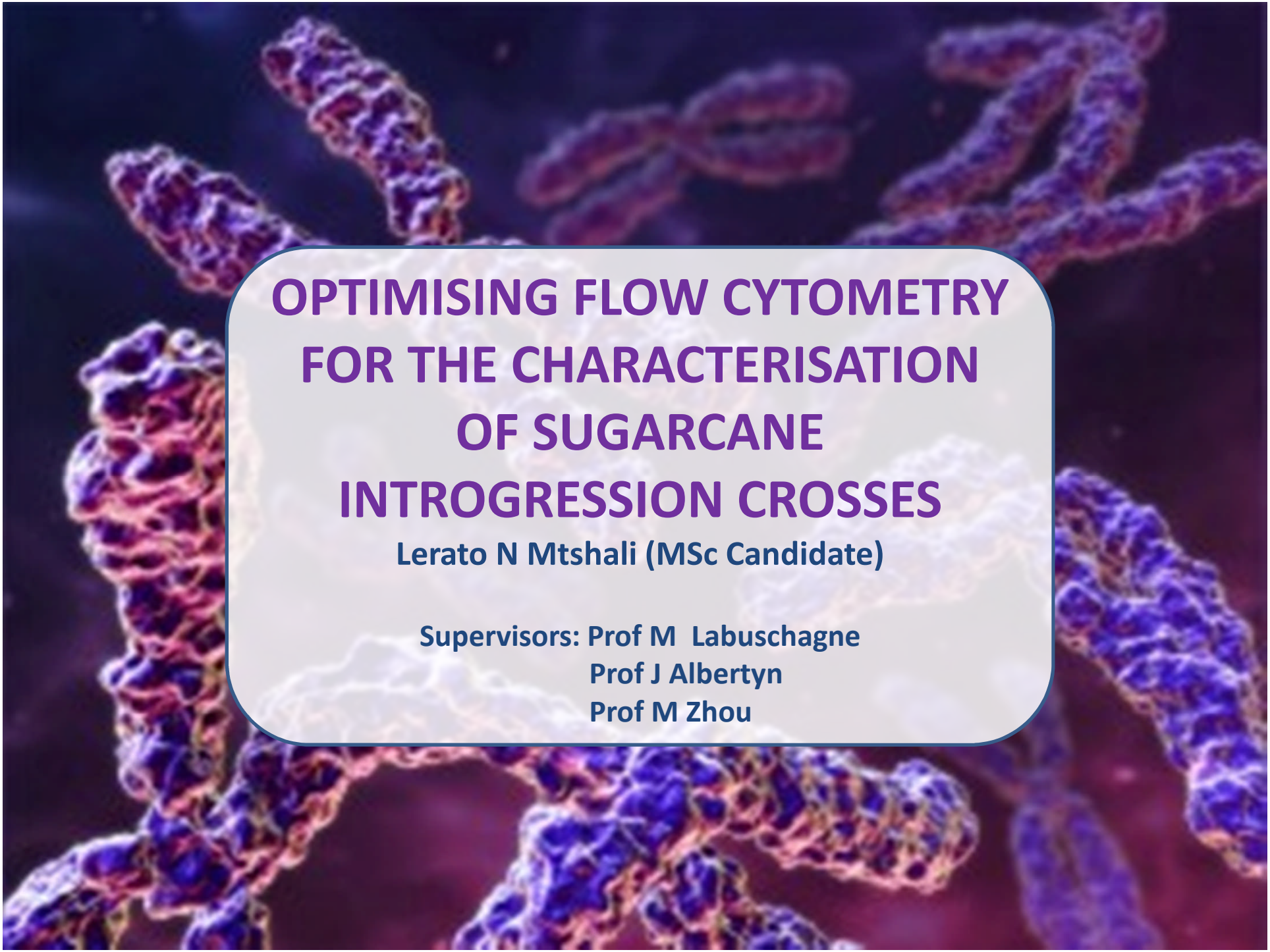
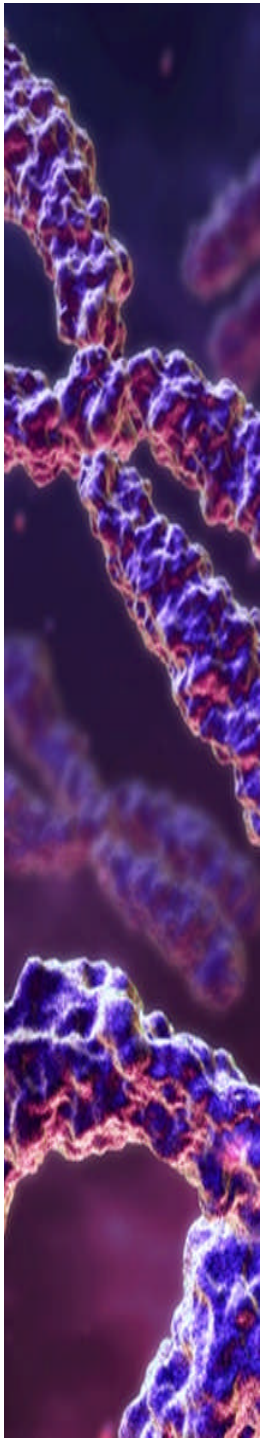


OPTIMISING FLOW CYTOMETRY FOR THE CHARACTERISATION OF SUGARCANE INTROGRESSION CROSSES

Lerato N Mtshali (MSc Candidate)

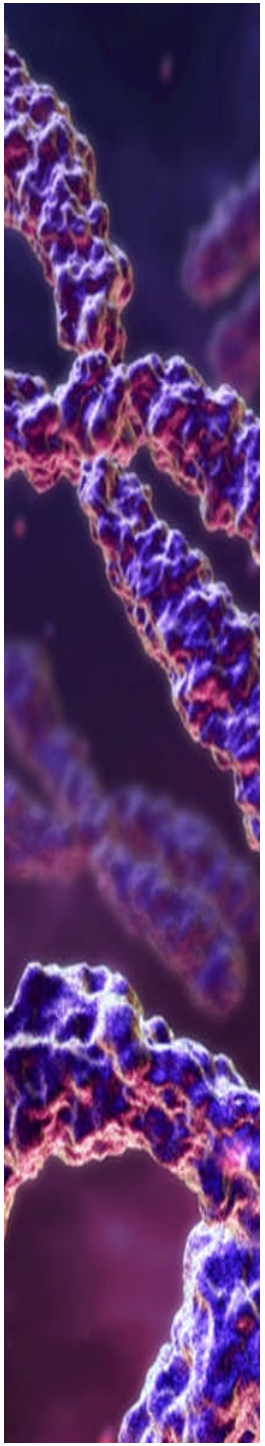
Supervisors: Prof M Labuschagne
Prof J Albertyn
Prof M Zhou



Introduction

- Commercial varieties: *S. officinarum* X *S. spontaneum*
- Limited clones of parental genotypes in initial hybridisation
  - Narrowing genetic base
- Introgression breeding
  - Widen the genetic base
  - Introduce novel traits

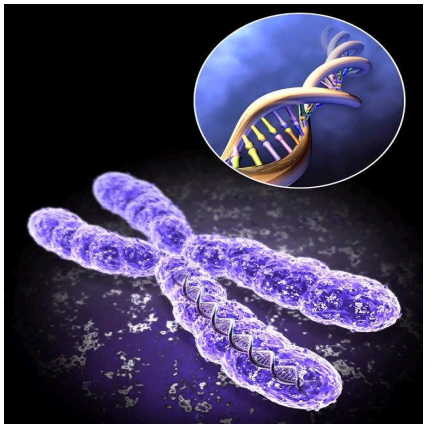




Introduction

- Knowledge of sugarcane cytogenetics can enhance introgression

- **Cytogenetics:** Is the branch of biology that deals with heredity and the structure and function of cellular components, particularly chromosomes



Introgression breeding

Introgression challenges

- Sterility
- Chromosome transmission

S. Officinarum or Cultivar **x** ** *Erianthus* or * *S. spontaneum*

Cultivar + F1 hybrid

Cultivar + BC1 hybrid (first back-cross)

Cultivar + BC2 hybrid (second back-cross)

Cultivar + BC3 hybrid (third back-cross)

SELECTION (phenotypic & molecular)

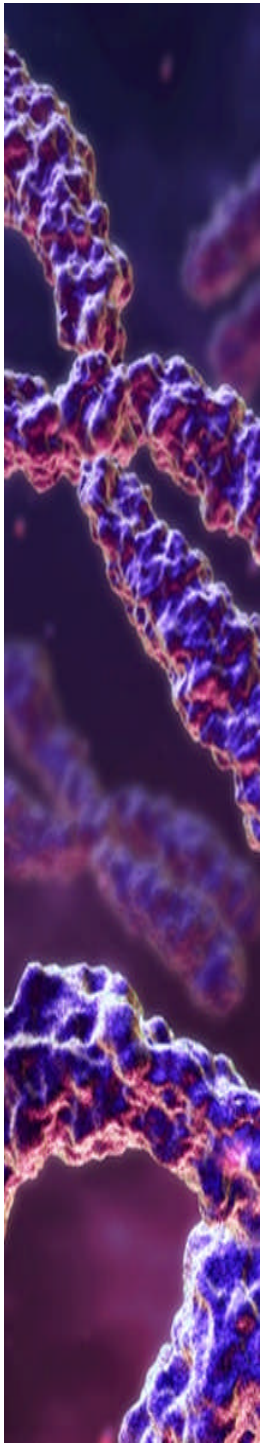
Improved genotype to feed into commercial breeding programme

(source of parents for crossing)



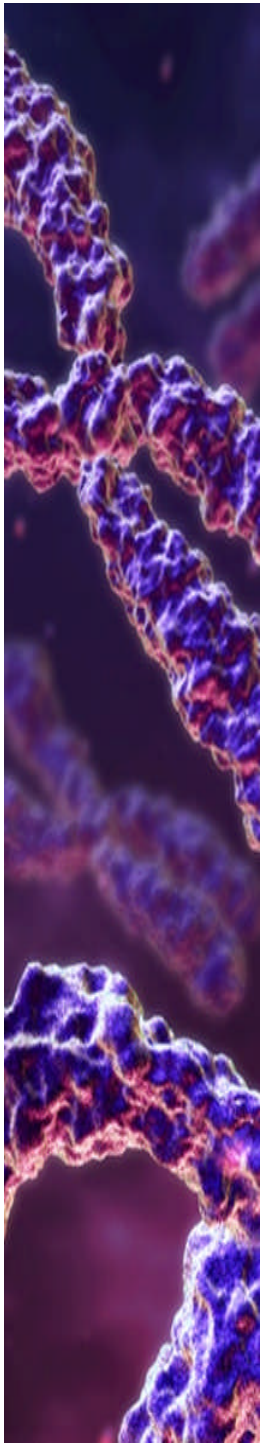
****Erianthus***: high fibre, high biomass, tolerance to drought and water logging conditions, pest and disease resistance

**S. spontaneum*: disease resistance, tolerance to drought conditions



Flow cytometry

- Cytogenetic technique
- Popular for:
  - Hybrid verification
  - Ploidy screening
  - Cell cycle analysis
  - Estimation of nuclear DNA content
  - Genome size
- Most studies focused on animal cells, few on plant cells and even less on sugarcane



Objectives

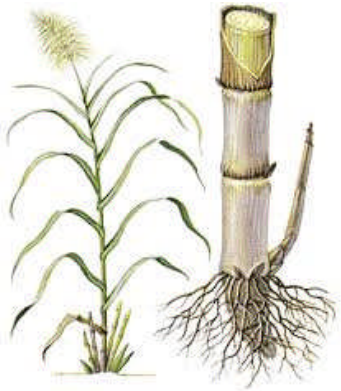
- Optimise tissue source, flow rates and nuclear counts for accurate sugarcane characterisation
- Determine sugarcane DNA content, chromosome numbers and DNA ploidy levels
- Predict progeny chromosome numbers from parental data
- Validate prediction model amongst progeny

Optimisation

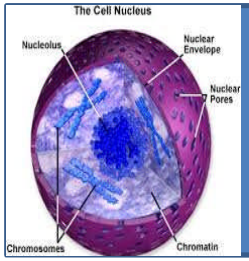
6 varieties

- 2 wild species (*S. spont* and *S. off*)
- 4 commercial type varieties

Tissue source

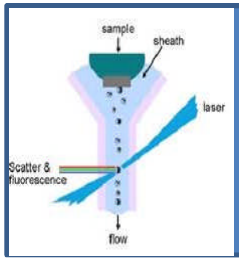


Nuclear count

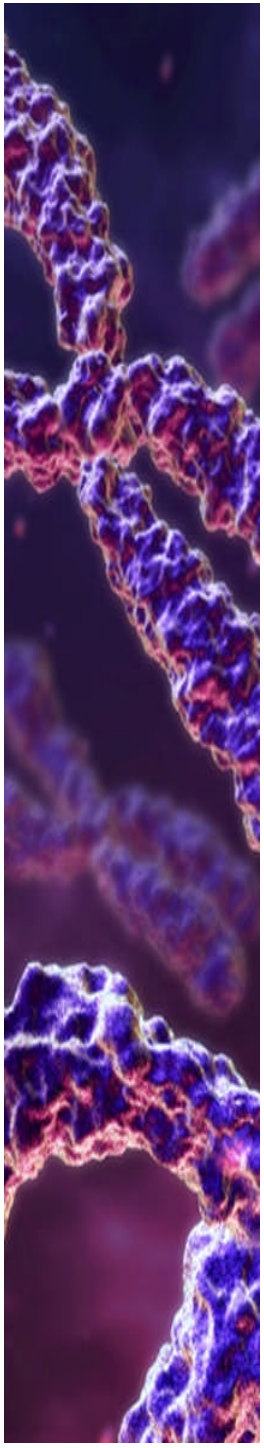


- 1000
- 1500

Flow rates (µl/sec)



- 0.2
- 0.5
- 0.8
- 2.0



Sugarcane characterisation

DNA content and chromosome number determination

Saccharum spp. interspecific hybrids used in the study

Progeny	Parent	
	Female	Male
VV0164 (BC_1)	04X0018 (F_1)	CB3839 (Comm)
VV0105 (BC_1)	04X0022 (F_1)	CB3839 (Comm)
WW0857 (BC_1)	04X0026 (F_1)	04K0993 (Comm)
VV0185 (F2)	04X0027 (F_1)	04X0022 (F_1)
VV0842 (BC_1)	04X0027 (F_1)	N48 (Comm)
VV0535 (BC_1)	04X0033 (F_1)	05U0114 (Comm)
WW0175 (BC2)	WI82498 (BC_1)	04G0031 (Comm)

- Two progenies selected per cross
- Internal standards:
  - *Zea mays* CE-777 (DNA content = 5.43 pg)
  - *S. officinarum* Black Cheribon(2n = 80, x = 8)

Methodology

Sample Preparation



Sampled leaf tissue



Nuclear extraction by chopping



Filtration and staining using DAPI



Analysis by flow cytometry

Data analysis



Cytometric histogram

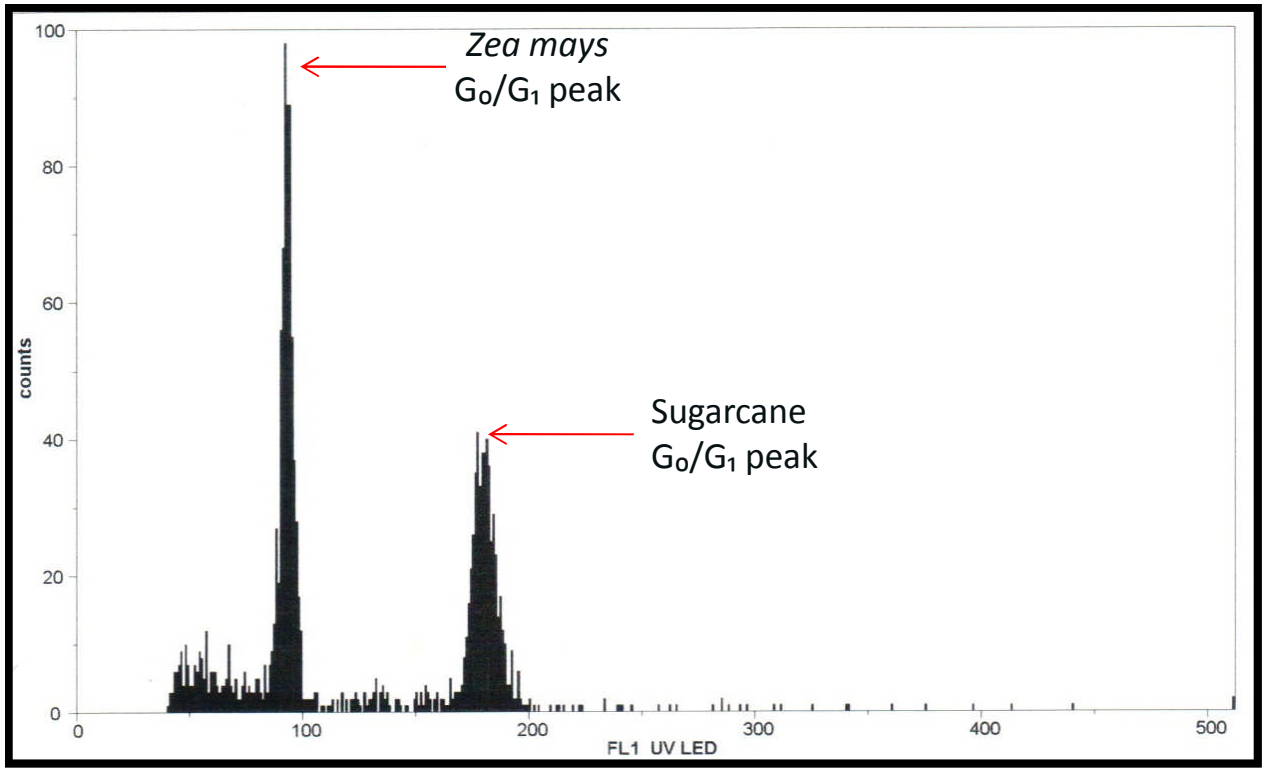


Figure 1 Mean fluorescent ratios (mean G_o/G_1 peak values) obtained after cytometry analysis. DNA content is calculated following the equation:

$$\text{Sample DNA content} = \text{Std DNA content} \times \frac{\text{Sample peak position}}{\text{Std peak position}}$$

Effect of tissue source (Leaves vs roots)

Table 1 ANOVA table for the leaf vs root comparison

Source	DF	Sum of Squares	Mean of Squares	F Value	Pr > F
Genotype	5	33.049	6.610	38.71	<.0001
Tissue	1	0.525	0.525	3.08	0.0974
Genotype*Tissue	3	0.773	0.258	1.51	0.248

Table 2 Comparison of nuclear DNA content obtained from leaf and root tissue

Genotype	N (L)	Leaves (pg/2C)	N (R)	Roots (pg/2C)
04X022	3	10.764 ± 0.078	3	10.466 ± 0.088
Black cheribon	3	9.301 ± 0.214	2	9.528 ± 0.065
UU0461	3	12.517 ± 0.128	3	12.295 ± 0.069
97B0740	3	12.535 ± 0.028	1	11.472
Mandalay	3	10.424 ± 0.041	-	*
VV0185	3	12.302 ± 1.169	-	*
Tissue source mean	18	11.307	9	10.979

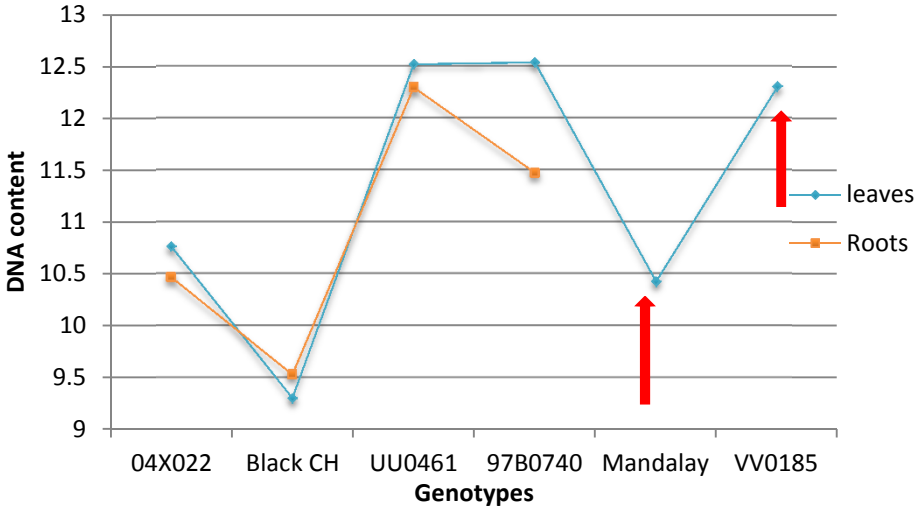
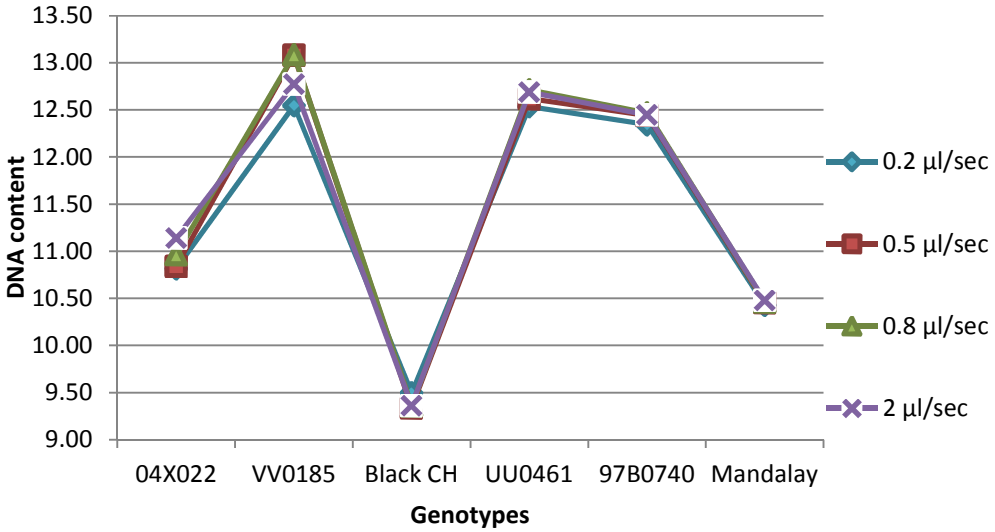


Figure 2 Graphical representation of the Sidak mean separation test

Effect of Flow rates (FR) and nuclear counts (NC)



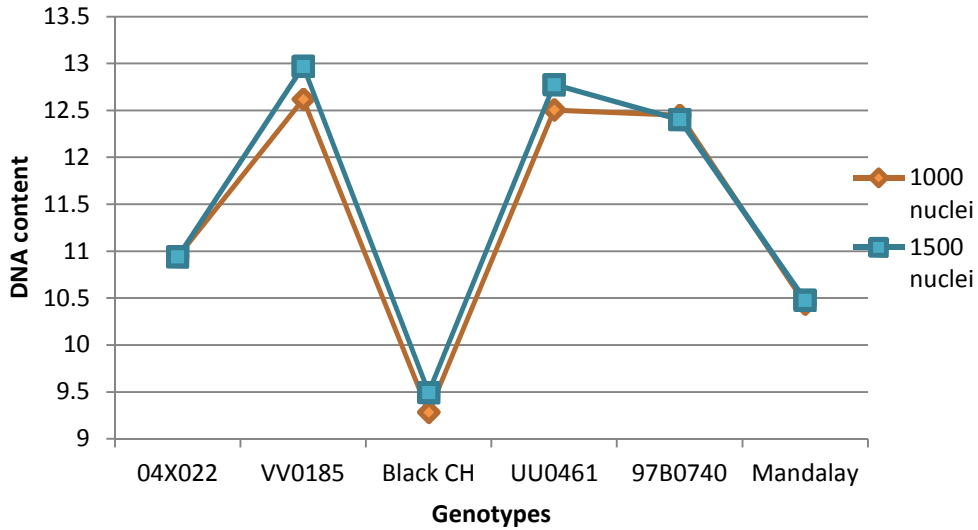


Table 3 ANOVA analysis of the effect of flow rates, nuclear counts and their interaction on DNA content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
G	5	230.5549390	46.1109878	351.09	<.0001
FR	3	0.3122639	0.1040880	0.79	0.5010
G*FR	15	1.2284510	0.0818967	0.62	0.8489
NC	1	0.6734041	0.6734041	5.13	0.0258
G*NC	5	0.7841033	0.1568207	1.19	0.3180
FR*NC	3	0.3660435	0.1220145	0.93	0.4299
G*FR*NC	15	1.5617012	0.1041134	0.79	0.6827

Prediction model

Table 4 Observed chromosome numbers and calculated 2C nuclear DNA content of parents involved in introgression crossing

genotype	Nuclear DNA content (pg)	Chromosome numbers	Ploidy level
04X0018 (F_1)	14.013 ± 0.1 ef	122	12x
04X0022 (F_1)	10.763 ± 0.05 a	90	9x
04X0026 (F_1)	13.710 ± 0.02 de	117	12x
04X0027 (F_1)	14.839 ± 0 f	129	13x
04X0033 (F_1)	13.850 ± 0.04 def	122	12x
WI82498 (BC_1)	13.830 ± 0.03 def	119	12x
CB3839 (comm)	13.154 ± 0.03 de	119	11x
04G0031 (comm)	11.768 ± 0.06 ab	103	10x
04K0993 (comm)	12.082 ± 0.58 bc	111	11x
N48 (comm)	12.871 ± 0.07 cd	112	11x
05U0114 (comm)	10.861 ± 0.07 a	94	9x

$$\text{Sample ploidy} = \text{Std ploidy} \times \frac{\text{sample peak position}}{\text{Std peak position}}$$

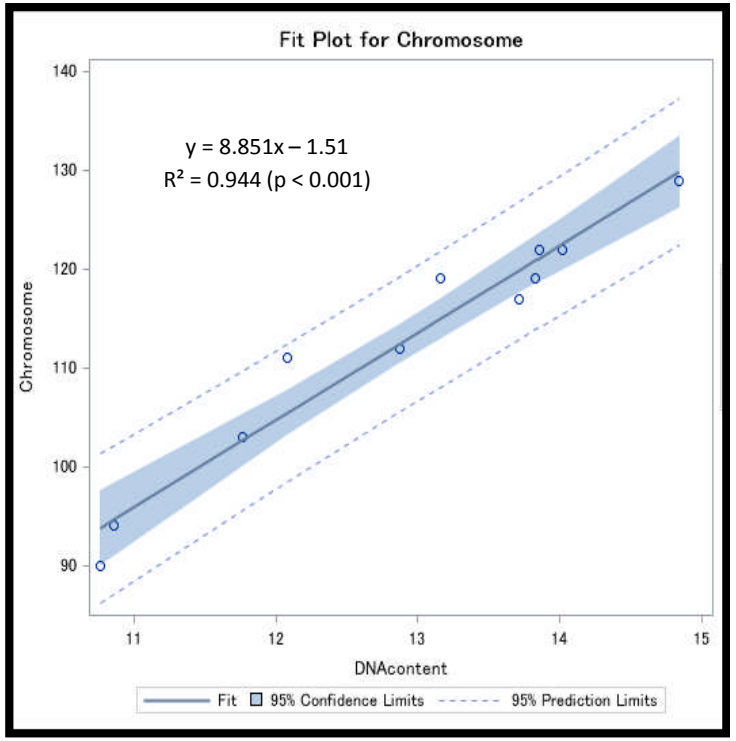


Figure 5 Regression analysis and prediction model produced using parental data

Chromosome prediction

Table 5 Observed and predicted chromosome numbers amongst progenies

Progenies	Cross	DNA content (pg)	Observed Chromosome number	Predicted Chromosome number	Ploidy level
1	VV0164	13.188 ± 0.02 a	115	108 ≤ 115 ≤ 122	11x
	VV0105	11.452 ± 0.02 c	100	92 ≤ 100 ≤ 107	10x
	WW0857	12.992 ± 0.02 a	120	107 ≤ 113 ≤ 120	12x
	VV0185	11.975 ± 0.13 b	105	97 ≤ 104 ≤ 111	11x
	VV0842	13.135 ± 0.06 a	121	108 ≤ 115 ≤ 122	12x
	VV0535	11.997 ± 0.03 b	104	98 ≤ 105 ≤ 112	10x
	WW0175	11.326 ± 0.03 c	106	91 ≤ 99 ≤ 106	10x
2	VV0164	12.894 ± 0.05 a	112	106 ≤ 113 ≤ 119	11x
	VV0105	11.533 ± 0.03 c	104	93 ≤ 101 ≤ 108	10x
	WW0857	13.014 ± 0.04 a	119	107 ≤ 114 ≤ 121	12x
	VV0185	12.302 ± .067 b	108	100 ≤ 107 ≤ 114	11x
	VV0842	*	*	*	*
	VV0535	12.589 ± 0.02 b	110	103 ≤ 110 ≤ 117	11x
	WW0175	11.228 ± 0.03 c	100	91 ≤ 98 ≤ 105	10x

Table 6 ANOVA analysis of significant differences between progenies and progenies within a cross

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Progeny	6	18.77858968	3.12976495	26.65	<.0001
Progenies (cross)	1	0.09610000	0.09610000	0.82	0.3727

Validation model

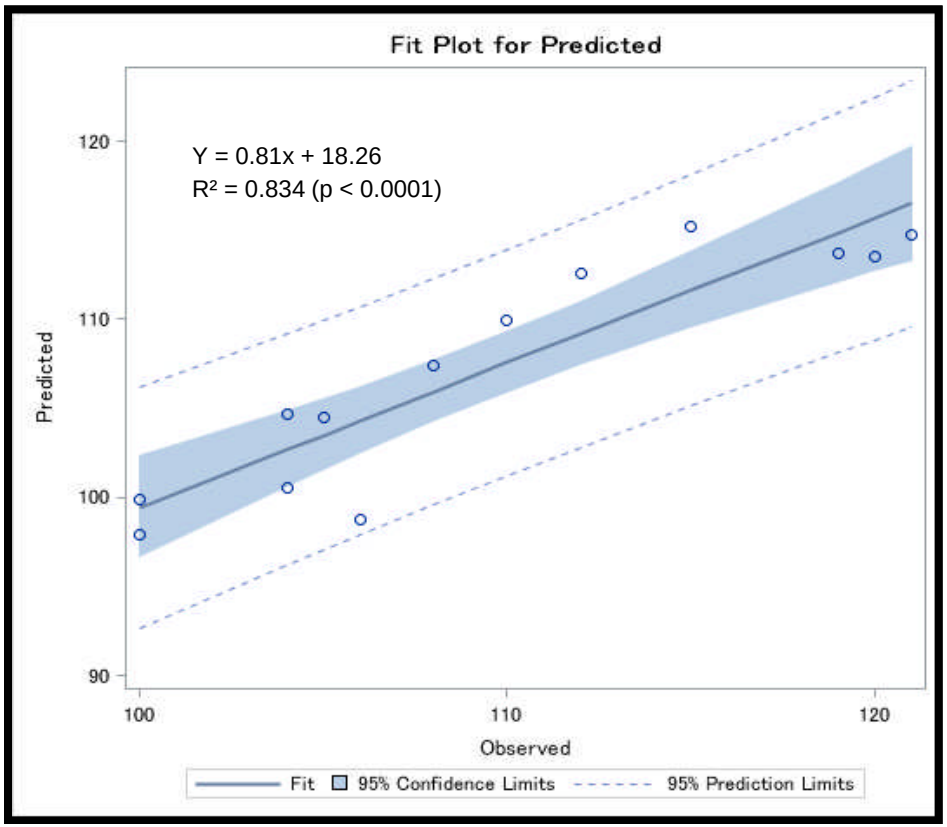
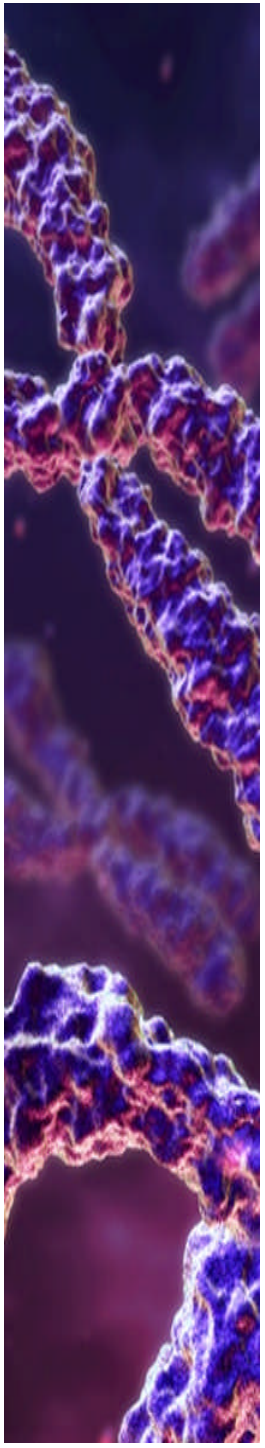


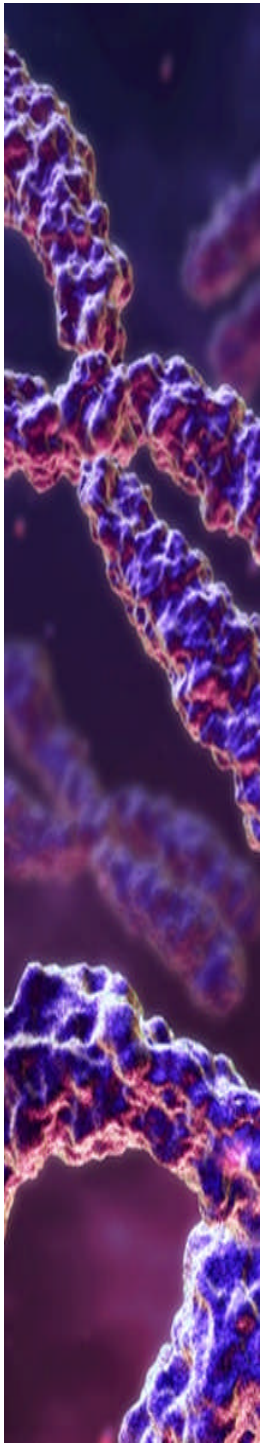
Table 7 T-test comparing observed and predicted chromosome numbers

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	24	0.87	0.3938



Conclusions

- Strong association between DNA content and chromosome numbers
- Prediction model reduces sample cost
- Strong correlation between observed and predicted values was revealed
- Tissue source, flow rates and nuclear counts do not significantly affect DNA content of genotypes



Conclusions

- Leaves are a more reliable source of genetic material than roots
- Ploidy elucidation has potential in preventing sterility in hybrid progenies and inferring chromosome transmission from parent to progenies.
- **Future studies** will aim at understanding the relationship between the chromosome numbers and phenotypic traits of sugarcane

Acknowledgements

