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SSR based Molecular Diversity and Marker-trait Association in Mungbean (*Vigna radiata* L.) under Deficit Moisture Stress

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ABSTRACT

Background: In recent years, breeding of climate resilient varieties particularly for deficit moisture stress is the major focus for sustainable crop production. Molecular markers being independent of environmental influence, SSR based genotyping and marker-trait association can address such a complex trait with utmost precision for molecular breeding in mungbean.

Methods: In the present study, ten SSR primer based genotyping was carried out in a set of elite germplasm lines to gauge genetic variation and explore marker-trait association under deficit moisture stress.

Result: The DNA amplification resulted as high as nine amplicons with 52.8% polymorphism. The primer GmDREB-2 was shown to be highly informative while TM-VF displayed high discriminative power for varietal identification. The wild accession *Vigna glabrescens* followed by Dhauli and Phulbani local produced unique molecular finger print by a number of primers and as such turned to be highly divergent genotypes. Only thirteen significant marker-trait associations were explored among which a 1610bp CodA_3 marker had shown strong association ($p<0.001$) with stress tolerance score and germination percentage under deficient moisture stress. While, GmDREB2_2, CodA_1 and TAA170_1 exhibited significant MTA with root length, ICCM 0249_5 with clusters/plant and both TAA 170_2 and RD 22_2 with seed yield/plant. The above trait-specific molecular markers may be considered useful for screening of parental lines and marker assisted selection in mungbean breeding program for deficit moisture stress tolerance.

Key words: Deficit moisture stress, Genetic diversity, Marker-trait associations, Mungbean, SSR primers.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek, 2n= 22] is the most important short duration pulse crop of India. It seems to be originated from *Vigna sublobata* in South Asia and belongs to the family Fabaceae. Nutritionally, mungbean is often preferred owing to its higher level of easily digestible protein (22.63-25.84%) (Idris *et al*., 2025), iron (5.9-7.6 mg/ 100g) (Dahiya *et al*., 2015), crude fiber (4.4%) (Offia and Madubuike, 2014) and low levels of oligosaccharides (Ihsan *et al.,*2013). It also harbors higher amount of vitamin A, vitamin B and phosphorous as compared to other grain legumes. Besides, sprouted mung bean is rich in vitamin C and bears traces of antinutritional factors without any health hazards.

Among a variety of abiotic stresses, deficit moisture stress alone affects more than 20% of agricultural lands world-wide (Rojas, 2020). It drastically affects plant growth (Jincya *et al*., 2019) by limiting the nutrient uptake (nitrogen, phosphorous, potassium and sulphur) and disrupts nitrate assimilation leading to alter amino acid concentrations and decrease in protein content in seeds. The tolerant cultivars show increased expression of transporters and aquaporins to maintain growth and metabolism under drought stress (Barzana *et al*., 2021). However, the progress of breeding for drought tolerance is indeed limited owing to its highly complex mechanism, time consumable selection of tolerant plants and expensive cost involved (Athar and Ashraf, 2009). Mungbean is considered sensitive to deficit moisture stress among the pulses (Dutta and Bera, 2008) in the order of lathyrus >horsegram> cowpea >pigeonpea> chickpea > lentil >mungbean>urdbean> fieldpea> rajmah (Pratap *et al*. 2019). It is reported to have narrow range of optimum osmotic adjustment (0.3-0.4MPa) as compared to other legumes (urdbean: 0-0.5, chickpea: 0-1.3, pigeonpea: 0.1-1.3 and ground nut: 0.2 - 1.6 MPa) and it may not be possible to revive the mungbean plants once it reaches lethal leaf water potential nearly to “1.9MPa (Pratap *et al*., 2019). Elucidation of the molecular basis for moisture stress tolerance is still not clear as compared to

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other important crops. A few candidate genes regulating production of osmolytes like proline, starch content, soluble sugars etc. have been reported for stress tolerance in legumes (Dar *et al*., 2016). Molecular finger prints, more specifically SSR (Simple Sequence Repeat) markers (Panigrahi *et al*. 2013) those linked to deficit moisture stress are worth trying for genotyping and marker-trait association. Therefore, we carried out molecular profiling based on gene specific SSR primers to explore allelic variation, genetic diversity and marker-trait association (if any) in a set of selected deficient moisture stress tolerant mungbean genotypes.

MATERIALS AND METHODS

In the present study, ten diverse mungbean core genotypes including susceptible checks (Dhauli and T34-1-5) for deficient moisture stress were selected from a set of 200 germplasm accessions comprising standard ruling varieties, important pre-released cultures and popularly adapted local land races (Ravada, 2023). These were rigorously screened twice based on germination percentage, drought tolerance score and seedling traits (shoot and root length and their fresh and dry weight) (Supplementary Table 1) under deficient moisture stress (-1.5 bar or -0.15 MPa corresponding to LD50) in growth chamber (25±1°C, RH: 68%, photoperiod 16/8h, light intensity 2000 lux) using PEG6000 (Ravada, 2023). The amount of PEG (in gram) required to impose deficit moisture stress at -1.5 bar was calculated as per Michel and Kaufmann (1973). Besides, the selected genotypes were replicated thrice in Randomized Block Design (RBD) and studied for ten agronomic traits (Supplementary Table 1) including seed yield over two years under deficit moisture stress (65% of field capacity) (in poly house) during late winter season.

Genomic DNA of each of the above genotypes was isolated following standard CTAB method (Doyle and Doyle, 1990). The crude genomic DNA was purified by DNase free RNase-A (GeNei), quantified using agarose electrophoresis followed by UV-VIS Nanodrop-2000 spectrophotometer at 260 nm and finally diluted to a working concentration of 10 ng/µl for PCR analysis.

Each individual RNA-free gDNA sample was primed and amplified using ten gene specific SSR primers (Table 1) in a reaction volume 20 µl. Amplifications were set up in a Gene Amp PCR (Applied Biosystems), programmed for 5 min at 94°C, 40 cycles of 1 min at 92°C, 1 min at varying annealing temperature specific to primer (53-58°C, Table 1) and 2 min at 72°C and final extension for 10 min at 72°C. The amplified DNA was electrophoresed in an agarose gel at a constant voltage of 50V and scanned by gel doc system (Fire Reader-Uvtec, Cambridge, UK) for detection of gene specific SSR alleles. The amplifications were checked at least twice for their reproducibility and the size of the amplicons was determined by comparing with the lambda DNA ladder (100 bp) with known size (bp) fragments.

Gels were scored for the presence (1) or absence (0) of bands. Polymorphism information content (PIC) and

Supplementary Table 1: Seedling and agronomic traits.

Genotype	Mean phenotypic (over two years) data (under drought stress)																		
	DT score	Germn. %	Shoot length	Root length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	DT score after PEG	DF	DM	PHT	NC	NP/C	NP/pl	PL	NS/P	SW	Y/pl.
GP 122= PUSA 9672	0	60.0	15.6	9.8	0.42	0.24	0.24	0.10	0	31.50	40.65	28.95	3.60	3.20	8.46	6.50	10.28	3.46	3.28
GP 134= RCM 14	1	80.0	14.2	9.2	0.438	0.12	0.22	0.09	1	31.77	41.27	31.88	3.21	2.14	6.91	6.47	8.83	3.55	2.43
GP 179= TARM 1	0	78.5	15.7	9.7	0.46	0.19	0.25	0.10	0	31.91	39.26	32.58	3.46	3.24	6.87	6.05	10.05	2.66	2.66
GP 129=RCM 5	0	73.3	9.5	9.5	0.24	0.11	0.20	0.08	0	31.77	38.27	31.90	3.28	2.21	6.74	5.32	9.02	3.45	2.85
GP 173=T 34-1-5	1	80.0	4.05	9.3	0.32	0.09	0.26	0.09	1	31.51	39.76	32.97	5.28	2.23	10.50	5.22	10.19	2.97	2.96
GP 227=SML 668	1	76.5	4.02	9.7	0.22	0.09	0.21	0.05	0	33.75	41.00	33.71	3.33	3.27	11.53	5.25	11.03	2.40	3.05
GP 62=OUM 99-4	2	66.7	14	9.7	0.438	0.12	0.23	0.10	1	30.69	40.69	41.25	6.78	2.35	14.36	6.05	10.58	2.33	3.60
GP 16= Dhauli	7	26.7	3.5	2.6	0.22	0.08	0.14	0.05	9	28.75	36.15	28.65	3.20	2.80	7.20	5.90	8.80	2.11	1.63
Phulbani Local	2	65.5	5.2	3.82	0.33	0.08	0.18	0.06	4	27.70	38.20	26.58	2.76	2.83	7.48	5.18	8.68	1.96	1.92
Vigna glaberescence	1	80.0	10.5	8.2	0.3	0.09	0.20	0.10	2	59.05	75.25	81.58	14.88	4.38	33.08	5.63	9.93	1.90	6.03

DT Score : Drought tolerance score.
DF: Days to 50% flowering, DM: Days to maturity, PHT: Plant height(cm), NC: No. of clusters per plant, NP/C: No. of pods per cluster, NP/Pl.: No. of pods per plant.
PL: Pod length(cm), NS/P: No. of seeds per pod, SW: 100-seed weight(g), Y/Pl.: Seed yield per plant(g).

Table 1: Amplified products and polymorhic information of a set of SSR primer pairs used in the study.

Primer	Source	Primer Sequence (3′-5′)	GC Content (%)	Tm (°C)	Tan (°C)	No. of monom--orphic alleles	No. of polym--orphic alleles	Total No. of alleles	% of polymo--rphism	MAF	PIC	Rp	Range of ampli-con size (bp)
ICCM-0249	Chickpea	5″-TTTCTTCGCATGGGCTTAAC-3″	45.00	55.25	54°C	0	8	8	100.0	0.90	0.806	5.4	120-1200
	(Nayak *et al.,* 2010)	5″-GGAGATTTGTTGGGTAGGCTC-3″	52.38	59.82									
TAA170	Chickpea	5″-TATAGAGTGAGAAGAAGCAAAGAGGAG-3″	40.74	61.93	53°C	1	3	4	75.0	1.00	0.505	4.8	180-800
	(Winter *et al.*,1999)	TATTTGCATCAATGTTCTGTAGTGTTT-3″	40.74	61.93									
TM-VF	*Vicia faba*	5″- CTTGCAGTCAAGCTCCCTTC-3″	55.00	59.35	54°C	2	5	7	71.4	1.00	0.48	8.8	300-1180
	(Amar *et al.*, 2016)	5′-CCTTGTCCCAAGACAGACCA-3″	55.00	59.35									
RD20A	Soya bean	5″- GTGGCACATGACTGAAGGAA	50.00	57.3	56°C	1	0	1	0.00	1.00	0	1	300
	(Neves-Borges	5″- ATCTTTCCAGCAGCACCTCT-3”	50.00	57.3									
	et al., 2012)												
RD22	Soya bean	5″- AATGCCGAAAGCCATTACAG	45.00	55.25	53°C	2	0	2	0.00	0.20	0.487	0.6	460-1110
	(Neves-Borges	5″- GCTTTGTTTTCCCTGCGTTA-3″	45.00	55.25									
	et al., 2012)												
GmDREB2	(Soya bean)	5″- GGGGAATTCATGGAAGAACCGGGTTTA-3″	48.15	64.97	58°C	0	2	2	100	0.10	0.99	0.4	820-1140
	(Savitri *et.al*., 2019)	5″- GGGCTCGAGATCTTCAGGTTTGGGATA- 3″	51.85	66.48									
codA gene	Mungbean	5′- CCACCATGATATTCGGCAAC-3′	50.00	57.3	53°C	0	9	9	100	0.70	0.088	7.2	380-2800
	(Baloda *et al*., 2017)	5′- GTGGAGGCTATTC GGCTA -3′	55.56	55.97									
HSP20	Mungbean	5′- AGGTGGAAGTGGAAGATGGG-3′	55.00	59.35	57°C	0	1	1	100	1.00	0	2	130
	(Tian *et al.,* 2016)	5′- GGAAGCCAGAACCTCCTCAT-3′	55.00	59.35									
Ap2	Mungbean	5′- TTGATGGAAGCTGGGGTGAT-3′	50.00	57.3	56°C	0	1	1	100	1.00	0	2	140
	(Tian *et al*., 2016)	5′- CTCAGAAAACGCCTGCCATT-3′	50.00	57.3									
VrUBC1	Mungbean	5′- GGCCTCTAAGCGGATTCTGAAG-3′	50.00	62.72	57°C	0	1	1	100	1.00	0	2	78
	(Chung *et al*., 2013)	5′- CTAGCCCATTGCATACTTCTGAGTCC-3′	50.00	64.8									

Note : Tm: Primer melting temperature, Tan: Primer annealing temperature, MAF: Major allele frequency, PIC: Polymorphic information content, Rp: Resolving power.

resolving power (Rp) of each primer was estimated as per Prevost and Wilkinson (1999). Besides, the binary data matrix of the unweighted paired 1/0 score was analyzed for estimation of similarity coefficients and construction of Dendrogram as per Jaccard (1908) using computer program for Numerical Taxonomy and Multivariate analysis system (NTSYS-PC), Version 2.02 (Rohlf, 2002). Similarity matrix was also used for principal coordinate analysis (PCA) comprising first three Eigen vectors (PCA 1, PCA 2 and PCA 3) to reveal spatial distribution of test genotypes in three dimensional spaces.

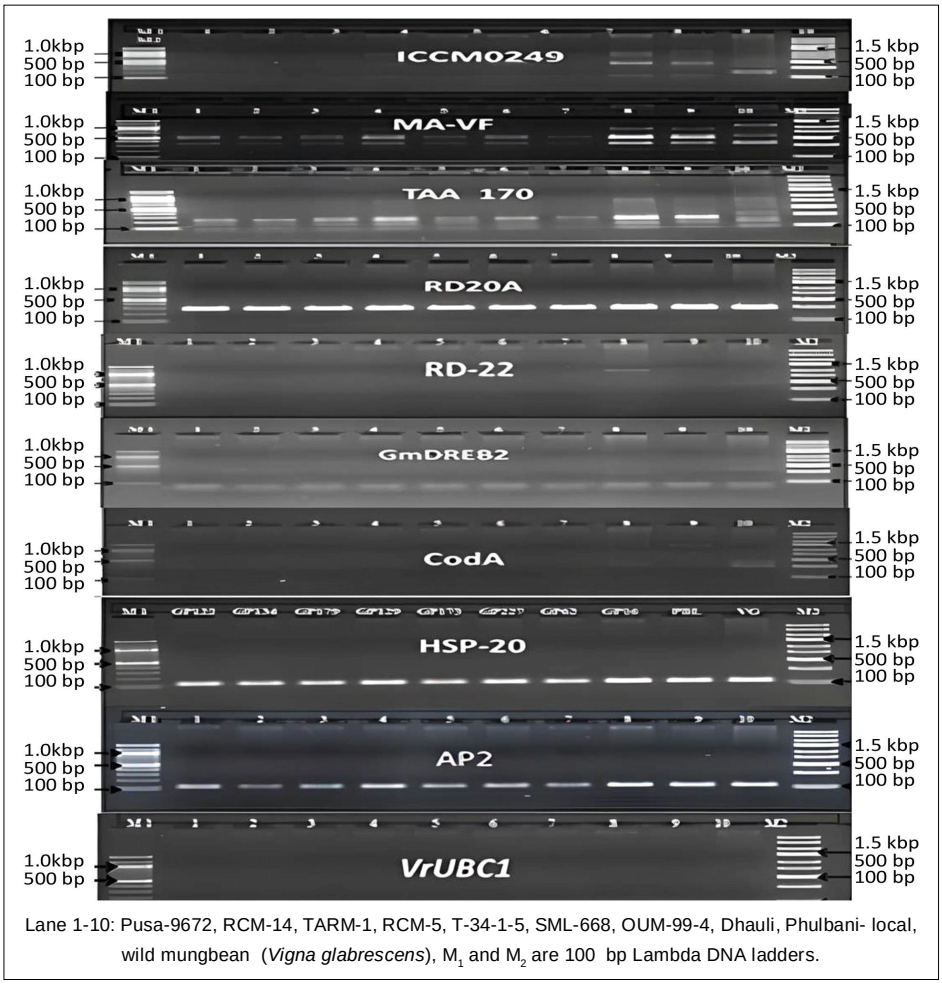
Besides, the Marker-trait Association (MTA) of different molecular markers was established using regression analysis (Elakhdar *et al*., 2016) as per SPSS (Statistical Package for Social Sciences) Software, version 16. The Marker-Trait Association with probability value at least less than 0.05 is considered a statistically significant relationship between a marker with the trait concerned.

RESULTS AND DISCUSSION

Deficit moisture stress is indeed a global problem for crop production. Ample genetic and genomic resources are now available in mungbean and related *Vigna* crops, which can be exploited for the development of climate smart mungbean cultivars. There is a need to explore suitable diverse genotype (s) with assured level of deficit moisture stress tolerance. In this context, study of genetic variation based on molecular markers (Tripathy *et al.,* 2015; Baisakh *et al.*, 2021) and follow-up tagging of appropriate marker(s) with stress tolerant trait (s) using marker-trait association (Dash *et al*., 2022) can be a way forward for identification of elite genotypes.

SSR based genotyping

Simple sequence repeat (SSR) markers are excellent for genotyping and assessment of stress tolerance (Younis *et al.,* 2020) with the advantages of being codominant, abundant, highly reproducible, highly polymorphic and easy to assay. In the present set of materials, only ten primers out of 50 SSR primers (Supplementary Table 2), amplified 36 amplicons (Supplementary Table 3) with an average of 3.6 alleles per primer. Chen *et al*. (2015) got amplified products only in 58 among 500 SSR primers while evaluation of genetic diversity in a panel of 157 cultivated and wild mungbean accessions. In contrast, 2-3 primers



Lane 1-10: Pusa-9672, RCM-14, TARM-1, RCM-5, T-34-1-5, SML-668, OUM-99-4, Dhauli, Phulbani- local, wild mungbean (*Vigna glabrescens*), M_1 and M_2 are 100 bp Lambda DNA ladders.

Fig 1: DNA profile of a set of selected moisture stress tolerant mungbean genotypes including susceptible checks.

Suppl. Table 2: List of SSR primers screened.

Markar	Forward primer seq	Reverse primer seq	crop	Reference
Satt383	CGATTACACCACG ATAATTTCCTCTG	CTTTCCATAATTTGGCAACCTCTTAG	soybean	Mishra *et al.*, 2022
Satt557	GCGGGATCACCA TGTAAATAGTG	GCGCTCAGCCTTAAATT TGAA	soybean	Mishra *et al.*, 2022
ICCM0249	TTTCCTGCAGGGCTAAC	GGAGATTTGTGGGTAGCCTC	chikpea	Nayak *et al.*, 2010
CEDG144	CAGTTACGAGTCTTGAACTTCAGC	CAGCTCTGTACAAAGCTGTAACTG	Mungbean	Suman *et al.*, 2019
STMS11	GTAACTTGTAATAATTCCTCCTCT	ATACATAACCCCCAAC	chikpea	Winter *et al.*, 1999
Satt009	CAACTTGAAATTACTAGAGAAAT	CTTACTAGCGT ATTAACCCTTG	Soybean	Cregan *et al.*, 1999
Satt197	CACTGCTTTTTCCCCTCTCT	AAGATACCCCCAACATTATTTGTAA	Soybean	Cregan *et al.*, 1999
SoySSR9.1	GGAAGATTGAT TCAAAAGTCA	CGAGAAAGTGATTGTGAGAA	Soybean	Nugroho *et al.*, 2020
Satt540	CTGGCGAATCAAGCTTTGTAAC	CCGTGATTGCGAAGAGGATATT	Soybean	Mishra, 2022
Ts72	CAAACAATCACTAAAAGTATTTGCTCT	AAAAATTGATGGACAAGTGTTATTATG	Chikpea	Winter *et al.*, 2000
TA47	TTTTTATAGGTGTCTTTTTGTTGTCTTT	TCTGAATAGGAAATAAGAAAGGTAGGTT	Chikpea	Winter *et al.*, 2000
RD20A	GTGGCACATGACTGAAGGAA	ATCTTTCCAGCAGCACCTCT	Soya bean	Neves-Borges et.al.,2012
TA37	ACTTACATGAATTATCTTTCTTGGTCC	CGTATTCAAATAATCTTTCATCAGTCA	Chikpea	Winter et al.,2000
TA25	AGTTTAATTGGCTGGTTCTAAGATAAC	AGGATGATCTTTAATAAATCAGAATGA	Chikpea	Winter et al.,2000
VrDREB2A	CTGCTCTTGCTTATGATGAA	ATGTAGTGGTAGTAGTAGTAGTG	Mungbean	Alsamadany, 2022
VrbZIP17	GGCATCATCATCTCCATG	GAGTTTGAGGTCGTTCCA	Mungbean	Alsamadany, 2022
VrHsfA6a	GCTTGCTATGAACATGGAGGA	TCATTATCATCATTTTCCAATGC	Mungbean	Alsamadany, 2022
Ap2	TTGATGGAAGCTGGGGTGAT	CTCAGAAAACGCCTGCCATT	Mungbean	Tian *et al.*, 2016
CEDG066	AGTAAAACAAGAACCCTCCCAAG	GTATTAAAATTTGGGGTGGTGG	Mungbean	Suman *et al.*, 2019
CEDG071	GGTCCATTGAGACGGATCGAG	TCCCACCTCAGCGGAATCC	Mungbean	Suman *et al.*, 2019
RD29B	AGCTGGAAGAGCCATCATG	CTCTGTCAGGGGACTGAGCAA	Soybean	Neves Borges *et al.*, 2012
ERD1	CGTCCAGAATTTGCTCAACAG	TGGGGTTATAGCCTTGTTGG	Soybean	Neves Borges *et al.*, 2012
FBOX	CTAATGGCACAATTGCAGCTCTC	AGATAGGGAAGATGGTGCAGGT	Soybean	Neves Borges *et al.*, 2012
LEA	CAGTGGCAGTGGGTGGTG	CAGGGTTATGGGACGCAAGG	Mungbean	Tian *et al.*, 2016
CP47	TGGGTGTCTGATCCTTATGGAC	CCTGCCGCAATATGATGAGAAG	Mungbean	Tian *et al.*, 2016
codA gene	CCACCATGATATTCGGCAAC	GTGGAGGCTATTC GGCTA	Mungbean	Baloda *et al.*, 2017
HSP20	AGGTGGAAGTGGAAGATGGG	GGAAGCCAGAACCTCCTCAT	Mungbean	Tian *et al.*, 2016
SUT	TGTCTCCGTCACAGCACCAC	GGAGGAGCCAACCAAGTTACAG	Mungbean	Tian *et al.*, 2016
ADH	CGAACCTGAAGCGTGTCC	CGAGTTGCGGAAGATGCC	Mungbean	Tian *et al.*, 2016
FAH	TGACAGCAGCGACGAGGTAGG	AGGACCAGGAGCAGCCATCAC	Mungbean	Tian *et al.*, 2016
AP2	TTGATGGAAGCTGGGGTGAT	CTCAGAAAACGCCTGCCATT	Mungbean	Tian *et al.*, 2016
SMP	GTCACCGCCTTATCAGCTTG	CCACACATAACACACGCACT	Mungbean	Tian *et al.*, 2016
VgActin	TTCCTTTTCTTCGTTTTTCATGCT	AAACCGAGGCACCTAATTCTTG	Mungbean	Tian *et al.*, 2016
ICCM0249	TTTCTTCGCATGGGCTTAAC	GGAGATTTGTTGGGTAGGCTC	Chickpea	Nayak *et al.*, 2010
TAA170	TATAGAGTGAGAAGAAGCAAAGAGGAG	TATTTGCATCAATGTTCTGTAGTGTTT-3"	Chickpea	Winter *et al.*, 1999
TM-VF	CTTGCAGTCAAGCTCCCTTC	CCTTGTCCCAAGACAGACCA	Vicia faba	Ammar *et al.*, 2017
SODC	ACAAGACCAGAAACACGAACAG	CCACCTCCTCCTCAATCCATAG	Mungbean	Tian *et al.*, 2016

Supplementary Table 2: Continue....

Supplementary Table 2: Continue....

RD22	AATGCCGAAAGCCATTACAG	GCTTTGTTTTCCCTGCGTTA	Soya bean	Neves-Borges *et al.*, 2012
GmDREB2	GGGGAATTCATGGAAGAACCGGGTTTA	GGGCTCGAGATCTTCAGGTTTGGGATA	Soya bean	Savitri *et al.*, 2019
ACT11	CGGTGTTTCATCTTGGGATC	GTCTTTCGCTCAATAACCCTA	Soybean	Neves-Borges *et al.*, 2012
HSP20	AGGTGGAAGTGGAAGATGGG	GGAAGCCAGAACCTCCTCAT	Mungbean	Tian *et al.*, 2016
SoySSR5.1	ATGAAGCCCAGAGAGTACAAC	CCCAAGTCTGAACATTACTCA	Soybean	Nugroho *et al.*, 2020
VrUBC1	ATGGCCTCTAAGCGGATTCTGAAG-3'	CTAGCCCATTGCATACTTCTGAGTCC	Mungbean	Chung *et al.*, 2013
DMB-SSR182	TAGAGCCTTCTGGTTTTTCACA	AGGAGGAGGATTTTGATGATGA	Mungbean	Suman *et al.*, 2019
CEDG068	TCTCCATAGGAACCCCTGAAAG	TGGGATCAGTGAATTCGCCAG	Mungbean	Suman *et al.*, 2019
CEDGAG001	CTCATCAGGGACATCCTCCC	GATCGTGATCGATCCAACGGTC	Mungbean	Suman *et al.*, 2019
CEDG008	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC	Mungbean	Suman *et al.*, 2019
CEDG056	TTCCATCTATAGGGGAAGGGAG	GCTATGATGGAAGAGGGCATGG	Mungbean	Suman *et al.*, 2019
CEDG091	CTGGTGGAACAAAGCAAAAGAGT	TGCGTCTTGGTGCAAAGAAGAAA	Mungbean	Suman *et al.*, 2019
CEDG264	GATTCCCTTCCTAGCTATGG	CTGCTGGACATGAAGATTCAG	Mungbean	Suman *et al.*, 2019

Supple. Table 3: Genotyping data

	Amplicons	ICCM 0249_1	ICCM 0249_2	ICCM 0249_3	ICCM 0249_4	ICCM 0249_5	ICCM 0249_6	ICCM 0249_7	ICCM 0249_8	TAA 170_1	TAA 170_2	TAA 170_3	TAA 170_4	MA-VF_1	MA-VF_2	MA-VF_3	MA-VF_4	MA-VF_5	MA-VF_6	MA-VF_7	RD-20A_1	RD 22_1	RD-22_2	GmDREB2_1	GmDREB2_2	Cod A_1	Cod A_2	Cod A_3	Cod A_4	Cod A_5	Cod A_6	Cod A_7	Cod A_8	Cod A_9	HSP 20_1	Ap 2_1	Vr UBC1_1
Genotype	Size (bp)	1200	1150	980	740	600	490	400	120	800	320	250	180	1180	1000	950	610	520	350	240	300	1110	460	1140	820	2800	2700	1610	1350	810	750	630	490	380	130	140	78
GP122= PUSA 9672		0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1
GP134= RCM14		0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
GP179= TARM 1		0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1
GP129=RCM5		1	0	1	0	0	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	1
GP173=T 34-1-5		0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
GP227=SML668		0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1
GP62=OUM 99-4		0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
GP16= Dhauli		1	0	1	0	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1
Phulbani Local		1	1	0	1	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0	0	1	1	0	1	0	0	1	1	1	1	1	1
Vigna glaberescence		0	0	1	0	1	0	1	0	1	1	0	1	1	0	0	1	0	1	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1

considered to be sufficient to distinguish between cultivars of broccoli (Hu and Quaros, 1991).

CodA revealed maximum number of polymorphic bands (9) with the widest size variation of amplicons (380-2800 bp) followed by the Primer ICCM-0249 (Fig 1, Table 1), while HSP-20, AP-2 and Vr UBC1 produced only one fragment each. Higher polymorphism of the above marker may be attributed to appreciable variation in number of tandem repeats within the core sequence in the genome across the panel of genotypes under study. Overall, 19 polymorphic bands were recorded out of total 36 bands resulting 52.8% polymorphism indicating subtle response of some primers towards genetic variation. While, Mathivathana *et al.* (2018) reported polymorphism only in primers: VrSSR_6_746 and VrSSR_1_31 out of ten primers used across six mungbean genotypes.

Presence or absence of specific band is the inherent feature of each genotype. Primer GmDREB 2 and ICCM-0249 produced 820 bp and 740 bp amplified products which were specific to Dhauli and Phulbani local respectively (Supple. Table 3). *Vigna glabrescens* produced unique molecular finger print by a number of primers *e.g.*, ICCM-0249, TAA-170, MA-VF, RD 22, GmDREB-2 and Cod-A which amplified 600 bp, 320 bp, 1180 bp, 460 bp, 1180 bp, 460 bp, 1140 bp and 810 bp amplicons respectively. In contrast, all test genotypes except *Vigna glabrescens* amplified 250 bp and 520 bp allele by primer TAA-170 and MA-VF respectively. These results suggest that SSR markers can be used as an accurate and efficient tool for identification of mungbean genotypes (Liu *et al.*, 2013).

Polymorphism information content (PIC) is a measure of allelic diversity (Table 1). The primers ICCM-0249 and GmDREB 2 may be considered highly informative as they revealed 100% polymorphism and PIC value more than 0.5 and the maximum value (0.99) being recorded by the later. Major allele frequency (MAF) being the distinct feature of a primer, ranged from 0.1 for GmDREB-2 to 1.0 for the SSR primer TAA-170,TM-VF,RD-20-A, HSP-20, AP-2 and VrUBC1 (Table 1). While, resolving power (Rp) was estimated to be as high as 8.8 in TM-VF indicating its immense discriminative power for varietal identification.

Molecular diversity

Available literature revealed use of SSR markers for study of genetic diversity in germplasm (Liu *et al.,* 2013; Ganguly and Bhat, 2012). *Vigna glabrescens* maintained high genetic dissimilarity with TARM-1(0.32), OUM-99-4(0.34), Phulbani local (0.35) and RCM-14(0.36) (Table 2) indicating better scope of using them in hybridization to achieve transgressive segregants. Similar molecular diversity has

Table 2: Similarity coefficient values between paired test genotypes for DNA profiles using SSR markers.

Genotype	Pusa-9672	RCM-14	TARM-1	RCM-5	T 34-1-5	SML -668	OUM-99-4	Dhauli	Phulbani Local	*V. glabrescens*
	1	2	3	4	5	6	7	8	9	10
PUSA -9672	1.0									
RCM -14	0.76	1.0								
TARM-1	0.70	0.92	1.0							
RCM-5	0.62	0.59	0.54	1.0						
T-34-1-5	0.65	0.84	0.77	0.50	1.0					
SML-668	0.94	0.72	0.66	0.60	0.61	1.0				
OUM-99-4	0.59	0.77	0.83	0.45	0.91	0.58	1.0			
Dhauli	0.63	0.48	0.44	0.75	0.41	0.66	0.37	1.0		
Phulbani local	0.55	0.46	0.42	0.68	0.38	0.59	0.34	0.79	1.0	
V. glaberesence	0.52	0.36	0.32	0.43	0.39	0.50	0.35	0.41	0.35	1.0

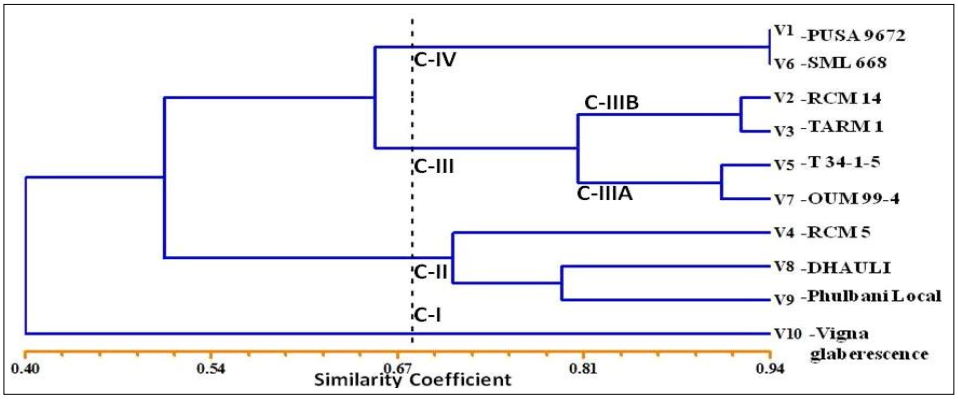


Fig 2: UPGMA based clustering of a set of 10 mungbean test genotypes using SSR data.

been also reported in many legume crops e.g., mungbean (Saini *et al*., 2010), common bean (Galvan *et al*., 2003) and chickpea (Rakshit *et al*., 2003).

The grouping of genotypes using UPGMA analysis (Fig 2) was found to be more or less consistent with that of three dimensional scaling based on PCA values (Fig 3). The erstwhile mentioned divergent genotypes (*Vigna glabrescens* with TARM-1, OUM-99-4 and RCM-14) which were initially separated from rest of the test genotypes in the UPGMA clustering were also shown to be sorted out to diverse extreme positions in case of three- dimensional scaling with vectors (Fig 3). This supports the findings of Tripathy *et al.* (2015) in mungbean and Baisakh *et al.* (2021) in urdbean. However, the deficit moisture stress tolerant genotypes *e.g*., SML-668 and PUSA-9672 with inherent high yield potential (> 3.0 g/plant) (Ravada, 2023) formed a distinct cluster (Fig 3). Therefore, the above divergent and high yielding test genotypes may serve as valuable materials for further genetic improvement in mungbean using recombination breeding.

Marker-trait association (MTA) under deficit moisture stress

Deficient moisture stress tolerance is a highly complex trait having involvement of a number of genetic factors. Breeding for the trait in mungbean can be accomplished by using MTA (Elakhdar *et al*., 2016), which enables indirect selection on markers avoiding the phenotypic assessment of traits. But, untill now, little information is available on MTA for abiotic stresses except cold tolerance for 230 germplasm lines in mungbean (Dash, 2021) and frost tolerance for

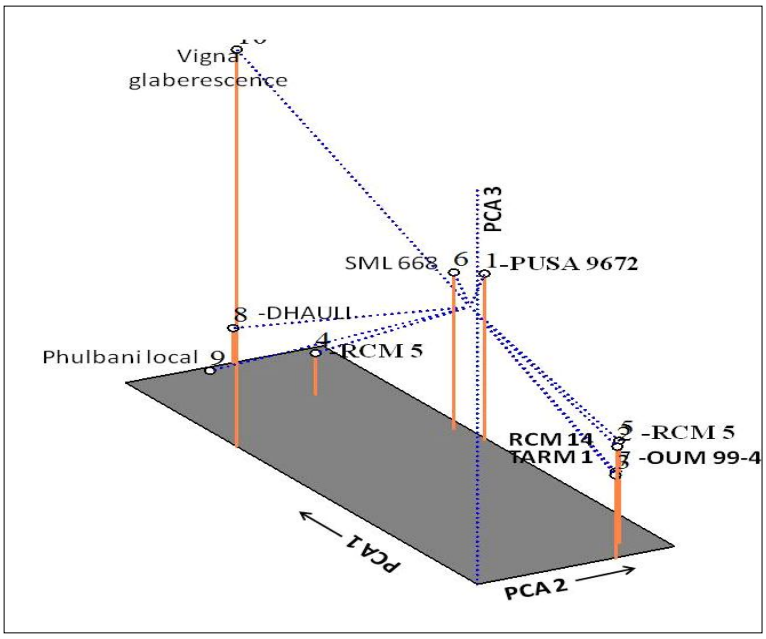


Fig 3: 3D -PCA plot of ten mung bean test genotypes using SSR data.

Table 3: Marker trait association using deficit moisture stress related SSR markers.

Phenotypic trait	Primer	Molecular marker	b-value	P-value	R^2
Deficit moisture stress tolerance score	ICCMO249	ICCMO249_6	2.619**	0.06	0.374
	CodA	CodA_3	6.1**	<0.001	0.873
Germination %	RD 22	RD 22_1	-28.2**	0.017	0.530
	CodA	CodA_3	-46.69**	<0.001	0.812
Root length	GmDREB2_2	GmDREB2_2	-6.169**	0.016	0.537
	CodA	CodA_1	-4.065**	0.015	0.544
	TAA170	TAA170_1	-3.537**	0.028	0.471
No. of Clusters/pl	ICCM0249	ICCM0249_5	11.002**	<0.001	0.890
	CodA	CodA_4	-5.717**	0.013	0.561
No. of seeds/pod	CodA	CodA_1	-1.294**	0.014	0.554
Seed yield/pl	TAA170	TAA170_2	3.321**	0.001	0.757
	RD 22	RD 22_2	3.321**	0.001	0.757
	CodA	CodA_4	-1.65**	0.038	0.437

672 worldwide pea (*Pisum sativum* L.) collections (Liu *et al*., 2017). The MTA can be performed either using ANOVA based on marker genotypes or a regression testing for a linear trend of marker alleles. In addition, because of the lack of distinct trait similarities, MANOVA seems to be not an ideal option to test for marker-trait associations (Bodah *et al*., 2017). Therefore, MTA was established based on regression analysis which is considered as more reliable approach. In the present study, linear regression analysis of each SSR allele on ten agronomic traits including seed yield, seedling parameters (germination % and seedling growth traits) and deficient moisture stress tolerance score (under stress using PEG) was carried out to explore significant MTA. Out of several possible combinations, we revealed thirteen significant MTAs (Table 3) under deficit moisture stress.

CodA amplified nine amplicons within the range of 2800 bp to 380 bp position in the electrophoregram. Out of these, the 1610 bp marker designated as CodA_3 was shown to be strongly associated ($p<0.001$) with deficient moisture stress tolerance score (1-9 scale) and germination percentage in a panel of 10 selected mungbean core germplasm lines comprising tolerant, moderately tolerant and sensitive genotypes with 87.3% and 81.2% of phenotypic variation respectively (Table 3). Similarly, Dash *et al.,* (2022) tagged few molecular markers with shoot and fruit borer resistance in brinjal. In our study,the markers ICCMO249_6 and RD-22_1 also revealed significant association with deficient moisture stress tolerance score and germination percentage. Besides, root trait is an important parameter which was affected significantly under deficit moisture stress using PEG (Ravada, 2023). Primer GmDREB2_2 revealed a significant marker-trait association with root length explaining 53.7% phenotypic variation for the trait. Hence, such above molecular fingerprints seem to be useful for validation and screening of mungbean germplasm under deficit moisture stress. The amplicon designated as CodA_1 produced by the primer CodA had shown significant marker-trait association with root length as well as number of seeds/pod and explained about 55% of the phenotypic variation for the traits. Available literature for MTA with regard to seed traits in mungbean is indeed scanty. Lucas *et al*., (2013) revealed haplotypes determining seed size in *Vigna unguiculata* using association studies and legume synteny. The marker CodA_4 had shown significant association with cluster/plant. Such component trait being an important determining factor for seed yield, the said marker (CodA_4) eventually also maintained significant association with the trait. Besides, the markers TAA170_2 and RD-22_2 also had shown significant association with seed yield and explained around 75% of the phenotypic variation. MTA Analysis of seed traits in accessions of common bean (*Phaseolus vulgaris* L.) has been investigated using genome-wide association studies (GWAS) (Lei *et al*. 2020), They identified 21 significantly associated markers for four seed traits including 100-seed dry weight.

CONCLUSION

The present investigation displayed genotype-specific polymorphism based on SSR based genotyping. *Vigna glabrescens,* Dhauli and Phulbani local emerged as highly divergent from high yielding cultivars cv. SML-668 and PUSA-9672. A few molecular markers strongly associated with seed yield as well as seedling traits related to moisture deficit stress tolerance. Thus, the MTA established in the present study would serve as immense value for marker assisted back cross breeding in mungbean to screen high yielding plants with deficit moisture stress tolerance.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

All authors declare that they have no conflicts of interest.

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