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SSR Marker Based DNA Fingerprinting for Cowpea Varieties of Tamil Nadu [*Vigna unguiculata* (L.) Walp.]

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A B S T R A C T

Morphological traits viz., flower colour, leaf shape, seed colour and seed shape are available to distinguish the new cowpea variety VBN 3 from the ruling varieties Vamban 1, and CO (CP) 7. However, all these varieties cannot be distinguished with a single character alone. A total of 25 SSR primers were used to differentiate VBN 3 from Vamban 1 and CO(CP) 7. Among these six primers *viz*., CEDG156, CP09781, CEDG171, CEDG127, CEDG008 and CEDG 305 had polymorphism among varieties. Primers *viz*., CEDG156, CP09781, CEDG171 and CEDG008 had polymorphism between Vamban 1 and VBN 3. Likewise, primers CEDG 156, CP09781, CEDG127 and CEDG305 had polymorphism between VBN 3 and CO(CP) 7. One primer, CP09781 was able to differentiate all three varieties. Hence these primers may be useful to differentiate these varieties at DNA level. These markers will be a potent tool in seed certification.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.], Fabaceae, (2n = 2x = 22) is an essential leguminous crop in less-developed countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia and Latin America (Singh *et al.*, 1997). Cowpea plays a major role in human nutrition not only because of its good protein quality with a high nutritional value. It provides strong support to the livelihood of small scale farmers through its contributions to their nutritional security, income generation and soil fertility enhancement. Worldwide about 6.5 million metric tons of cowpea are produced annually on about 14.5 million hectares. The low productivity of cowpea is attributable to numerous abiotic and biotic constraints. The abiotic stress factors comprise drought, low soil fertility and heat while biotic constraints include insects, diseases, parasitic weeds, and nematodes.

Progress has been made through conventional breeding at international and national research institutions in the last three decades towards the development of new varieties. Several morphological characters are available to differentiate the varieties. However appropriate growth stage is necessary to observe the differentiation. To overcome this

problem some of the techniques have currently in the utilization. One among the proven technique is DNA fingerprinting technology. DNA fingerprinting technique was invented by Alec Jeffreys during 1985. While it is very difficult task to tell the differences in a crop variety simply just by the morphological descriptors, DNA fingerprinting makes it possible for researchers to pinpoint specific fingerprint and accurately identify crop varieties. Several research organizations in India and across the globe have started offering DNA testing for plant varietal identification. DNA fingerprinting is used by plant breeders of both private and public sectors for identification for crop varieties. More importantly, Protection of Plant Variety and Farmers Right Authority (PPV & FRA, 2001), Govt. of India made a DNA fingerprint as an additional mandatory requirement for the release of new crop varieties released by the Variety Release Committee. With this background, an attempt was made in this study to identify a molecular marker to differentiate the newly released variety VBN 3 from the other ruling varieties of cowpea.

Materials and Methods

The experimental material comprises the new variety VBN 3 released from National Pulses Research Center, Vamban (Fig. 1) and check varieties *viz.,* Vamban 1 and CO(CP)7. The new variety VBN 3 is a cross derivative between TLS 38 x VCP 16-1. The duration ranges from75-80 days and suitable for cultivation in June – July and September – October season in Tamil Nadu. DNA fingerprinting work has carried out with a total of 25 markers. These were randomly selected from the SSR markers reported by Isemura *et al.,* (2012) in mungbean (Table 1).

DNA extraction was performed using CTAB procedure suggested by Doyle and Doyle (1987) with modifications. Young leaves were collected and ground using CTAB buffer of 500 µl with pinch of Poly Vinyl Pyrrolidone. Then the tubes were kept in water bath at 65˚C for 30 min. The sample were added with 500 µl of P: C: I (Phenol: Chloroform: Iso-amyl alcohol) in the ratio of (25:24:1), centrifuged for about 10 min at 10000 rpm and collected the supernatant. Again the supernatant was added with 400 µl of C: I (Chloroform: Iso-amyl alcohol in the ratio of 24:1). The tube was centrifuged for 5 min at 10000 rpm and collected the supernatant without disturbing the bottom layer. Then it was added with 300 µl of 100% ethanol, kept in deep freezer at 4˚C for 30 min and centrifuged for 10 min at 8000 rpm. The supernatant was discarded and allowed the pellets to air dry. The pellets were added with 50µl of TE Buffer and 3µl of RNAase. The samples were kept in water bath for 30min at 65˚C. Further the DNA was stored under -20˚C for future use.

The PCR profile starts with 94°C for 4min followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 1 minute. A final extension at 72°C for 20 minutes was included. The PCR was performed using thermocycler (Eppendorf, Germany). The final PCR product was electrophoresed using 3.5% agarose gel with 100 bp ladder. Agarose gel was documented using GELSTAIN 4x advanced gel documentation unit (Medicare, India).

Results and Discussion

The major task in the varietal release program is the identification of distinguishable morphological characteristics for the newly released varieties. Distinguishing morphological traits for VBN 3 and check varieties were presented in Table 2 and Figure 2. Among the cowpea varieties, VBN 3 is clearly distinct from CO(CP)7 for its leaf shape and seed shape.

Table.1 List of primers and its sequences

Primer codes	Forward/Reverse	Primer sequence 5'-3'
CEDG093	F	AAAACCCATGTAAAAGTTCA
	R	CAATCCATTCCCTTCTTAAT
CEDG128	F	CTGCCAAAGATGGACAACTTGGAC
	R	GCCAACCATCATCACAGTGC
CEDG254	F	CGATGTCTCTTGCTTCAAGG
	R	GTGAAGGACTAGCCAAGTTTG
CEDG136	F	GTTCCAAGTCTCCAATCCGTAC
	R	CACTTCACTAGAACTGGTTCAG
CEDG244	F	GCATATAAGAAAAGCTTATCC
	R	CTCTTGGAGTGATTTGATC
CEDG275	F	CACACTTCAAGGAACCTCAAG
	R	GTAGGCAACCTCCATTGAAC
CEDG117	F	GTACACTTCCACTAATCCAAAATT
	R	TGGTACCTTCCTTATCTGAAATTA
CEDG305	F	GCAGCTTCACATGCATAGTAC
	R	GAACTTAACTTGGGTTGTCTGC
CEDG139	F	CAAACTTCCGATCGAAAGCGCTTG
	R	GTTTCTCCTCAATCTCAAGCTCCG
CP04320	F	GTTTTCCAGTTTTCTGCATTCCAAC
	R	AACCATCAGCTTTCCTTTCAGACA
CEDG132	F	GGGTGTAATCCGTCAGAGGC
	R	CTTCCCCCTCTTCCGTTCTC
CEDG171	F	CTTGAGAACCAACTCGAACTTC
	R	GGGAAATCGAAGAGGGACAG
CEDG191	F	CAATAAGCAATCTGTGGAGAG
	R	CTGCAGGAAACTTGGAATTGC
CEDG245	F	GATAGAGCTTAAACCCTC
	R	CTTTTGATGACAAATGCC
CEDG174	F	GAGGGATCTCCAAAGTTCAACGG
	R	GAAGGCTCCGAAGTTGAAGGTTG
CEDG111	F	TGGAAGTTTCCAAGAGGGTTTTC
	R	TCTCACCACCTTTTACCTTCTCA
CEDG176	F	GGTAACACGGGTTCAGATGCC
	R	CAAGGTGGAGGACAAGATCGG
CEDG156	F	CGCGTATTGGTGACTAGGTATG
	R	CTTAGTGTTGGGTTGGTCGTAAGG
CEDG092	F	TCTTTTGGTTGTAGCAGGATGAAC
	R	TACAAGTGATATGCAACGGTTAGG
CP09781	F	CTGACGCATTCAGCATTTTACAGC
	R	GGAAATACGGTTGCGTCCATGTAT
CEDG008	F	AGGCGAGGTTTCGTTTCAAG
	R	GCCCATATTTTTACGCCCAC
CEDG141	F	CCAGGCATCCATGATGACC
	R	GAAGTTGTTGGTAATGGTTGCCTC
CEDG198	F	CAAGGAAGATGGAGAGAATC
	R	CCTTCTAAGAACAGTGACATG
CEDG127	F	GGTTAGCATCTGAGCTTCTTCGTC
	R	CTCCTCACTTGGTCTGAAACTC
CEDG020	F	TATCCATACCCAGCTCAAGG
	R	GCCATACCAAGAAAGAGG

Table.2 Distinguishing morphological traits for cowpea varieties

S. No	Morphological traits	Vamban 1	VBN 3	CO(CP)7
1.	**Flower colour**	Yellowish white	Light purple	Light purple
2.	**Leaf shape**	Sub globose	Sub globose	Sub hastate
3.	**Seed colour**	Creamy white	Light brown	Brown
4.	**Seed shape**	Kidney	Kidney	Rhomboid

Table.3 Polymorphic primers for the varieties Vamban 1, VBN 3 and CO(CP)7 with base pair differences

Primers/varieties	Base pair differences (bp)		
	Vamban 1	VBN 3	CO(CP)7
CEDG156	185	195	185
CP09781	290	260	300
CEDG171	195	205	205
CEDG127	260	260	290
CEDG008	110	130	130
CEDG305	130	130	120

Fig.1 Field view of VBN 3

Fig.2 Morphological differences among cowpea varieties: a) Flower colour; b) Leaf shape; c) Seed colour and d) Seed shape

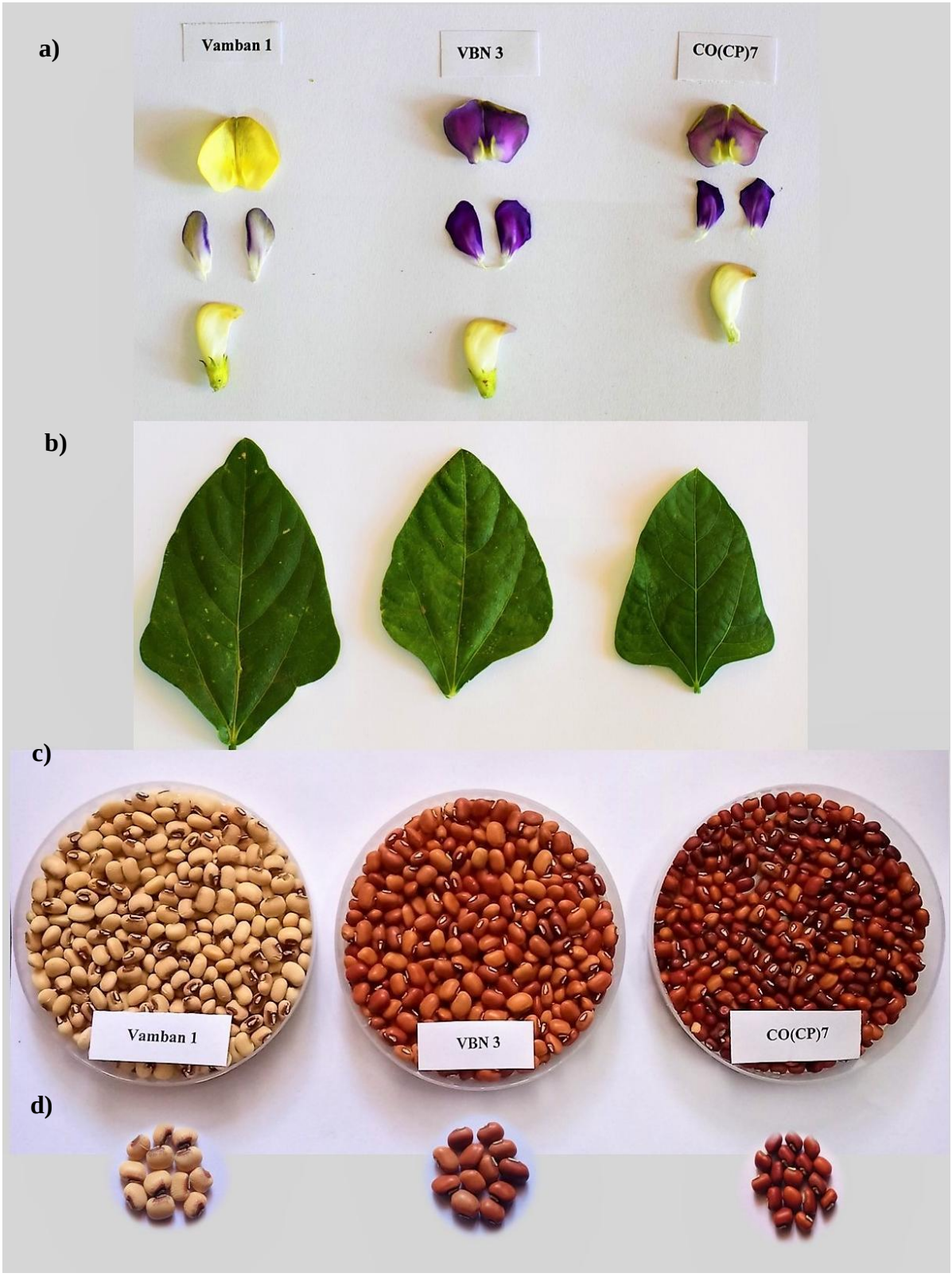
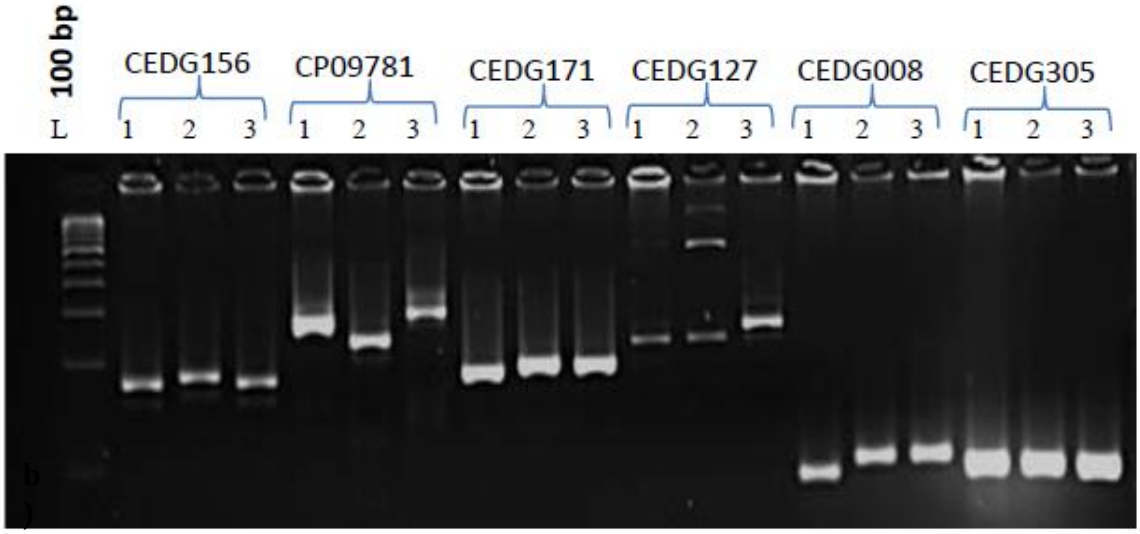


Fig.3 DNA fingerprinting for varieties Vamban 1, VBN 3 and CO(CP)7



1-Vamban 1; 2-VBN 3; 3-CO(CP) 7

The variety VBN 3 has sub globose leaf shape and kidney seed shape. However the variety CO(CP)7 has sub hastate leaf shape and rhomboid seed shape. Vamban 1 has similar leaf shape and seed shape of VBN 3. Likewise, VBN 3 can be differentiated from Vamban 1 for flower colour and seed colour. Variety VBN 3 has light purple and light brown as flower and seed colour respectively. While the variety Vamban 1 has yellowish white flower and creamy white seed colour. VBN 3 and CO(CP) 7 has similar flower colour and CO(CP) 7 has brown seed colour.

Distinguishing the closely related varieties is a difficult task. In that case morphological descriptors will lose its utility as it has limited classifications. Hence molecular level varietal differentiation has gained its importance for the varietal identification. DNA fingerprinting will be a better choice of obtaining clear differences to pinpoint the particular variety.

A total of 25 SSR primers were used to differentiate VBN 3 from Vamban 1 and CO(CP)7. These primers were selected from 11 linkage groups of mungbean reported by Isemura *et al.*, (2012). Among the primers six primers *viz*., CEDG156, CP09781, CEDG171, CEDG127, CEDG008 and CEDG 305 had polymorphism among varieties (Table 3 and Fig. 3). Primers CEDG156, CP09781, CEDG171 and CEDG008 had polymorphism between Vamban 1 and VBN3. Likewise, primers CEDG 156, CP09781, CEDG127 and CEDG305 had polymorphism between VBN3 and CO(CP)7. One primer, CP09781 was able to differentiate all three varieties. Hence these primers may be useful to differentiate these varieties at DNA level. This can be a potent tool in seed certification to find out the genetic purity at seed lot inspection itself.

To conclude, morphological descriptors are available to distinguish the varieties Vamban 1, VBN 3 and CO(CP)7. However, all these varieties cannot be distinguished with a single character alone. The DNA marker will be an added tool to distinguish varieties. Out of 25 primers tested, six primers were useful to aid in DNA fingerprinting to differentiate these varieties. Among these, CP09781 can able to

differentiate all the three varieties. These markers will be a potent tool in seed certification.

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