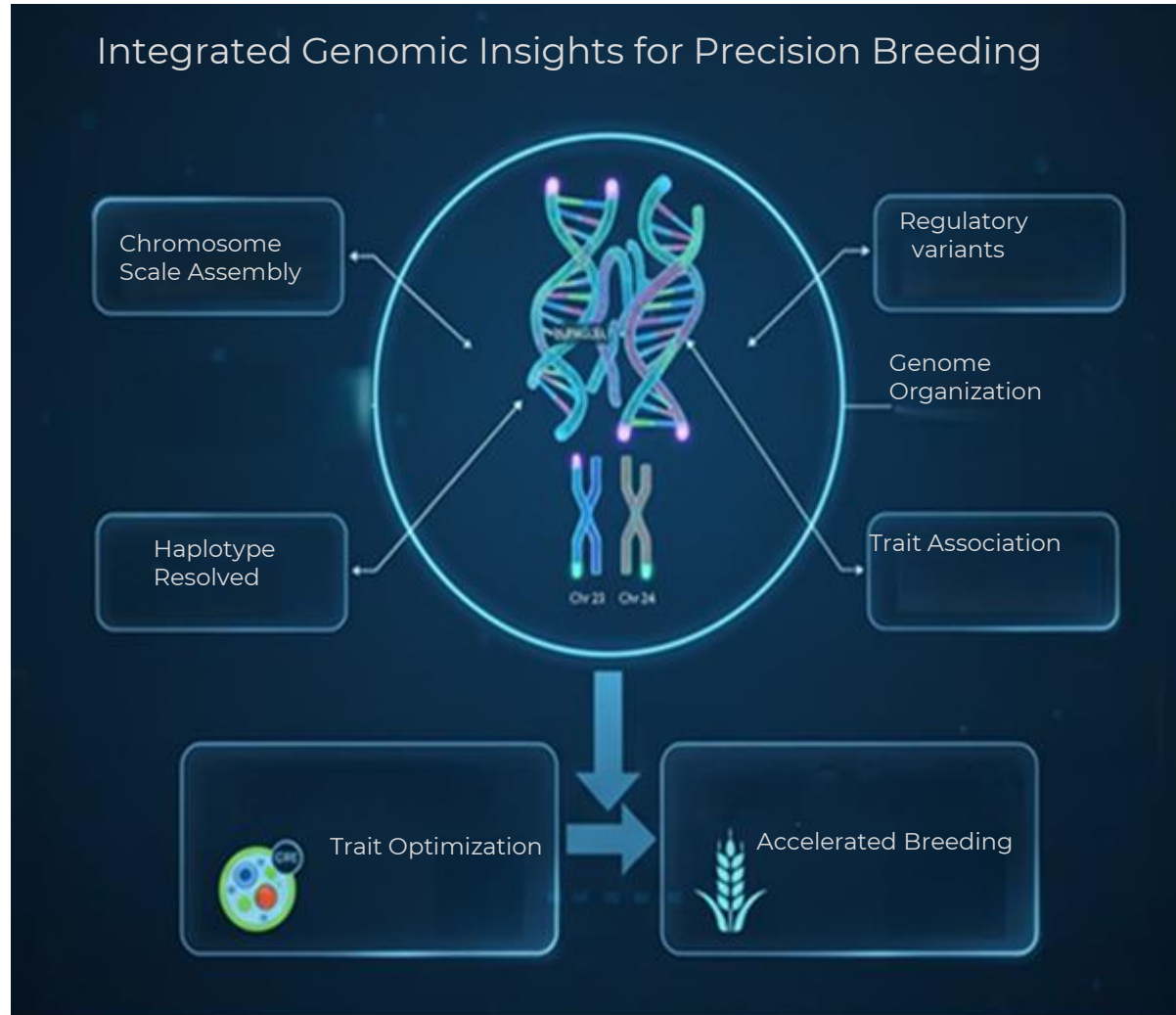
- 8,042 homoeologous gene pairs were identified and their expression profiles were assessed across 19 tissues.
- Tissues in different developmental stages, including shoot apex, shoot, root, bud, leaf, stem, seedling, and flower were utilized in the study.
- Expression bias between the S and T subgenomes 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.

Deep Dive into NitabV6...continued



- NitabV6, *N. sylvestris*, *N. tomentosiformis*, *N. tabacum* TN90 and *N. tabacum* Bx.
- The results highlighted that 93.2% of the genes were orthologous, while 6.8% remained unassigned to 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. tomentosiformis* and *N. sylvestris*) with 39,241 genes unique to the plant.

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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Thank you