RESEARCH ARTICLE

STUDIES ON CYTOLOGICAL CHARACTERIZATION OF *CISSUS QUADRANGULARIS* ECOTYPES FOR ASSESSMENT OF PLOIDY LEVEL

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Abstract: *Cissus quadrangularis*, commonly known as veldt grape, is one of the medicinally important perennial, climbing succulent, widely distributed in Africa, the Arabian Peninsula, Northern India, and Southeast Asia. The present investigation on cytological characterization of veldt grape was conducted at Department of Medicinal and Aromatics crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore from 2019 -2020. Fifty veldt grape ecotypes were collected from different geographical locations of Tamil Nadu and five morphologically superior ecotypes identified were subjected to ploidy level estimation bt using flow cytometry method. The mean FL1-H value varied from 1018.05 to 2896.2 for the five superior ecotypes selected. The highest mean value FL1-H were found in TNCq23 and the lowest mean value recorded in TNCq34 from the plot 4.CV value of the veldt ecotypes ranged from 217.38 (TNCq 23) to 334.00 (TNCq9). The histogram of the mean position of G1 phase of the selected veldt grape ecotypes using radish as the reference indicated that the mean position of G1 peak for all the five ecotypes (TNCq32, TNCq34, TNCq29, TNCq23 and TNCq9) exhibited diploid number (2n) of chromosomes.

Keywords: *Cissusquadrangularis*, Ecotypes, Ploidy level, Flow cytometry

INTRODUCTION

Commonly known as veldt grape, *Cissus* is the largest genus in the grape family Vitaceae with about 300 species (Wen, 2007). According to Bogar, a Tamil siddhar (sometime between 550 and 300 BCE), *Cissus* substantiates its name as Vajrangi / Vajjravalli, meaning makes body as strong as diamond, the hardest substance known to man. It is widely distributed all over hotter parts of Tamil Nadu, growing luxuriously in slopy elevations upto 500 MSL with annual rainfall of less than 700 mm. Maximum diversity is observed in Virudhunagar, Tirunelveli, TuticorinPerambalur, Salem and Erode districts of Tamil Nadu. Dry stems constitute high-quality material for the export to various pharmaceutical industries. Researchers believe that *Cissusquadrangularis* can help burn fat, increase lean muscle mass and reduce appetite. In conjunction with the benefits above, *Cissusquadrangularis* has also been directly linked to lower cholesterol levels, probably due to its strong impact on metabolic factors in the body. The plant extract serves as rich source of calcium ions and is known to accelerate healing of fracture, increase the bone strength and stimulate the production of osteoblasts, the cells in the body responsible for bone growth. *Cissusquadrangularis* being an under-utilized medicinal crop with enormous pharmaceutical significance, lack of knowledge on genetic wealth coupled with ignorance of cytological information, poses a main barrier for the commercial cultivation in Tamil Nadu. Effective formulation of a breeding programme for improvement of quantitative attributes depends upon the magnitude of genetic variability and the extent of heritability of desirable characters. With the above background information, the present investigation was taken up to determine the ploidy level of selected superior performing ecotypes using flow cytometry method.

MATERIALS AND METHODS

The present investigation entitled "Studies on evaluation, cytological and molecular characterization in veldt grape (*Cissusquad-rangularis*)" was conducted at Department of Medicinal and Aromatics plants, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore from 2019 - 2020. Morphologically five superior ecotypes were subjected to analysis the ploidy level by using the flow cytometry method**.** Three types of buffer solutions viz., LB01 lysis buffer, Galbraith's buffer, Tris.MgCl2 buffer were prepared using standard procedures. The flow cytometry analyses were carried out in a BD (Becton Dickinson) FACS Calibur™ flow cytometer four color.

- 0.5 cm young veldt grape leaves sample was fragmented with the help of a scalpel in petri dish containing 0.25 ml nuclei-isolation buffer A of the partec high resolution DNA kit (partecmunster). Then 0.25 mg RNAse added to the solution
- After adding 0.75ml propidium iodide (PI) solution (50mg/l in water) the suspension with nuclei was filtered through a 30-mesh nylon filter.
- The fluorescence of nuclei was measured using a partec CA-II flow cytometer
- DAPI (4,6-Diamidino2-Phenylindole Dihydro-chloride) fluorescence intensities of suspension

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were measure using partecploidyanalyser PA (partec GmbH) to determine relative DNA fluorescence (Dolezel*et al*., 1997).

The DNA content for each sample was proportional to the amount of PI intercalating in the double-stranded DNA. The DNA content per cell was also proportional to the fluorescence intensity of PI measured by the flow cytometer. The flow cytometer records the fluorescence intensity of cells in the G1 and G2 periods for the sample. The G1 period cells precede DNA synthesis (S), and the G2 period cells have accomplished DNA replication but have not split. Thus, the 2C DNA content was calculated based on the value of the fluorescence intensity of G1 peaks for both the internal standard and the sample.

Ploidy level was determined by comparing mean relative fluorescence of each sample with the 2C peak of unknown genome size. Radish (genome size of 2C=1.11pg) was used as an external standard.

DNA ploidy of the unknown sample with the external standard calculated as follows.

Mean position of the G1 sample peak

$$\text{Sample ploidy (integer)} = \frac{\text{Reference ploidyx}}{\text{Mean position of the G1 reference peak}}$$

Ploidy analysis with internal standard

The assay with external standard unless not sufficiently precise for aneuploidy detection then the internal standardization is used to detect the ploidy level in unknown sample. In internal standardization the Perfect overlap of peaks of the reference plant and the unknown sample indicates the same ploidy. Bifurcated or nonsymmetrical (skewed) peaks imply aneuploidy. Aneuploidy may only be reliably identified using simultaneous analysis of a reference and aneuploid plant if the CVs of the DNA peaks are lower than half of the difference between the DNA contents of both plants.

Experimental results

The morphologically superior five ecotypeswere subjected to cytological analysis the ploidy level by using BD (Becton Dickinson) FACS Calibur™ flow cytometer.Radish was used as the internal standard for examine the ploidy level of veld grape. The histogram of morphologically superior ecotypes such as TNCq32, TNCq34, TNCq29, TNCq23 and TNCq9 is shown in Fig 1 to 5 and the mean and cv value of the veldt grape ecotypes given in Table 1 to 5**.** The mean value FL1-H for the five superior ecotypes varied from 1018.05 to 2896.2. The highest mean value for FL1-H were found in TNCq23 and the lowest mean value recorded in TNCq34 from the plot 4.CV value of the veldt ecotypes ranged from 217.38 to 334.00 The highest CV value FL1-H were found in TNCq23 and the lowest mean value recorded in TNCq9 from the plot 4.Based on the histogram of the mean position of G1 phase of the selected veldt grape ecotypes, the mean position of the peak in plot number 4 for the ecotypes TNCq32, TNCq34, TNCq29, TNCq23,TNCq9 indicated that all the five ecotypes exhibited diploid number (2n) of chromosomes.

DISCUSSION

According to the classification by Soltis*et al*. (2003) the genome of *C. quadrangularis* falls within the group of plants with very small genome. The size of the *Cissusquadrangularis* genome is approximately in the same range as *Cissusantarctica* and *Cissustrifoliata* which are tetraploid in nature. The measurement of nuclear DNA content using flow cytometry (FC) has been an effective technique for estimating ploidy levels of many higher plant species. Polyploidy is defined as the occurrence of different ploidy levels within an organism generated either by endo-reduplication, primarily in plants, or by endomitosis, which occurs largely in animals (Barow, 2006). The exhibition of endo-polyploidy pattern is quite recurrent in xerophytic plants with small genome size with CAM metabolism. This mechanism facilitates the succulents to survive and reproduce in dry environments with minimum levels of water (De Rocher*et al*. 1990). In the current study, radish used as the reference standard 2C = 1.11 pg (Gichuki*et al*., 2019). Based on the histogram obtained from the flow cytometry method, all the five ecotypes (TNCq32, TNCq34, TNCq29, TNCq23 and TNCq9,) exhibited diploid number of chromosomes. The present study was supported by Robert *et al*. (2001) who evaluated the seven two variants (A (smooth stem angles) and B (rough stem angles)) of *C quadrangularis*from Kenya, reported their chromosome numbers to be 24 and 28 respectively.Karkamkar et al (2010) also reported the chromosome numbers of *Cissus* species as 2n = 24, 48. In addition, Chu *et al*. (2018) revealed the chromosome numbers for seven other *Cissus* species 2n = 24, 40, 48, or 66) and suggested a linear relationship between the chromosome numbers and genome size (1C = 0.37–1.03 pg).

Veldt genus is considered to accommodate large number of species making its genome size, chromosome numbers, and genome characteristics unfamiliar. The above studies suggest that, polyploidization and repetitive element alterations in the extended genome size of *Cissus* genus contributed to the existing chromosomal abnormalities. Due to variation in sample preparation, staining and analysis, the peak position may not exactly reflect the ploidy. While in low polyploids a small shift in peak position usually does not compromise reliability of ploidy estimates, whereas attention should be paid to the analysis of high polyploids.Further studies are needed for the confirmation of ploidy levels in veldt grape ecotypes.

Table 1. Mean and cv value of TNCq32

Plot 2: B01 1	Count	Events / µL	% of This Plot	% of All	Mean FL2-A	Mean FL3-A	CV FL2-A	CV FL3-A	Median FL2-A	Median FL3-A
All	5,000	263	100.00%	100.00%	12,954.86	343,377.33	174.23%	226.38%		
R1	2,098	110	41.96%	41.96%	12,980.90	119,591.07	32.63%	59.27%		
Plot 3: B01 1: Gated on R1	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
This Plot	2,098	110	100.00%	41.96%	12,980.90	119,591.07	32.63%	59.27%		
P2	1,796	95	85.61%	35.92%	13,707.87	96,890.07	25.95%	23.01%		

Plot 4: B01 1: Gated on (P2 in R1)	Count	Events / µL	% of This Plot	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	1,796	95	100.00%	35.92%	1,018.05	257.20%	

Table 2. Mean and cv value of TNCq34

Plot 1: B06 2	Count	Events / µL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5,000	250	100.00%	100.00%	1,404,019.59	1,560,562.94	159.06%	211.06%		
Plot 2: B06 2	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
All	5,000	250	100.00%	100.00%	10,235.56	401,603.79	193.96%	241.83%		
R1	2,898	145	57.96%	57.96%	9,439.54	99,362.32	33.67%	73.74%		
Plot 3: B06 2: Gated on R1	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
This Plot	2,898	145	100.00%	57.96%	9,439.54	99,362.32	33.67%	73.74%		
P2	2,540	127	87.65%	50.80%	9,395.89	77,062.16	29.89%	35.55%		

Plot 4: B06 2: Gated on (P2 in R1)	Count	Events / µL	% of This Plot	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	2,540	127	100.00%	50.80%	1,598.68	224.84%	

Table 3. Mean and cv value of TNCq29

Plot 1: B03 3	Count	Events / µL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5,000	200	100.00%	100.00%	1,773,591.21	2,141,977.99	150.45%	177.84%		
Plot 2: B03 3	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
All	5,000	200	100.00%	100.00%	11,985.57	392,138.28	347.71%	208.12%		
R1	1,983	79	39.66%	39.66%	9,284.49	98,436.68	40.64%	86.82%		
Plot 3: B03 3: Gated on R1	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
This Plot	1,983	79	100.00%	39.66%	9,284.49	98,436.68	40.64%	86.82%		
P2	1,668	67	84.11%	33.36%	9,445.68	70,081.16	37.50%	40.15%		

Plot 4: B03 3: Gated on (P2 in R1)	Count	Events / µL	% of This Plot	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	1,668	67	100.00%	33.36%	1,854.35	241.14%	

Table 4. Mean and cv value of TNCq23

Plot 1: B04 4	Count	Events / µL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5,000	119	100.00%	100.00%	1,680,431.86	1,945,181.36	161.38%	194.72%		
Plot 2: B04 4	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
All	5,000	119	100.00%	100.00%	14,739.30	323,439.66	188.22%	210.47%		
R1	1,601	38	32.02%	32.02%	13,680.00	144,641.66	45.30%	58.94%		
Plot 3: B04 4: Gated on R1	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
This Plot	1,601	38	100.00%	32.02%	13,680.00	144,641.66	45.30%	58.94%		
P2	1,200	29	74.95%	24.00%	15,495.37	109,712.71	35.51%	25.60%		

Plot 4: B04 4: Gated on (P2 in R1)	Count	Events / µL	% of This Plot	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	1,200	29	100.00%	24.00%	2,896.52	217.38%	

Table 5. Mean and cv value of TNCq9

Plot 1: B05 5	Count	Events / µL	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5,000	227	100.00%	100.00%	1,739,253.16	2,107,791.01	147.00%	175.00%		
Plot 2: B05 5	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
All	5,000	227	100.00%	100.00%	9,085.08	330,395.35	182.01%	231.59%		
R1	2,417	110	48.34%	48.34%	8,404.47	91,459.33	40.18%	87.24%		
Plot 3: B05 5: Gated on R1	**Count**	**Events / µL**	**% of This Plot**	**% of All**	**Mean FL2-A**	**Mean FL3-A**	**CV FL2-A**	**CV FL3-A**	**Median FL2-A**	**Median FL3-A**
This Plot	2,417	110	100.00%	48.34%	8,404.47	91,459.33	40.18%	87.24%		
P2	2,071	94	85.68%	41.42%	8,312.37	66,548.80	35.88%	40.94%		

Plot 4: B05 5: Gated on (P2 in R1)	Count	Events / µL	% of This Plot	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	2,071	94	100.00%	41.42%	1,455.90	334.00%	

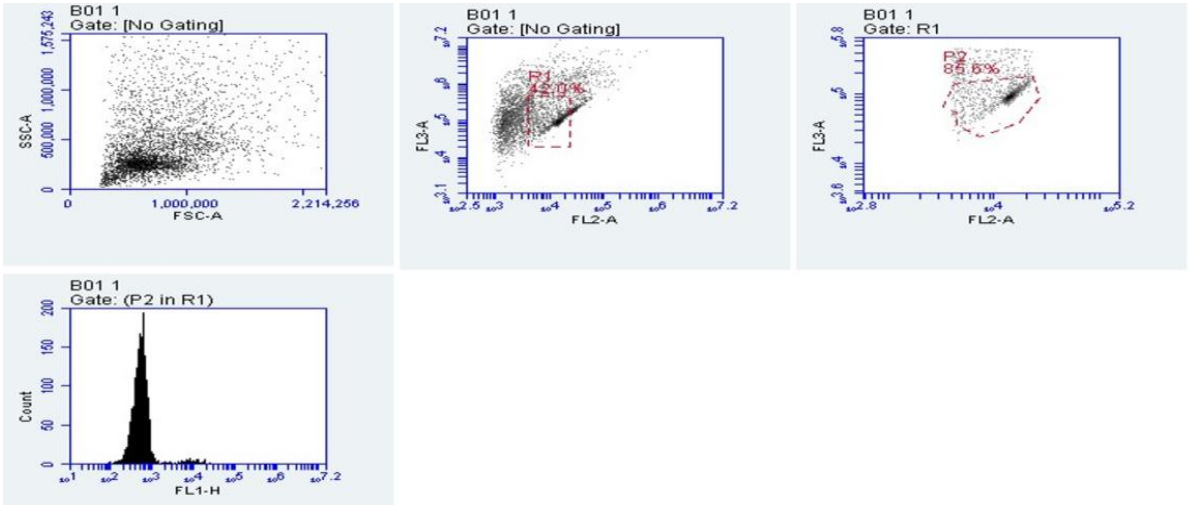

Fig 1: Histogram of veld grape ecotype TNCq32 by flowcytometry analysis

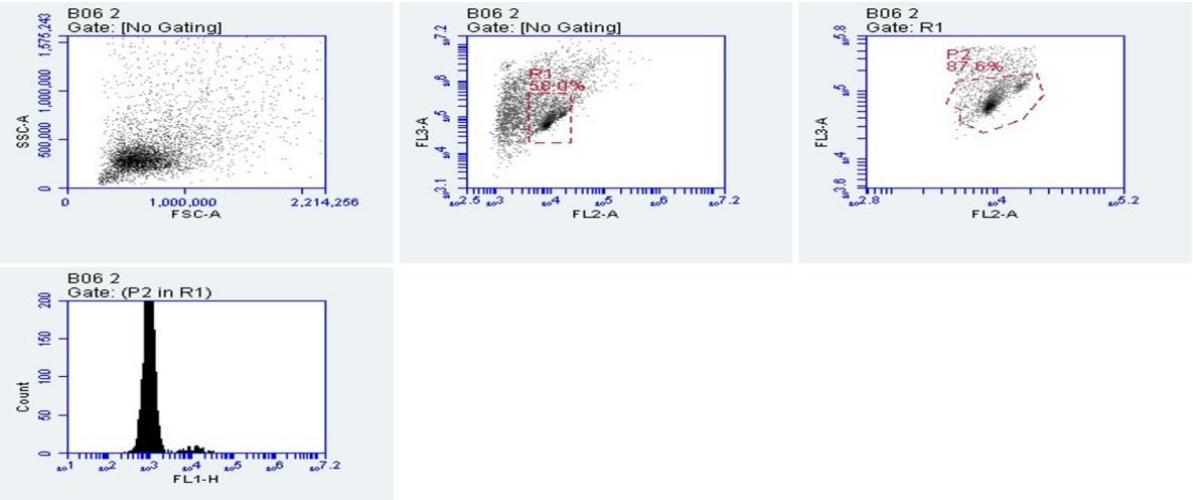

Fig 2: Histogram of veld grape ecotype TNCq34 by flowcytometry analysis

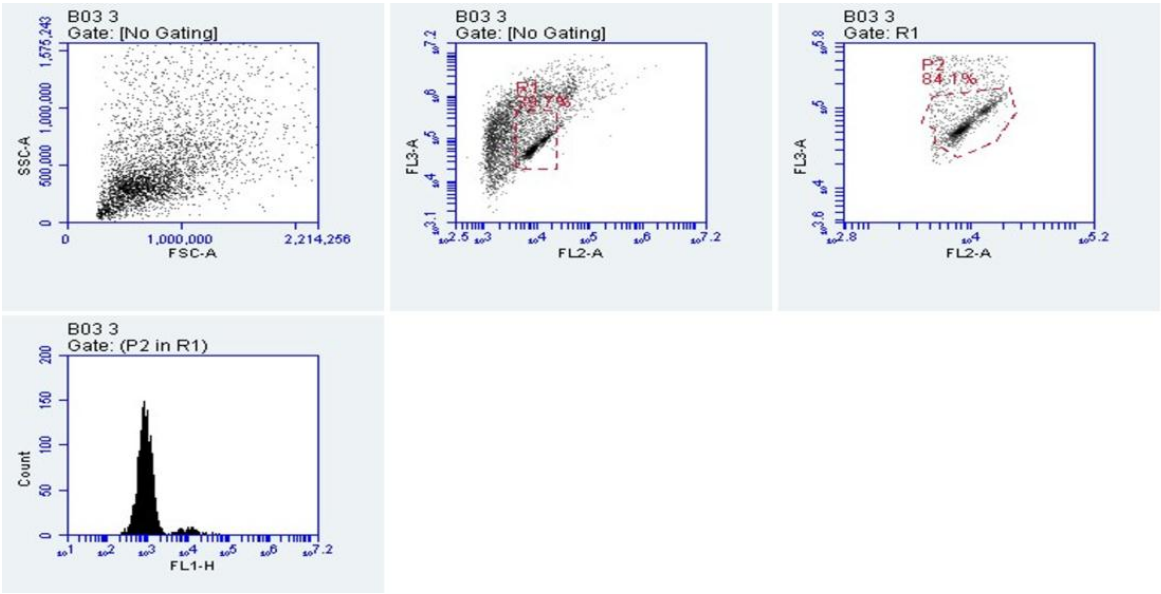

Fig 3: Histogram of veld grape ecotypeTNCq29 by flowcytometry analysis

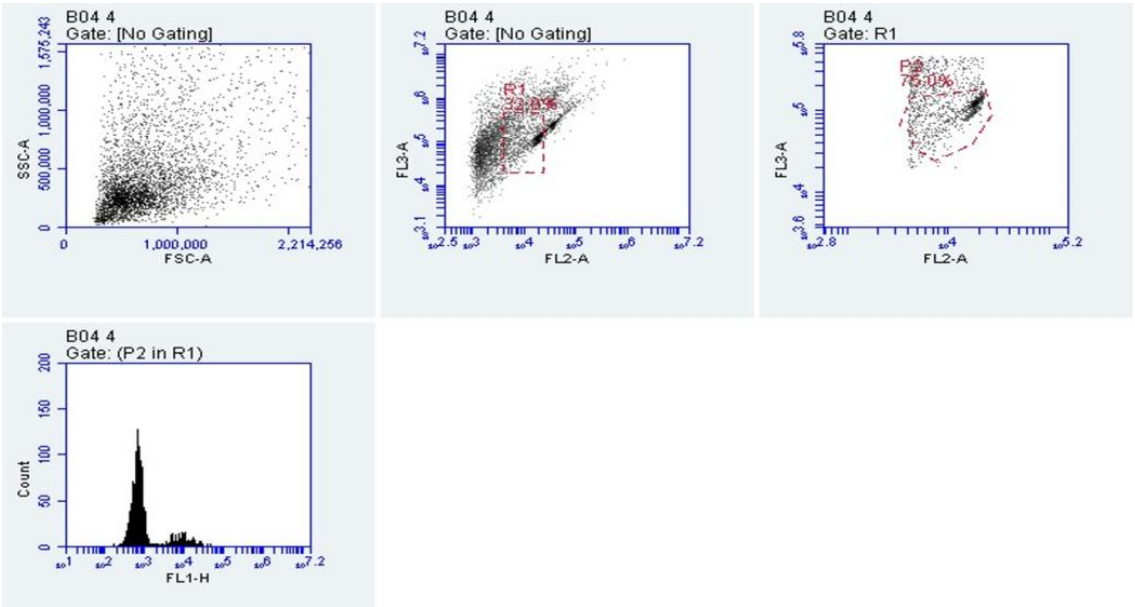

Fig 4: Histogram of veld grape ecotypeTNCq23 by flowcytometry analysis

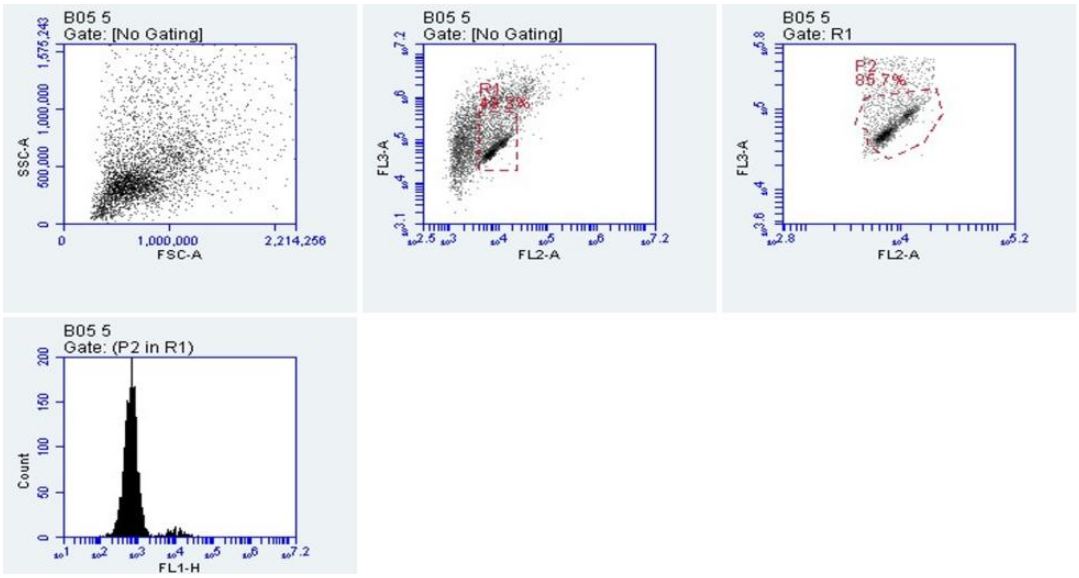

Fig 5: Histogram of veld grape ecotype TNCq9 by flowcytometry analysis

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