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Research Article

PHENOTYPING OF VASCULAR STREAK DIEBACK DISEASE (VSD) RESISTANT COCOA HYBRIDS AND ENDORSEMENT OF RESISTANT GENE IN AUSPICIOUS GENOTYPE BY EMPLOYING MOLECULAR MARKER SYSTEMS

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Abstract- Cocoa (*Theobroma cacao* L.) is native to humid tropical region of Central America and considered as important agro forestry tree species. Vascular streak dieback (VSD), a devastating disease with distinct symptoms of dieback, is one of the major threats for cocoa cultivation. The confounding part of this disease is that chemicals have little effect on disease control. The most tenable and economic technique to tackle this disease is by evolving resistant materials. When this disease began to assume unmanageable magnitudes in India, Kerala Agricultural University had initiated VSD resistant breeding since 1995. Initially, 566 hybrids which expressed tolerance in the nursery for two years were field established. After fifteen years of field screening, 46 hybrids which manifested field resistance were selected for further study. Examination on self-incompatibility position exhibited that majority of them (37) were self incompatible. When yield contributing characters were accessed VSD I 31.8 was found to be superior and out rated the checks, most popular varieties available. Disease resistance was further confirmed by budding and molecular markers.VSD I 31.8 (CCRP 15) can be consider as a novel hybrid released in the world with VSD resistance and considerable yield.

Keywords- *Ceratobasidium theobromae,* resistance breeding, *Theobroma cacao* L., VSD, hybrid.

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Introduction

Cocoa (*Theobroma cacao* L.) is native to humid tropical region of Central America and considered as important agro forestry tree species [1,2]. Vascular streak dieback (VSD), a devastating disease with distinct symptoms of dieback, is one of the major threats for cocoa cultivation. The disease was first delineated in Papua New Guinea, engendered by the fungus *Oncobasidium theobromae* [3]. Contemporary studies led to reclassification of the causal organism to another basidiomycete fungus, *Ceratobasidium theobromae* [4]. In India, the disease was first promulgated by Abraham, (1981) [5] and then by Chandramohan and Kaveriappa, (1982) [6]. The confounding part of this disease is that chemicals have little effect on disease control [7-10] and the only control method recommended is frequent pruning of infected branches below the visible symptoms [8,11,12]. However, incessant pruning will result in inhibition of cocoa growth and moreover it is labour - intensive and exorbitant [13].

The most tenable and economic technique to tackle this disease is by evolving resistant materials [14-16]. When this disease began to assume unmanageable magnitudes in India, Kerala Agricultural University had initiated VSD resistant breeding since 1995 [17,18] and this paper reveals the variability prevailing among identified resistant hybrids and endeavour to tag VSD resistant gene in most auspicious genotype of this breeding programme.

Materials and Methods

The hybridization programme to address VSD was initiated in KAU during 1995-96, deploying thirty-one females and four male parents [19]. Since the causal organism is obligate parasite, artificial inoculation of pathogen was not possible. Hence, inoculum was dispensed by keeping already infected seedlings around the experimental materials [20]. After nursery screening for two years, 566 seedlings which manifested resistance were planted in a separate block as VSD set I [16]. These plants were perpetuated under uniform level of management and scored for disease incidence as per the score developed by Abraham, *et al*., (2000) [8] [Table-1] for fifteen years during the peak period of infestation.

Table-1 *Score chart for vascular streak dieback infestation in cocoa*

Disease scale	Intensity of Infection
0	No infection
1	< 25 per cent of twig infected
3	25-50 percent infection
5	50- 75 percent infection
7	> 75 per cent infection
9	Mortality of the plant

Fifty hybrids were deduced with score 0 indicating no infestation during the entire period of investigation [19]. At present only forty-six hybrids are retained in the field and four were defunct due to natural calamities and details are given in [Table-2]. Incompatibility positions of these hybrids were figured out by selfing 100 flowers per tree, as per the procedure advised by Mallika, *et al*., (2002) [21].

Table-2 *List of hybrids identified as resistance to VSD*

Sl.No	Stand No	Hybrid
1	VSD I 2.3	MI3.12 X G VI 55
2	VSD I 4.1	GII 19.5 X G VI 55
3	VSD I 4.3	GII 19.5 X G IV 18.5
4	VSD I 4.6	G VI 4 X G IV 18.5
5	VSD I 4.11	G VI 4 X G IV 18.5
6	VSD I 4.12	G VI 4 X G IV 18.5
7	VSD I 5.11	G VI 4 X G VI 55
8	VSD I 9.17	G VI 126 X G IV 18.5
9	VSD I 10.6	G VI 126 X G IV 18.5
10	VSD I 10.7	G VI 126 X G IV 18.5
11	VSD I 10.8	G VI 126 X G IV 18.5
12	VSD I 10.10	G VI 126 X G IV 18.5
13	VSD I 10.18	G VI 126 X G IV 18.5
14	VSD I 11.10	G VI 126 X G IV 18.5
15	VSD I 11.12	G VI 126 X G IV 18.5
16	VSD I 11.22	G VI 126 X G IV 18.5
17	VSD I 11.23	G VI 126 X G IV 18.5
18	VSD I 13.11	G VI 126 X G VI 55
19	VSD I 13.12	G VI 126 X G VI 55
20	VSD I 13.13	G VI 126 X G VI 55
21	VSD I 13.17	G VI 126 X G VI 55
22	VSD I 14.6	G VI 126 X G VI 55
23	VSD I 14.14	G VI 126 X G VI 55
24	VSD I 14.15	G VI 126 X G VI 55
25	VSD I 15.7	G VI 137 X G VI 55
26	VSD I 15.10	G VI 137 X G VI 55
27	VSD I 15.15	G VI 137 X G VI 55
28	VSD I 16.4	G VI 140 X G IV 18.5
29	VSD I 16.6	G VI 140 X G IV 18.5
30	VSD I 16.10	G VI 140 X G VI 55
31	VSD I 16.11	G VI 140 X G VI 55
32	VSD I 17.2	G VI 140 X G VI 55
33	VSD I 17.9	G VI 140 X G VI 55
34	VSD I 18.12	G VI 143 X G VI 55
35	VSD I 18.15	G VI 143 X G VI 55
36	VSD I 19.6	G VI 143 X G VI 55
37	VSD I 21.6	G VI 143 X G VI 55
38	VSD I 21.27	G VI 143 X G VI 55
39	VSD I 22.1	G VI 167 X G IV18.5
40	VSD I 23.21	G VI 171 X G IV18.5
41	VSD I 23.24	G VI 172 X M 13.12
42	VSD I 29.8	G VI 188 X G VI 55
43	VSD I 29.9	G VI 188 X G VI 55
44	VSD I 29.11	G VI 188 X G VI 55
45	VSD I 30.6	G VI 188 X G IV 18.5
46	VSD I 30.7	G VI 188 X G IV 18.5
47	VSD I 30.8	G VI 188 X G IV 18.5
48	VSD I 31.7	G VI 189 X G VI 55
49	VSD I 31.8	G VI 189 X G VI 55
50	VSD I 31.9	G VI 189 X G VI 55

Yield data were recorded as number of pods per tree per year from 2004 to 2016 and pooled mean was estimated.

Yield contributing characters were registered from five pods per tree as per the standard procedure [22] following the design CRD. Pod length (cm), pod breadth (cm), pod weight (g), wet bean weight/ pod (g), number of beans, single dry bean weight (g) etc. were recorded for acquiring information about general performance of the hybrids.

Genetic parameters like Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV) [23], Heritability (H^2) and Genetic Advance (GA) [24] were computed for these hybrids.

The best performer was compared with the check varieties CCRP 1 and CCRP 8. Affirmation of VSD resistance was done with budding experiment, by adopting infected seedlings as rootstock. In addition to this, one ISSR (UBC 815) and one SSR (mTcCIR42) markers found to be tightly linked with VSD resistance in early studies [25,26] were also used to confirm the presence of disease resistant gene in the selected hybrid.

Results and Discussion

Mechanism of self incompatibility is exploited as a pollination control mechanism in many crops for hybrid production. This will help to evade the humdrum affair of emasculation [27,28]. Cocoa is also privileged with this mechanism and self-incompatibility in cocoa was first reported by Pound, *et* al., (1932) [29]. In the present investigation when self-incompatibility positions of forty-six hybrids were evaluated it was seen that thirty-seven hybrids turned to be self-incompatible, without setting fruits even after selfing 100 flowers. Hybrids, VSD I 4.3 and VSD I 4.11 were bracketed as self-compatible since they set fruit on selfing [Table-3]. Many workers have delineated that the self-incompatibility is the cardinal rule in a cocoa population [30,31] and the experimental population also showcased the same trend.

Table-3 *Incompatibility position of the VSD resistant hybrids*

Sl. No	Stand No	Hybrid	No. of flowers selfed	No. of fruit set	Incompatibility position
1	VSD I 2.3	MI3.12 X G VI 55	100		SIC
2	VSD I 4.1	GII 19.5 X G VI 55	100		SIC
3	VSD I 4.3	GII 19.5 X G IV 18.5	5	3	SC
4	VSD I 4.6	G VI 4 X G IV 18.5	100		SIC
5	VSD I 4.11	G VI 4 X G IV 18.5	9	2	SC
6	VSD I 4.12	G VI 4 X G IV 18.5	100		SIC
7	VSD I 5.11	G VI 4 X G VI 55	100		SIC
8	VSD I 10.6	G VI 126 X G IV 18.5	100		SIC
9	VSD I 10.7	G VI 126 X G IV 18.5	100		SIC
10	VSD I 10.8	G VI 126 X G IV 18.5	100		SIC
11	VSD I 10.10	G VI 126 X G IV 18.5	133	2	SC
12	VSD I 10.18	G VI 126 X G IV 18.5	100		SIC
13	VSD I 11.10	G VI 126 X G IV 18.5	100		SIC
14	VSD I 11.22	G VI 126 X G IV 18.5	100		SIC
15	VSD I 11.23	G VI 126 X G IV 18.5	100		SIC
16	VSD I 13.11	G VI 126 X G VI 55	100		SIC
17	VSD I 13.12	G VI 126 X G VI 55	100		SIC
18	VSD I 13.13	G VI 126 X G VI 55	100		SIC
19	VSD I 13.17	G VI 126 X G VI 55	100		SIC
20	VSD I 14.6	G VI 126 X G VI 55	100		SIC
21	VSD I 14.14	G VI 126 X G VI 55	100		SIC
22	VSD I 14.15	G VI 126 X G VI 55	100		SIC
23	VSD I 15.7	G VI 137 X G VI 55	100		SIC
24	VSD I 15.10	G VI 137 X G VI 55	25	1	SC
25	VSD I 15.15	G VI 137 X G VI 55	100		SIC
26	VSD I 16.4	G VI 140 X G IV 18.5	11	2	SC
27	VSD I 16.6	G VI 140 X G IV 18.5	100		SIC
28	VSD I 16.10	G VI 140 X G VI 55	100		SIC
29	VSD I 16.11	G VI 140 X G VI 55	100		SIC
30	VSD I 17.2	G VI 140 X G VI 55	100		SIC
31	VSD I 17.9	G VI 140 X G VI 55	35	4	SC
32	VSD I 18.12	G VI 143 X G VI 55	23	4	SC
33	VSD I 18.15	G VI 143 X G VI 55	100		SIC
34	VSD I 19.6	G VI 143 X G VI 55	100		SIC
35	VSD I 21.6	G VI 143 X G VI 55	100		SIC
36	VSD I 21.27	G VI 143 X G VI 55	100		SIC
37	VSD I 22.1	G VI 167 X G IV18.5	51	4	SC
38	VSD I 23.24	G VI 172 X M 13.12	100		SIC
39	VSD I 29.8	G VI 188 X G VI 55	100		SIC
40	VSD I 29.11	G VI 188 X G VI 55	100		SIC
41	VSD I 30.6	G VI 188 X G IV 18.5	100		SIC
42	VSD I 30.7	G VI 188 X G IV 18.5	28	1	SC
43	VSD I 30.8	G VI 188 X G IV 18.5	100		SIC
44	VSD I 31.7	G VI 189 X G VI 55	100		SIC
45	VSD I 31.8	G VI 189 X G VI 55	100		SIC
46	VSD I 31.9	G VI 189 X G VI 55	100		SIC

Yield of cocoa is summarized as number of pods per tree per year. Yield was registered in all forty-six hybrids for 13 continuous years (2004-2016) and pooled mean average is presented in [Table-4]. Yield varied across the genotypes and maximum yield (86.23) was recorded by VSD I 31.8. None of the individual expressed a yield above 100 pods per tree per year, which is categorized as high yielder [32]. "Immunity/ resistance are regarded as a double-edged sword" as opined by Xu, *et al*., (2017) [33]. The reason is that, defense protein generated by the plant is not only toxic to pathogen but also to the plant itself resulting in yield curtailment, and there are reports to support that disease resistance mechanism may result in reduction in yield [34-37]. However, hybrids with 80 pods per tree per year are-

Table-4 *Yield data (No. of pods/tree/year) of resistant hybrids for thirteen years after attaining the stable yield*

Sl. No.	Hybrids	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Pooled mean
1	VSD I 2.3	53	58	62	66	56	60	67	58	63	84	95	94	79	68.85
2	VSD I 4.1	49	56	44	49	53	41	47	47	50	55	57	67	73	52.92
3	VSD I 4.3	62	56	56	47	85	81	86	78	66	89	52	82	74	70.31
4	VSD I 4.6	43	55	51	44	55	59	66	54	51	64	57	69	77	57.31
5	VSD I 4.11	40	46	43	42	44	41	60	47	54	73	63	60	65	52.15
6	VSD I 4.12	46	55	58	54	58	61	48	59	62	58	56	54	60	56.08
7	VSD I 5.11	66	72	79	74	85	84	74	85	94	76	88	77	80	79.54
8	VSD I 10.6	50	79	95	58	50	56	46	53	44	48	54	87	78	61.38
9	VSD I 10.7	57	50	56	50	61	60	60	63	67	74	74	70	71	62.54
10	VSD I 10.8	48	41	54	58	47	55	49	52	56	52	50	58	55	51.92
11	VSD I 10.10	57	59	63	55	64	60	59	60	57	66	62	78	68	62.15
12	VSD I 10.18	52	53	44	63	59	48	54	53	50	54	58	61	67	55.08
13	VSD I 11.10	52	56	68	62	73	64	60	52	61	58	71	85	83	65.00
14	VSD I 11.22	76	85	79	86	81	84	82	83	87	76	80	78	81	81.38
15	VSD I 11.23	46	49	57	61	52	54	65	60	58	63	51	54	54	55.69
16	VSD I 13.11	48	52	52	65	61	53	57	51	50	20	59	65	68	53.92
17	VSD I 13.12	35	33	37	43	34	46	43	48	48	49	52	61	66	45.77
18	VSD I 13.13	41	37	47	46	44	46	50	51	51	49	51	72	65	50.00
19	VSD I 13.17	38	43	38	44	34	36	47	44	36	38	40	44	48	40.77
20	VSD I 14.6	17	24	33	16	13	28	33	42	28	79	22	68	82	37.31
21	VSD I 14.14	31	40	42	48	39	45	38	35	40	55	59	53	70	45.77
22	VSD I 14.15	20	32	43	36	32	30	21	31	24	51	51	64	55	37.69
23	VSD I 15.7	51	34	41	42	28	37	55	30	34	49	69	116	93	52.23
24	VSD I 15.10	25	29	24	32	33	23	35	22	38	55	47	74	62	38.38
25	VSD I 15.15	42	36	40	35	44	30	46	34	26	82	82	95	67	50.69
26	VSD I 16.4	21	49	15	23	54	24	19	40	39	49	30	77	70	39.23
27	VSD I 16.6	30	32	40	44	38	35	34	42	50	74	66	69	45	46.08
28	VSD I 16.10	32	45	41	58	68	50	41	28	49	87	78	81	95	57.92
29	VSD I 16.11	18	32	16	29	13	31	34	32	22	97	54	109	50	41.31
30	VSD I 17.2	16	36	32	16	78	44	48	41	39	87	53	106	76	51.69
31	VSD I 17.9	15	26	25	19	24	26	15	22	18	20	24	51	54	26.08
32	VSD I 18.12	30	47	35	42	55	50	48	49	60	71	84	98	62	56.23
33	VSD I 18.15	28	35	40	38	55	30	45	38	40	55	85	95	65	49.92
34	VSD I 19.6	33	42	38	35	42	50	48	56	55	95	80	104	84	58.62
35	VSD I 21.6	30	34	40	35	46	63	51	34	50	94	84	108	79	57.54
36	VSD I 21.27	25	32	35	42	50	89	55	45	56	95	55	97	108	60.31
37	VSD I 22.1	20	32	30	38	87	49	49	50	30	86	56	78	59	51.08
38	VSD I 23.24	32	43	29	38	31	20	55	61	65	57	60	95	68	50.31
39	VSD I 29.8	36	30	29	35	54	93	64	65	56	50	52	90	58	54.77
40	VSD I 29.11	27	45	35	30	42	25	32	45	52	49	61	80	62	45.00
41	VSD I 30.6	30	28	27	38	45	65	40	83	55	79	58	65	62	51.92
42	VSD I 30.7	16	32	34	36	30	50	35	40	55	76	52	79	74	46.85
43	VSD I 30.8	34	30	9	54	18	85	26	29	3	30	44	90	58	39.23
44	VSD I 31.7	21	20	25	26	17	20	22	34	36	29	58	62	58	32.92
45	VSD I 31.8	80	80	84	88	86	90	88	89	90	88	80	90	88	86.23
46	VSDI 31.9	20	49	44	44	49	45	60	67	39	56	38	67	53	48.54

considered to be an above average yielder [38] and can be selected for further evaluation.

Pod weight (g), wet bean weight per pod (g), number of beans per pod and single dry bean weight (g) are considered to be important parameters contributing to final yield [39]. Analysis of variance was done for these characters and result depicted in [Table-5]. VSD I 31.8 had the largest pod of 870.22 g with an agreeable wet bean weight of 200.12 g. This hybrid expressed less number of beans when compared to some other entries. However, number of beans alone cannot be considered as selection criteria, since large number with small size is not an advisable character. Reduction in size will in turn result in reduction of butter content [40], which is an essential ingredient for quality chocolate [41]. VSD I 31.8 manifested large bean size of 1.43 g, whereas International standard for dry bean is 1.00 g [42].

Quantum of variability available for exploitation and the extent to which the desirable characters are inherited to the next generation form the basis of success to any breeding programme. The best way to quantify the variability is by judging the genetic parameters [43, 44]. All the characters expressed high phenotypic and genotypic coefficient of variation as per the classification given by Sivasubramanian and Madhavamenon, (1973) [23], indicated that high amount of variability existed among the genotypes [Table-6] and the result was in tune with earlier workers [45,19]. High GCV value for single pod weight (59.38 %), wet bean weight per pod (51.13%), number of beans per pod (23.65%), single dry bean weight (41.38%), wet bean weight per plant per year (68.47%) dry bean weight per plant per year (70.69%) in contrast to their low ECV value, 2.71 per cent, 2.67 per cent, 8.93 per cent, 3.95 percent, 1.75 percent, 6.67 percent respectively, indicated that variability manifested by these characters are genetically controlled and least affected by the environment. High heritability along with high genetic gain was intimated by all characters besides number of beans per pod, pointing out that these characters are administrated by additive gene action and can be used as selection criteria [19]. Moderate heritability and genetic gain fabricated the character number of beans per pod as an undesirable one for selection.

In view of all the parameters included for evaluation, VSD I 31.8 was ranked as first. Performance of this hybrid was juxtaposed with CCRP 1 and CCRP 8, where CCRP 1 is the leading clone among the released varieties and CCRP 8 is the foremost hybrid. A remarkable percentage of increase was observed for all the characters when compared with clone. Percent increase over CCRP 8 was also high and recorded a value more than 50 percent [Fig-1].

Confirmation of disease resistance is of utmost important. The causal organism *Ceratobasidium theobromae* is an obligate parasite, hence cannot culture on artificial media. This makes the testing of resistance by artificial inoculation of the pathogen impossible. However, an attempt was made to assess the resistance by budding on diseased root stock. Details are given in [Table-7] and [Plate-1]. Out of 78 seedling budded, bud take was observed only in 20 plants. This is because of the week nature of root stock. However, none of the established budded plants experienced the symptom of VSD.

Molecular markers are strong tool in identifying genotypes with disease resistance

Table-5 *Pod and bean characters of VSD resistant hybrids*

Sl. No.	Hybrids	Single pod weight(g)	Wet bean wt/pod(g)	No. of beans /pod	Single dry bean weight(g)
1	VSD I 2.3	272.00	65.00	30.80	0.64
2	VSD I 4.1	228.00	53.10	32.20	0.92
3	VSD I 4.3	400.00	82.00	44.80	0.93
4	VSD I 4.6	220.00	53.90	40.60	0.81
5	VSD I 4.11	280.60	86.88	39.00	0.80
6	VSD I 4.12	270.00	90.50	45.00	0.94
7	VSD I 5.11	420.10	105.08	40.80	0.82
8	VSD I 10.6	350.90	91.55	40.00	0.91
9	VSD I 10.7	230.80	56.48	45.40	0.69
10	VSD I 10.8	372.00	80.08	47.40	0.77
11	VSD I 10.10	250.38	75.46	37.30	0.78
12	VSD I 10.18	219.10	63.20	46.40	0.66
13	VSD I 11.10	311.00	75.42	40.40	0.72
14	VSD I 11.22	286.00	91.18	36.80	0.63
15	VSD I 11.23	400.00	135.10	48.00	0.77
16	VSD I 13.11	450.00	105.10	45.32	0.86
17	VSD I 13.12	325.10	91.80	48.00	0.77
18	VSD I 13.13	360.20	96.40	50.60	0.63
19	VSD I 13.17	509.00	116.70	46.60	0.75
20	VSD I 14.6	300.00	78.90	50.40	0.74
21	VSD I 14.14	239.30	87.20	50.80	0.64
22	VSD I 14.15	458.30	144.70	51.40	0.79
23	VSD I 15.7	433.10	120.20	39.60	1.10
24	VSD I 15.10	500.00	121.50	53.40	0.88
25	VSD I 15.15	416.10	100.60	43.80	0.88
26	VSD I 16.4	325.10	86.35	48.40	0.87
27	VSD I 16.6	360.22	111.47	63.80	0.66
28	VSD I 16.10	183.10	60.20	48.20	0.54
29	VSD I 16.11	364.00	100.40	48.80	0.84
30	VSD I 17.2	309.00	98.50	48.00	0.68
31	VSD I 17.9	280.00	86.70	52.40	0.55
32	VSD I 18.12	550.40	110.20	41.90	0.93
33	VSD I 18.15	377.46	97.32	34.40	0.96
34	VSD I 19.6	404.00	120.60	45.80	0.68
35	VSD I 21.6	324.40	96.80	45.40	0.75
36	VSD I 21.27	404.00	75.20	47.60	0.75
37	VSD I 22.1	286.00	78.40	43.60	0.84
38	VSD I 23.24	210.00	67.88	33.60	0.56
39	VSD I 29.8	388.00	116.82	44.20	1.54
40	VSD I 29.11	204.00	69.00	40.40	0.77
41	VSD I 30.6	312.00	85.60	39.40	0.93
42	VSD I 30.7	297.70	100.00	46.40	0.73
43	VSD I 30.8	248.00	62.50	41.60	0.65
44	VSD I 31.7	380.00	98.60	55.60	0.65
45	VSD I 31.8	870.22	200.12	48.40	1.43
46	VSD I 31.9	416.00	139.90	56.40	0.73
	CV (%)	**10.24**	**9.44**	**12.64**	**10.22**
	CD (0.05)	**44.15**	**11.01**	**7.05**	**0.10**

Plate-1 Budding to confirm VSD resistance

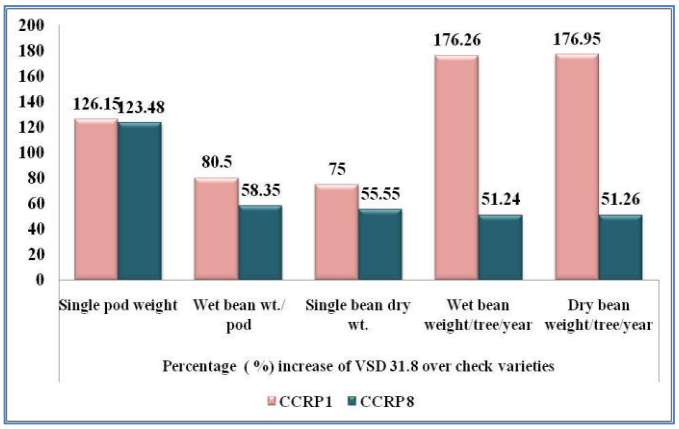

Fig-1 Percent increase in characters of VSD I 31.8 over check varieties (CCRP 1 and CCRP 8)

since it is least leveraged by the environment [46]. Significance of molecular markers in this study is that there is no other clear-cut validation procedure for quantifying disease resistance. ISSR and SSR primers are proved to be efficacious in tagging resistant genes by many workers [47-49]. The ISSR marker UBC857 and SSR marker mTcCIR42 was figured to be linked with VSD resistance by Chandrakant, (2014) [25] and further validated by Tulshiram, (2016) [26]. These markers when used to tag resistant gene in VSD I 31.8 they got expressed in the hybrid [Fig-2 and Fig-3] along with resistant genotype used in the early study but was absent in susceptible genotype. This confirmed that VSD I 31.8 is resistant to vascular streak dieback disease.

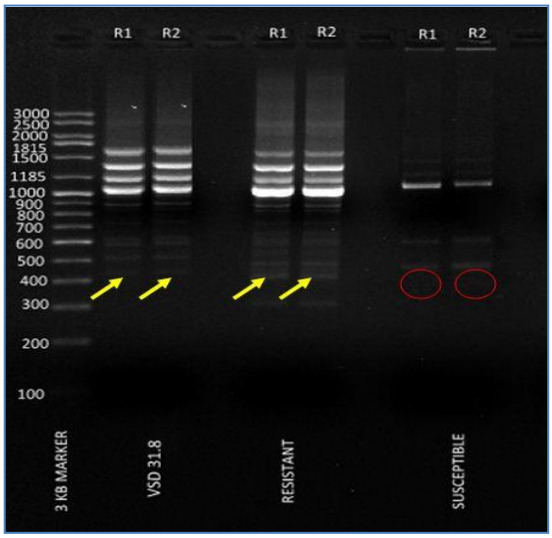

Fig-2 Profile of VSD I 31.8 using the ISSR primer UBC 857

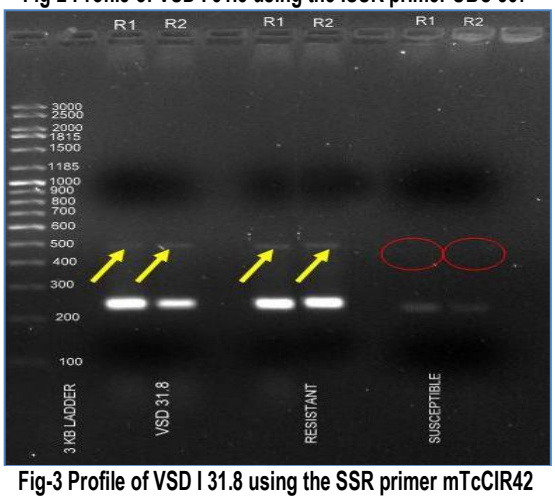

Fig-3 Profile of VSD I 31.8 using the SSR primer mTcCIR42

Table-6 *Genetic parameters of the hybrid population*

Characters	PCV (%)	GCV (%)	ECV (%)	H^2	GA (%)
Single pod weight (g)	62.09	59.38	2.71	91.45	37.23
Wet bean weight/ pod (g)	53.79	51.13	2.67	90.33	31.86
No. of beans/ pod	32.58	23.65	8.93	52.68	11.25
Single dry bean weight(g)	45.33	41.38	3.95	83.33	24.77
Wet bean weight/plant/year(kg)	70.22	68.47	1.75	95.09	43.78
Dry bean weight/plant/year(kg)	77.36	70.69	6.67	83.50	42.36

PCV and GCV [23]- Low: Less than 10%, Moderate: 10-20%, High: More than 20%
H2 [24]- Low: Less than 30%, Moderate: 30-60%, High: More than 60%
GG [24]- Low: Less than 10%, Moderate: 10-20%, High: More than 20%

Table-7 *Budding studies to confirm resistance in VSD I 31.8*

Hybrid	No. of plants budded	Bud take	No. of VSD affected plants
VSD I 31.8	78	20	0

Application of research: Evolving a hybrid demonstrating resistance to vascular streak die back disease is a pronounced achievement when the catastrophes engendered by this disease in other important cocoa growing countries are reviewed. In addition to this all the superior hybrids spotted out in this study will be advanced to establish polyclonal garden. Planting materials from this garden can take care of the spread of this disease in the whole country to a great extent.

Research Category: Resistance breeding, fungal disease, obligate parasite, hybrids

Abbreviations: VSD: vascular streak dieback disease, CCRP: Cadbury co-operative cocoa research project, CRD: completely randomized block design, PCV: phenotypic coefficient of variation, GCV: genotypic coefficient of variation, ECV: environmental coefficient of variation, GA: genetic advance, ISSR: inter simple sequence repeat, SSR: simple sequence repeat.

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