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Genetic Structure in FCV Tobacco Population as Assessed by Multi-locus Genotyping Using SSR Markers

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Abstract

Association mapping studies largely depend on the genetic nature of the population. An understanding of genetic structure of population assists in designing apt studies that accounts the dynamics of variability in population. Current study is aimed at analyzing genetic structure of tobacco (*Nicotiana tabacum* L.) population consisted of 135 FCV (Flue Cured Virginia) genotypes using multi-locus genotyping with mapped Simple Sequence Repeat (SSR) markers. 25 unlinked SSR markers delineated 135 genotypes revealing a total of 85 alleles with an average of 3.4 alleles per locus. Contrasting allelic frequencies were observed in most of the loci studied. Bayesian method of inference employed the genotyping data to derive genetic structure information from the population. High FST measures in the subpopulations (0.44, 0.09 and 0.29 at population level K=3) indicated high inbreeding coefficient signifying a strongly structured population. Evolutionary dissimilarity estimate using DARwin also supported the finding of resilient structure in the population. These findings were found highly useful in designing appropriate mapping strategies for tobacco breeding.

Keywords: *Nicotiana tabacum*; Population structure; SSR markers; Trait mapping

Introduction

Tobacco (*Nicotiana tabacum* L., 2n = 48) has been cultivated for thousands of years and has served as one of the major trade commodity in different cultures. It has been one of the most important commercial crops in the world. In the past several decades, this plant has found yet another use, serving as a widely utilized model system in plant-cell culture and genetic-engineering research [1]. Because of its economic importance and value as a biological research tool, numerous investigations have been undertaken to examine its evolutionary origin and genome structure and organization. Morphological, karyotypical, and physiological characters have already been used to study the genetic background of tobacco [2,3]. However, morphological characters usually vary with the environmental conditions in which they are cultivated. The number of karyotypical characters is limited and the study of genotypic diversity based on isozyme variation is restricted to a few polymorphic enzyme systems encoded by a small number of loci [4]. Among the different agricultural crops in India, tobacco has been little studied for genetic improvement. Globally available literature on tobacco genetics is also scanty.

Genetic improvement in crop plants including tobacco largely depends on the population dynamics such as genetic diversity and population structure. Though a large number of tobacco varieties of different commercial types have been developed in India, their genetic base is unknown [5]. Population genetic structure data empowers breeder for rational use of genetic resources to suitably design breeding strategies to develop superior tobacco varieties. Population subdivisions have critical effects on the dynamics of alleles in populations as well as on the statistical tests imposed on genetic data sampled from such individuals. It is well known that population subdivision affects the nature of alleles in a population under the influence of mutation, drift, and selection; hence, the eventual fate of an allele is affected by population subdivision [6]. Therefore, facts on genetic structure of the population are vital for development of appropriate and informative mapping populations for trait mapping and subsequent marker assisted breeding.

Microsatellite markers (also known as Simple Sequence Repeats; SSRs) have been established in different crop plants as highly informative markers. The fact that SSR markers are co-dominant, abundant in genome, multi-allelic and can be reliably automated to analyze genetic parameters in *Nicotiana* makes them attractive as genetic markers. Availability of mapped SSRs from tobacco [7,8] in Solanaceae Genomics Network (SGN) aids in efficient use of these microsatellite markers and facilitates the integration of genetic divergence information in tobacco breeding applications.

With this background, we focused to analyze the genetic structure of FCV tobacco population to enable adoption of an appropriate mapping strategy for maker based trait tagging. Particulars on population genetics are critical for effective utilization of genetic variability in tobacco breeding for sustainable crop improvements.

Materials and Methods

Plant material and populations

Core germplasm collection of FCV tobacco population consisting of 135 genotypes (Appendix I) representing the total available FCV tobacco gene pool was used for the study. Leaf materials from these genotypes were collected from the field plantation maintained at Indian Leaf Tobacco Development (ILTD) germplasm at Rajahmundry in Andhra Pradesh, India. For those genotypes in which

leaves were not readily available, seedlings were raised in vermiculite media under laboratory conditions and emerging young leaves were used for DNA isolation. During field sampling, leaves were immediately placed in dry ice, transported to lab and stored at -80^{0}C until further analysis. Disease-free healthy leaves were cleaned thoroughly with sterile water for isolating good quality DNA.

GP No	Genotype	GP No	Genotype	GP No	Genotype	GP No	Genotype
1	960 ET	38	Gold Dollar	75	Nc 89	112	V 72 Meagaint
2	961 ET	39	Golden Harvest	76	NC 98	113	Vinca
3	American Joiner	40	Golden Wilt	77	No.301	114	Virgin aurea
4	Bell-61-11-No.1	41	Island Gold	78	No.401	115	Virginia 0131
5	Black shank res (973ET)	42	Kentucky Pryor	79	No.402	116	Virginia 0192
6	Bright Cospalia	43	Kotari Hicks	80	Nopy-10	117	Wild fire res Orinoco
7	C 139	44	Kuaka 860	81	Nordeal	118	ZZ100
8	C 86	45	Kutsaga 51	82	N-Tabacum	119	LV1
9	Cock Tobacco	46	Kutsaga E1	83	Ovens 62	120	LV2
10	Coker 128	47	Kutsaga E2	84	Oxford 1	121	LV4
11	Coker 187	48	Kutsaga Mammoth	85	Oxford 26	122	LV5
12	Coker 213	49	Lonibow	86	Oxford 3	123	UV3
13	Coker 253	50	Mammoth Gold	87	PD 611	124	UV4
14	Coker 254	51	Maryland	88	PD-4	125	SL21
15	Coker 298	52	Maryland 59	89	Proctor Special	126	EN-NEW
16	Coker 316	53	Maryland 64	90	Pulawaska 63	127	K326
17	Coker 347	54	Mc 1610	91	R.76.8 MR	128	SBS 1
18	Coker 411	55	Mc 64 1	92	Rhomas-7	129	KST 28
19	Coker-76 51-MM	56	Mc Nair 10	93	Riwaka-3	130	Georgia 1469
20	Coley's Special	57	Mc Nair 1040	94	Riwaka-4	131	Harrison Special
21	CSIRO - 3T	58	Mc Nair 12	95	SC 58	132	NC 628
22	Davis Special	59	Mc Nair 121	96	SC 71	133	No.400
23	DB 101	60	Mc Nair 133	97	SCR	134	LV3
24	DB 28	61	Mc Nair 135	98	Shamboon	135	SL17
25	Delcrest	62	Mc Nair 14	99	Speight G 140		
26	Delhi 61	63	Mc Nair 192	100	Speight G 23		
27	Delhi 76	64	Mc Nair 944	101	Speight G 33		
28	Delray	65	MRS 2	102	Speight G 36		
29	EC 11083	66	NC 1071	103	Speight G 58		
30	EC 15103	67	NC 12	104	Speight G 7		
31	F 105	68	NC 13	105	Speight G 70		
32	F 106	69	NC 2326	106	Strain 205		
33	F 108	70	NC 2512	107	T.1.448		

34	F 109	71	NC 3150	108	T.1.448A		
35	F 207	72	NC 6129	109	V 156		
36	F 210	73	NC 67	110	V 168		
37	F 212	74	NC 744	111	V 184 NFT		

Appendix I: List of 135 FCV tobacco genotypes used for analysis

DNA isolation and SSR genotyping

DNA from plant material was isolated using DNeasy Plant Mini Kit 250 (Qiagen, USA). Protocol was suitably optimized to isolate good quality DNA from all the lines. DNA quality was tested under agarose electrophoresis at 0.8% gel concentration and also quantified using NanoDrop 8000 (Thermo Fisher Scientific Inc., USA). SSR markers representing all the linkage groups were selected from SGN database (www.sgn.cornell.edu) based on tobacco linkage map published by Bindler *et al.* [7]. Oligonucleotide primers were synthesized from Sigma Genosys and their suitable PCR amplification conditions were optimized.

PCR conditions were optimized with minor modifications to Don *et al.* [9] protocol in 25 µl reaction with ~10 ng of template DNA, 0.2 µM of each primer, 100 µM of each dNTPs, 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 unit of Taq polymerase (Sigma Inc.). PCR amplification conditions were as follows; Initial denaturation at 94°C for 5 minutes, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at annealing temperature of respective primer pair and an extension of 1 minute at 72°C. The final extension was at 72°C for 5 min. A two-stage touchdown amplification profile was designed where specific PCR products were not found. Reactions were conducted with a 1°C reduction in annealing temperatures at each cycle from 65°C to 55°C, followed by 25 cycles at an annealing temperature of 55°C. PCR products were subjected to electrophoresis on 1.5% agarose gel stained with ethidium bromide (0.5 µl/10 ml of gel) at 150 V to identify the optimum annealing temperature for each of the SSR primers. For high resolution separation of SSR amplicons, a reliable Polyacrylamide Gel Electrophoresis (PAGE) protocol was suitably developed for tobacco. The samples were prepared for PAGE electrophoresis by adding 4.0ul of Gel loading buffer (0.25% bromophenol blue; 0.25% xylene cyanol; 30% Glycerol) to the reaction mixtures. Samples were electrophoresed on 10% non-denaturing polyacrylamide gels (gels were prepared in Tris-borate buffer, pH 8.0) using a mini PAGE apparatus. Each gel was run for 1.5 hrs at a 150-V with constant current. The DNA bands were visualized by staining with ethidium bromide. The sizes of the PCR products were estimated by comparison to an accompanying standard size 50bp and 20 bp ladder. With this optimized PCR and PAGE conditions, all the SSR primers were screened to test their suitability for genotyping. The sequences of SSR primers and their amplicons sizes are presented in Appendix II. Final genotyping was done on 15 µl reaction volume and amplicons were analyzed on PAGE for ideal fragments analysis. Profiles were scored for each marker across all the genotypes and the data was analyzed.

Sl no.	Primer Name	Linkage Group	Forward sequence (5' - 3')	Reverse sequence (5' - 3')	Target TNR
1	IMN 3034	1	AAAGAAGCACGGTCAAATAGG	GCAACAACAAGGTGTCATGG	TAA
2	IMN 3038	2	GAGGTGGAAGTCATCGGAAA	CGTCTGTCATACACGCGAAA	CAT
3	IMN 3043	3	CGCCACAACAACTCACCTTA	TCATGCATGTTTCTCCTCCTT	AAG
4	IMN 3049	4	CGCAATCTCCTAATGCGACT	TTGCATTAATCAGCTTCTTCTCC	GAA
5	IMN 3054	5	GTCTGTACCTTCGCCAAAGC	TCCTCAGAGAACTCCAGCGT	GAA
6	IMN 3060	6	TGTCTCGTGAAGCATGAA	GGAAATGGAGGATCTCGT	CT/CTT
7	IMN 3066	7	AGTTGCAGGATTGTTCGCTT	CGACTGCAAGAGTTGGCAAT	GAA
8	IMN 3072	8	ACCATGTCCTTCCAACCT	AAGAACGAGGTTTGCCTT	CTT
9	IMN 3076	9	CCGAGTCTGTTTTGGTTG	GCGAGCATCTCTCATTTC	CTT
10	IMN 3084	10	ATAGTCTAAGCCCCACGG	AGCCATTGTAAGGGGATG	AAG
11	IMN 3086	11	AAATTACTTGTGCTTGTAAGTAGCG	TCATTTCAGAAAGCATATATTGGTG	TA
11	IMN 3089	11	CTTATGTCCAAACGCCCACT	CGCATCGATAAATTCCTACCA	TAA
12	IMN 3091	12	TCCAAAGTTGGACCAGAA	GTCCTACATGGGGCTCTT	AGG

13	IMN 3095	13	GACAACAATCAGTAAAGGAAACGA	AATGCAAGACCCTGTCAACC	TAA
14	IMN 3097	14	TGCTTCCAACCTGAGAAA	TGAGGAGGTAAGGGGTTC	GT
15	IMN 3099	15	AGCCCGATCCAACCAC	CATTGCGGAGTAGATTTTCGT	CA/AT
16	IMN 3101	16	TGATTTGTATTGACAGCGTGAAG	TTGTTTAGTTACCCTATTTGACTTGC	TA
17	IMN 3104	17	AATGCCACTTACGACATCTATCA	TGAAGCGTTAACGGCAATTT	TA
18	IMN 3105	18	AGAAGGCAAACTTTCTTGCTT	TTCCAAATACCACACTTCTTACTCA	TA
19	IMN 3107	19	GGTCACCGTCTTCTTCTCCA	CCCAAATTTATCACGCAACC	TAG
20	IMN 3109	20	ACGATGGAGATGGTGTTG	CCCAAGATACCCAACCTT	GT
21	IMN 3111	21	TCAAATGAGGGTTGTAGCCA	TGCAATGGCTACACAAGAAGA	CGA
22	IMN 3114	22	TTGAACACCAATTGCGGTAA	AAATTCTTGGGTCATGGTGG	TA
23	IMN 3116	23	GCCGAATTAAACCAACCAAA	ACCGGATTGCTTAATTGTCG	TAAAAA
24	IMN 3118	24	AATGTCTGCCCAATCGAAAG	CGAATAACGACACTCGAACG	CA
Note: TNR: Tandem Nucleotide Repeat					

Appendix II: Primer sequences, their target TNRs and corresponding linkage groups.

Data Analysis

Bayesian model-based program STRUCTURE (10,11) and DARWin 5.0 [12] were used to analyze the genotyping data. Using a burn-in length of 20,000 and a run length of 100,000, STRUCTURE was run with admixture model with correlated allele frequencies. Ten independent simulations were run for each level of K (the number of populations). Statistical estimation of the K value was possible with the LnP(D) output value from the STRUCTURE [13]. LnP(D) is the logarithmic likelihood of the observed genotype distribution in K clusters and it is maximum at optimal K level [10]. Phylogenetic analysis based on neighbor-joining model using Nei's distances was also performed to understand the genetic dynamics of the population. The polymorphism information content (PIC) of each microsatellite locus was determined as described by Weir [14] as PIC = 1-Σ Pi2, where Pi is the frequency of the ith allele in the genotypes examined. Allele frequencies were compared between populations using Wright's F statistics to calculate the fixation index FST. FST is a measure of between population variance and gives the proportion of overall diversity which is attributable to differences between populations [15].

The distribution of genetic variation within and among the subpopulations was analyzed using F statistics [16], where 1 - FIT = (1 - FIS) (1 - FST). FIT is the correlation between individuals within the total sample (i.e. total fixation index), and is made up of two components; FIS, which describes deviations from Hardy-Weinberg expectations within subpopulations, and FST which measures differentiation between subpopulations. FST was calculated for each allele at each locus, from the relationship FST= σ2/p(l - p), where σ2 is the total variance among samples, and p is the mean allelic frequency. To determine the extent to which any deficits of heterozygotes could be attributed to variance in allelic frequencies among the subpopulations (the Wahiund effect), the relationship between heterozygote deficits and variance of allelic frequencies among subpopulations was calculated as σ2/He - H0 (where σ2 is the variance summed for each locus, He is the expected heterozygosity, and H0 is observed heterozygosity for the total population as detailed by Johnson and Black [17]).

Results

SSR markers were successfully amplified in accordance with their reported amplicon sizes. Most of the tandem nucleotide repeats selected for study were of three base repeats as they are easy scorable for their allelic variations upon polyacrylamide gel based genotyping as used by many researchers [18]. A total of 85 alleles were detected at 25 unlinked loci as assessed by SSR primer pairs spanning all the 24 linkage groups [7] of tobacco. The PIC values ranged from 0.26 to 0.73 with an average of 0.48. The number of alleles per SSR locus ranged from 2 to 7 with an average of 3.4 alleles per locus. Microsatellite loci IMN3099 was the most informative locus with 7 alleles detected across the population. The data on allelic frequencies at different loci tested are presented in Figure 1.

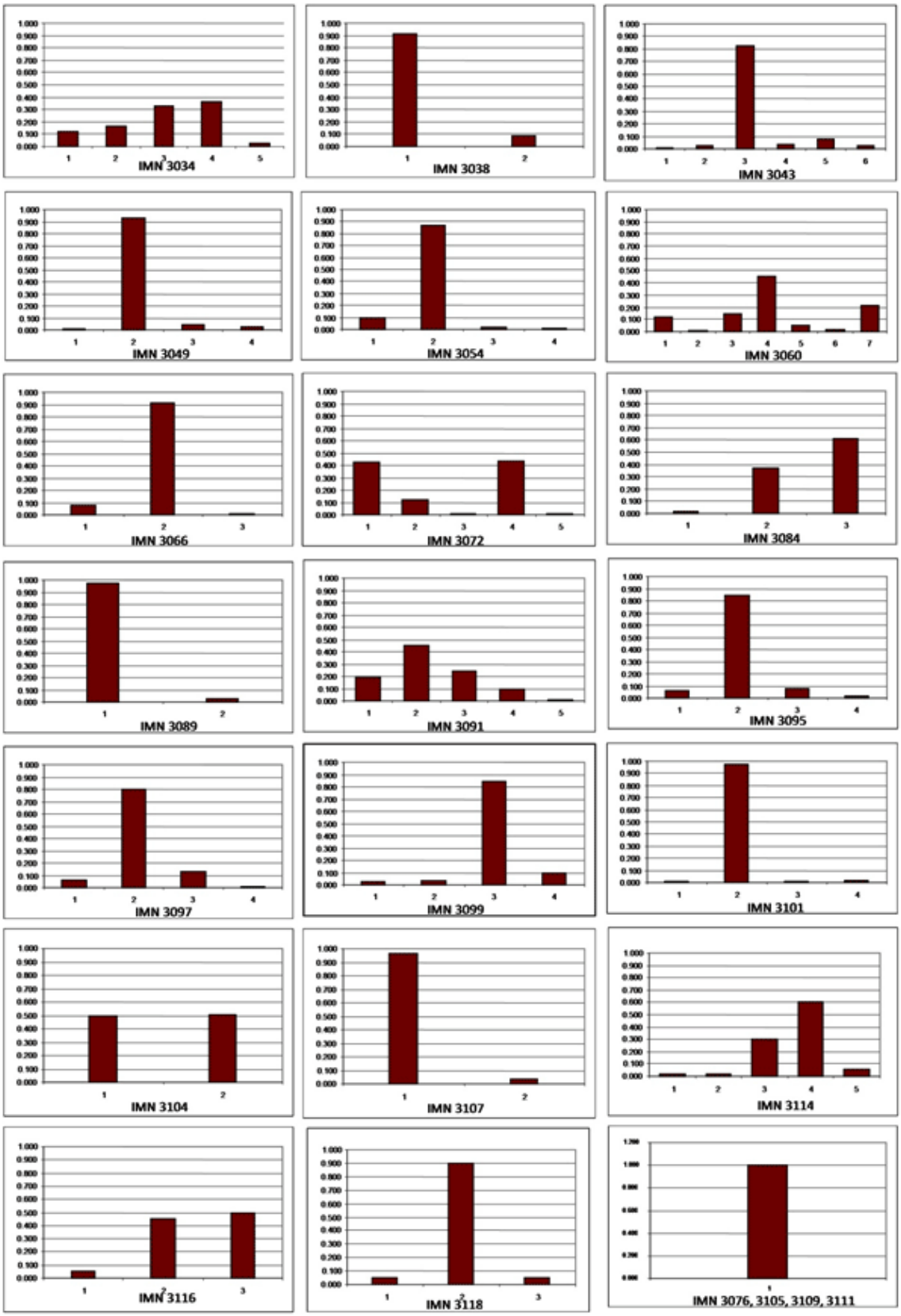

Figure 1: Allele frequencies at different microsatellite loci in FCV tobacco population (X axis is alleles by respective SSR primers and Y axis is allele frequency)

Allelic data when analyzed revealed significant information on population heterozygosity levels and FST values. At a metapopulation level (assigned K= 1), the average distances (expected heterozygosity) between individuals in entire population was 0.2941 with a mean FST value of 0.1078 (Figure 2). This signifies the deficit in heterozygosity in the metapopulation.

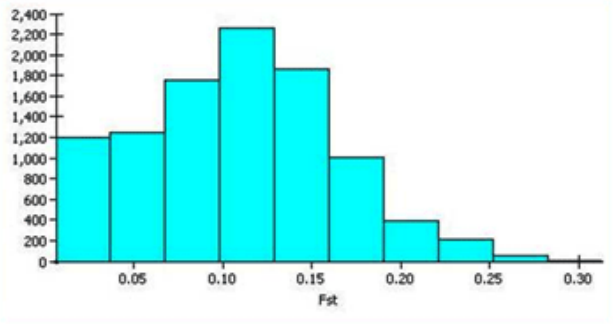

Figure 2: Histogram of distribution of F_{ST} values in metapopulation.

Different levels of K were statistically assigned to the population to understand the genetic nature of the population variation. Structure model analyzes the data on the basis of posterior probability of goodness of fit. At varied levels of assigned K, the maximum LnP(D) output determines the optimum levels for consideration. Data was analyzed for varied levels of K up to 15 and highest LnP(D) was observed at K = 3. Average expected heterozygosity between individuals in same cluster for these three populations were 0.1887, 0.3589 and 0.2606 with mean FST values of 0.4415, 0.0859 and 0.2934 respectively (Figure 3). These three subpopulations showed intense levels of inbreeding within in the clusters as supported by these Strong FST values.

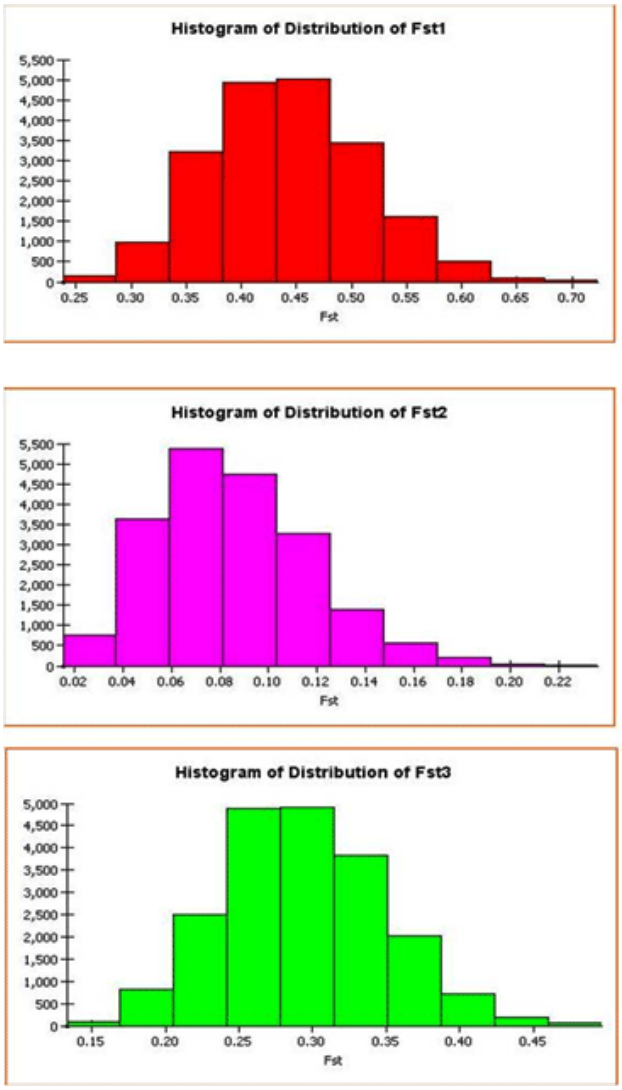

Figure 3: Histograms of the distributions of F_{ST} values in three subpopulations.

Additionally, Cluster analysis was performed on 15 subdivisions based on the allele- frequency divergence among those subpopulations. It was very evident from this analysis that there was a high degree of inbreeding within this population as evidenced by inbreeding driven subdivisions (Figure 4). Estimating admixture proportions can be particularly challenging if there are very few representatives of the parental populations. Grouping of all the 135 individuals based on their estimated population membership coefficient Q matrix with three prevailing subdivisions is presented in Figure 5. The data was further tested with another model of analysis to validate our results. This included analysis with DARwin (DARwin 5.0: Dissimilarity Analysis and Representation for Windows) as used in rice research [19]. This model assumes the basis of evolutionary dissimilarities using microsatellite derived allelic data and performs clustering of individuals. The narrow clustering of the genotypes with limited divergence further supported our findings that the present populations possess a strong genetic structure (Figure 6).

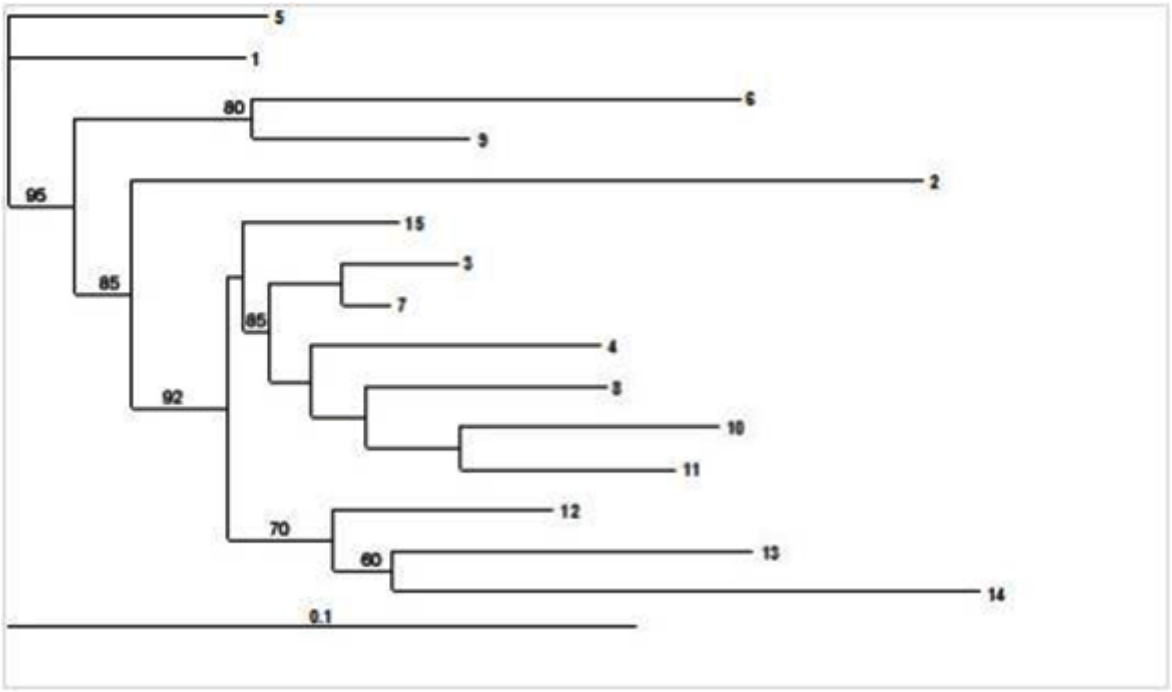

Figure 4: Clustering of 15 subdivisions based on their allele-frequencies (K=15)

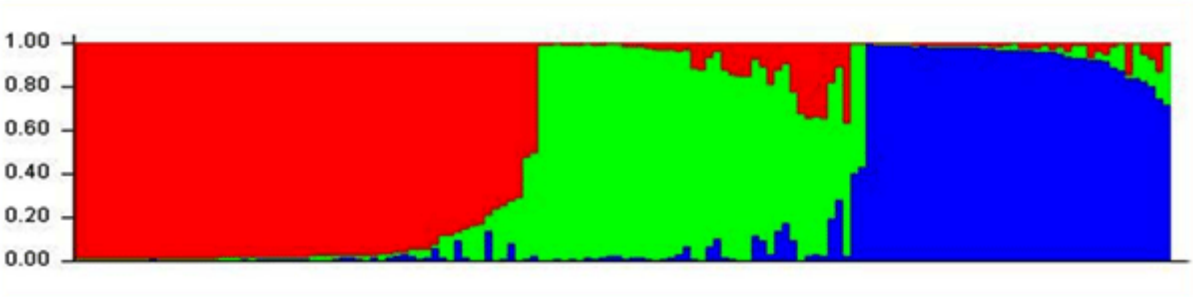

Figure 5: Estimated Q matrix of 135 tobacco genotypes at population level K=3. (X axis: Genotypes and Y axis: population membership coefficient for three subdivisions represented by red, green and blue colors)

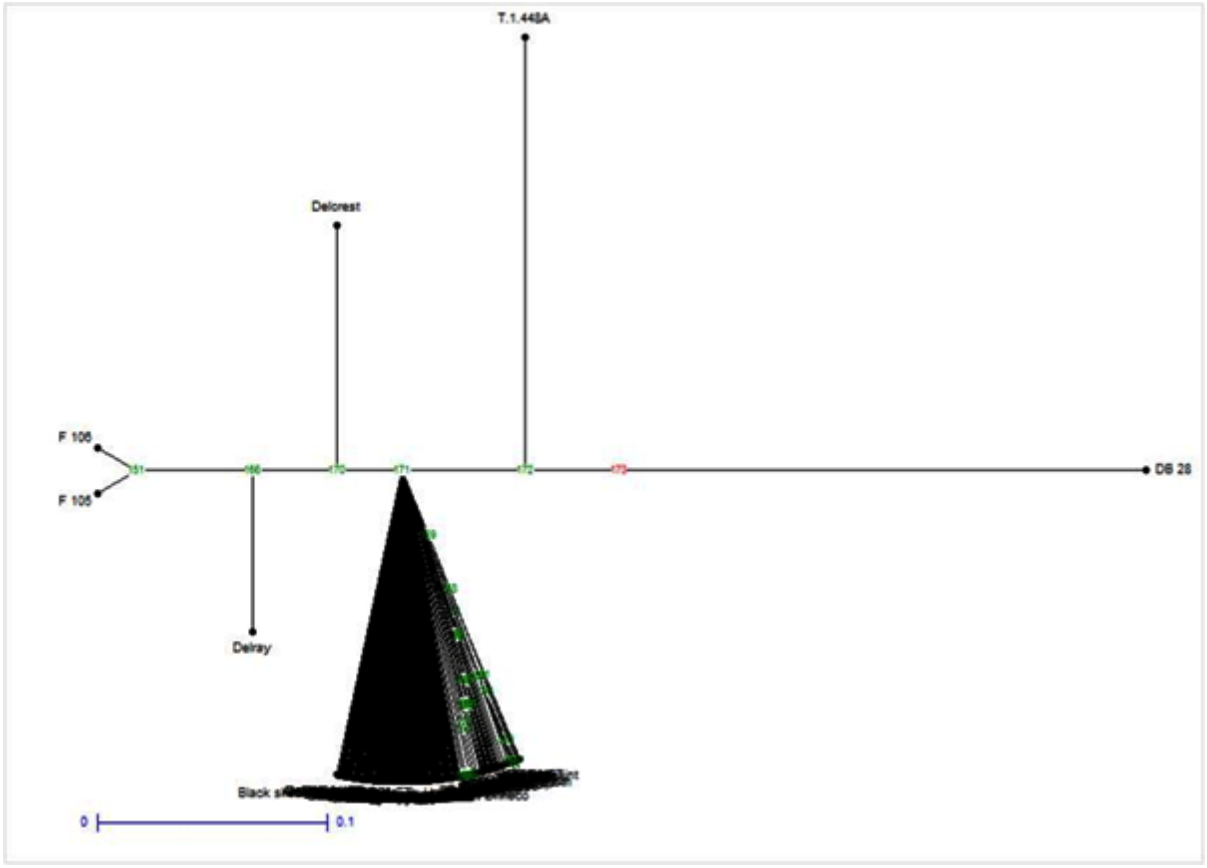

Figure 6: Clustering of individuals in the populations as analyzed by DARwin 5.0 indicating highly structured population.

Discussion

The current population genetic analyses using STRUCTURE was the most common approach and found to be highly suitable to address the issue of characterizing population differentiation. Inappropriate a priori grouping of individuals into populations may diminish the power of such analyses to elucidate biological processes, potentially leading to unsuitable mapping strategies or trait management research approaches. Population structure in 113 tomato accessions was also estimated in similar lines by employing SSR markers to identify markers linked to late blight resistance in Solanum phureja for breeding programs [20]. In another study similar approach with multilocus sequence data was conducted to infer population structure and demographic history in several wild tomato species (Solanum section Lycopersicon) by Thomas *et al.* [21]. Hence our current analysis of genetic structure in FCV tobacco population was critical to establish substantial evidences on the genetic structure in existing population of tobacco germplasm.

Multi-locus genotyping of FCV tobacco population consisting of 135 genotypes using mapped SSR markers successfully led to the identification of a strong genetic structure. Bayesian method of inference employed the genotyping data to derive genetic structure information on the population. It showed high FST measures in the subpopulations indicative of high inbreeding coefficient and high heterozygote deficit signifying an intensely structured population. With the data indicating that current population was not ideal for association mapping approach, alternative mapping options were worked out and accordingly biparental mating derived mapping population was developed for the trait mapping exercise. Our current results are in line with results of Fricano et al. [22] who also highlighted that the global population of tobacco is highly structured. Though other reports on similar findings can be found on population genetics of tobacco, germplasm specific genetic structure details are helpful to breeders for effective utilization of resources. The strong genetic structure in the current population suggest that tobacco might have experienced a series of selection followed by high inbreeding for several decades leading to a genetic bottleneck and limited divergence during its commercial cultivation.

It is well-realized in the past with various crops, that unsuited population when used in mapping results in spurious marker-trait associations. Hence the current results are very important in FCV tobacco molecular breeding for designing suitable mapping strategy to tag markers for traits of economic significance. While one continue to breed FCV tobacco, it is critical here that the genetic resources of the wild relatives of tobacco should be systematically evaluated and carefully utilized in commercial breeding. These sources will supplement and might even challenge the established lines in their effectiveness in breeding programs. The impact of this population genetics component can be rationally used to handle genetic diversity strategies and by breeders for effective use of existing variation in

various breeding programs for enabling sustainable genetic improvements for yield and quality in tobacco.

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References

1. Zhang HY, Liu XZ, Wei L, Zhou LY, Yang YM (2007) Insect-resistant Transgenic Tobacco Plants Containing both B.t and GNA Genes. Biologia Plantarum 51: 746-748.
2. Zhang DM (1994) Study on Surface Structure Scanning of Some Tobacco Seeds with Electrical-glass Observing. Chin Tobacco Sin 2: 12-15.
3. Lei YH, Xu ML, Huang XY (1997) Tobacco Collection in the Yunnan Province. Science and Technology publishing company of Yunnan, Yunnan. 1-20.
4. Lu JP (1997) The Application of PAGE in the Cultivars Identification of Flue-cured Tobacco. Seed 5: 30-32.
5. Siva Raju K, Madhav MS, Sharma RK, Murthy TGK, Mohapatra T (2008) Genetic polymorphism of Indian tobacco types as revealed by amplified fragment length polymorphism. Curr Sci 94: 633-639.
6. Wright S (1943) Isolation by distance. Genet 28: 114-138.
7. Bindler G, Rutger VDH, Irfan G, Plieske, J, Martin G, et al. (2007) A microsatellite marker based linkage map of tobacco. Theor Appl Gen 114: 341-349.
8. Bindler G, Plieske J, Bakaher N, Gunduz I, Ivanov N, et al. (2011) A high density genetic map of tobacco (Nicotiana tabacum L.) obtained from large scale microsatellite marker development. Theor Appl Genet. 123: 219-230.
9. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) Touchdown PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 19: 4008.
10. Pritchard JK, Matthew S, Peter D (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics 155: 945-959.
11. Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587.
12. Perrier X, Jacquemoud-Collet JP (2006) DARwin software.
13. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14: 2611-2620.
14. Weir BS (1990) Genetic data analysis. Methods for discrete genetic data. Sunderland Massachusetts: Sinauer Associates.
15. Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. Annals of Human Genetics 47: 253-259.
16. Wright S (1978) Evolution and Genetics of Populations: Variability Within and Among Populations. Vol. 4, University of Chicago Press, Chicago.
17. Johnson MS, Black R (1984) The Wahlund effect and the geographical scale of variation in the intertidal limpet Sip honaria sp. Marine Biology 79: 295-302.
18. Mackill DJ (2003) Applications of genomics to rice breeding. A mini review IRRN 28.1: 9-15.
19. Kenneth LM, Kevin LC, Regina B, Rebecca MD, Keyan Z, et al. (2009) Genome-wide SNP variation reveals relationships among landraces and modern varieties of rice. Proc Natl Acad Sci 28: 12273-12278.
20. Teresa MV, Carolina B, Gustavo G (2009) Identification of diagnostic markers to late blight resistance in Solanum phureja for breeding programs. The 6th Solanaceae Genome Workshop, New Delhi.
21. Thomas S, Aurelien T, Carlos M, Wolfgang S (2009) Using multi-locus sequence data to infer population structure and demographic history in several wild tomato species (Solanum section lycopersicon). The 6th Solanaceae Genome Workshop, New Delhi.
22. Fricano A, Bakaher N, Corvo MD, Piffanelli P, Donini P, et al. (2012) Molecular diversity, population structure, and linkage disequilibrium in a worldwide collection of tobacco (Nicotiana tabacum L.) germplasm. BMC Genetics 13: 18.