



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 Dhauri 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/100 g) (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 (Jincy *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

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>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 -1.9 MPa (Pratap *et al.*, 2019). Elucidation of the molecular basis for moisture stress tolerance is still not clear as compared to

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 (Dhauri 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 16/8 h, 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	DT score	Germn. %	Shoot length	Root length	Mean phenotypic (over two years) data (under drought stress)										NP/pl	PL	NS/P	SW	Y/pl.
					Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	DT score after PEG	DF	DM	PHT	NC	NP/C					
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= Dhauri	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 glabrescence	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 polymorphic information of a set of SSR primer pairs used in the study.

Primer	Source	Primer Sequence (3'-5')	GC content (%)	T _m (°C)	T _{an} (°C)	No. of monomorphic alleles	No. of polymorphic alleles	Total No. of alleles	% of polymorphism	MAF	PIC	R _p	Range of amplicon size (bp)
ICCM-0249	Chickpea (Nayak <i>et al.</i> , 2010)	5'-TTTCTTCGCATGGGCTTAAAC-3'	45.00	55.25	54°C	0	8	8	100.0	0.90	0.806	5.4	120-1200
TAA170	Chickpea (Winter <i>et al.</i> , 1999)	5'-GGAGATTTGTTGGGTAGGCTC-3'	52.38	59.82	53°C	1	3	4	75.0	1.00	0.505	4.8	180-800
		5'-TATAGAGTGAGAAAGACAAAGAGGAG-3'	40.74	61.93									
TM-VF	<i>Vicia faba</i> (Amar <i>et al.</i> , 2016)	TATTTGCATCAATGTTCTGTAGTGTTC-3'	40.74	61.93	54°C	2	5	7	71.4	1.00	0.48	8.8	300-1180
		5'-CTTGCACTCAAGCTCCCTTC-3'	55.00	59.35									
RD20A	Soya bean (Neves-Borges <i>et al.</i> , 2012)	5'-CCTTGTCCTCAAGACAGACCA-3'	55.00	59.35	56°C	1	0	1	0.00	1.00	0	1	300
		5'-GTGGCACATGACTGAAGGAA	50.00	57.3									
RD22	Soya bean (Neves-Borges <i>et al.</i> , 2012)	5'-ATCTTTCCAGCAGCACCTCT-3'	50.00	57.3	53°C	2	0	2	0.00	0.20	0.487	0.6	460-1110
		5'-AATGCCGAAAGCCATTACAG	45.00	55.25									
GmDREB2	Soya bean (Savitr <i>et al.</i> , 2019)	5'-GCTTTGTTTCCCTGCGTTA-3'	45.00	55.25	58°C	0	2	2	100	0.10	0.99	0.4	820-1140
		5'-GGGGAATTCATGAAGAACCGGTTTA-3'	48.15	64.97									
codA gene	Mungbean (Baloda <i>et al.</i> , 2017)	5'-GGGCTCGAGATCTTCAGGTTTGGGATA-3'	51.85	66.48	53°C	0	9	9	100	0.70	0.088	7.2	380-2800
		5'-CCACCATGATATTCGGCAAC-3'	50.00	57.3									
HSP20	Mungbean (Tian <i>et al.</i> , 2016)	5'-GTGGAGGCTATTC GGCTA -3'	55.56	55.97	57°C	0	1	1	100	1.00	0	2	130
		5'-AGGTGGAAGTGGAAGATGGG-3'	55.00	59.35									
Ap2	Mungbean (Tian <i>et al.</i> , 2016)	5'-GGAAGCCAGAACCTCCTCAT-3'	55.00	59.35	56°C	0	1	1	100	1.00	0	2	140
		5'-TTGATGGAAGCTGGGGTGAT-3'	50.00	57.3									
VrUBC1	Mungbean (Chung <i>et al.</i> , 2013)	5'-CTCAGAAAACGCCCTGCCATT-3'	50.00	57.3	57°C	0	1	1	100	1.00	0	2	78
		5'-GGCCTCTAAGCGGATTCTGAAG-3'	50.00	62.72									
		5'-CTAGCCCATTCGATACCTTCTGAGTCC-3'	50.00	64.8									

Note : T_m: Primer melting temperature, T_{an}: Primer annealing temperature, MAF: Major allele frequency, PIC: Polymorphic information content, R_p: 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

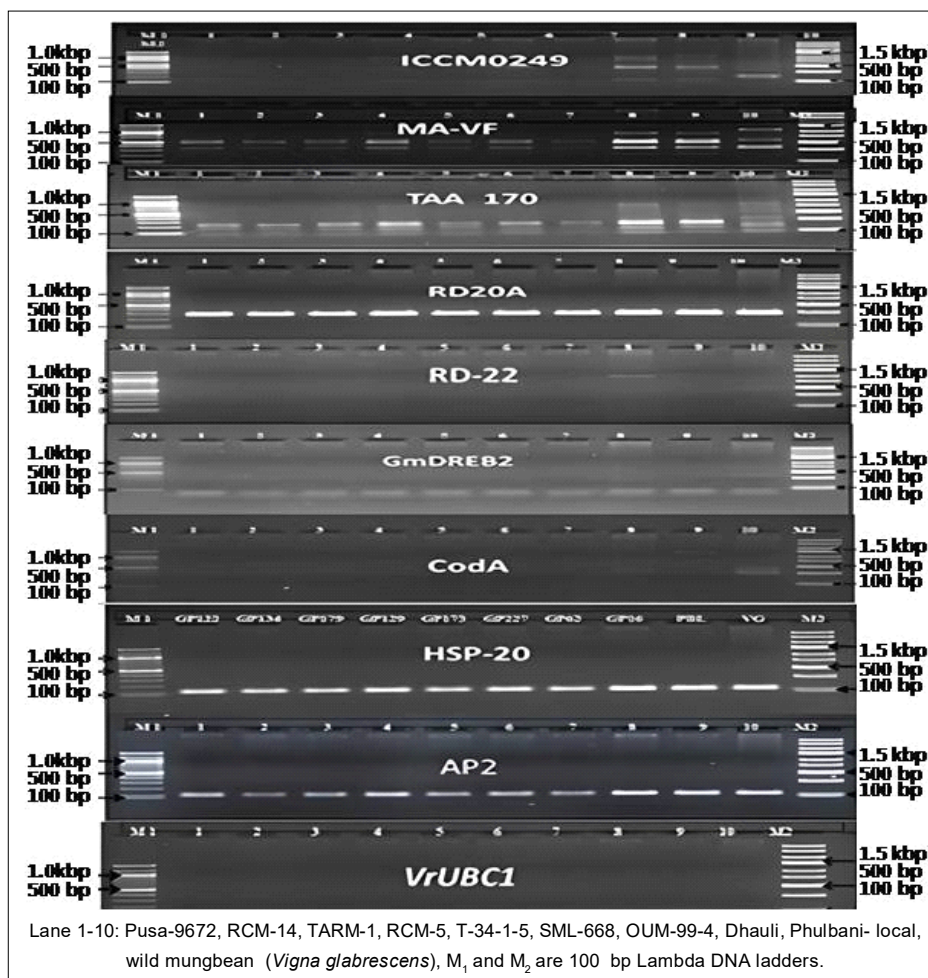


Fig 1: DNA profile of a set of selected moisture stress tolerant mungbean genotypes including susceptible checks.

Suppl. Table 2: List of SSR primers screened.

Marker	Forward primer seq	Reverse primer seq	crop	Reference
Satt383	CGATTACACCCAGATAAATTCCTCTG	CTTTCATATAATTTGGCAACCTCTTAG	soybean	Mishra et al., 2022
Satt557	GCGGGATCACCATGTAATAATAGTG	GCGCTCAGCCTTAAATTTGAA	soybean	Mishra et al., 2022
ICCM0249	TTTCTGTCAGGGCTAAC	GGAGATTGTGGGTAGCCTC	chikpea	Nayak et al., 2010
CEDG144	CAGTTACGAGTCTTGAACCTCAGC	CAGCTCTGTACAAAGCTGTAAGTG	Mungbean	Suman et al., 2019
STMS11	GTAACCTTGTAATAATTCCTCTCT	ATACATAACCCCAAC	chikpea	Winter et al., 1999
Satt009	CAACTTGAAATTAAGAGAAAT	CTTACTAGCGTATTAAACCCTTG	Soybean	Cregan et al., 1999
Satt197	CAGTCTTTTCCCCTCTCT	AAGATACCCCAACATTATTTGTAA	Soybean	Cregan et al., 1999
SoySSR9.1	GGAAGATTGAT TCAAAAGTCA	CGAGAAAGTGATTGTGAGAA	Soybean	Nugroho et al., 2020
Satt540	CTGGCGAATCAAGCTTTGTAAC	CCGTGATTGCGAAGAGGATATT	Soybean	Mishra, 2022
Ts72	CAAAACAATCACTAAAGTATTTGCTCT	AAAAATTGATGGACAAGTGTTATTATG	Chikpea	Winter et al., 2000
TA47	TTTTATAGGTCTTTTTTTGTTGCTTT	TCTGAATAGGAAATAAGAAAGGTAGGTT	Chikpea	Winter et al., 2000
RD20A	GTGGCACATGACTGAAGGAA	ATCTTCCAGCAGCACCTCT	Soya bean	Neves-Borges et al., 2012
TA37	ACTTACATGAATTATCTTCTTGGTCC	CGTATTCAAATAATCTTTTCATCAGTCA	Chikpea	Winter et al., 2000
TA25	AGTTTAATTGGCTGGTTCTAAGATAAC	AGGATGATCTTTTAATAAATCAGAATGA	Chikpea	Winter et al., 2000
VrDREB2A	CTGCTCTTGCTTATGATGAA	ATGTAGTGGTAGTAGTAGTAGTG	Mungbean	Alsamadany, 2022
VrZIP17	GGCATCATCATCTCCATG	GAGTTTGAGGTCGTTCCA	Mungbean	Alsamadany, 2022
VrHsfA6a	GCTTGCTATGAACATGGAGGA	TCATTATCATCATTTTCCCAATGC	Mungbean	Alsamadany, 2022
Ap2	TTGATGGAAGCTGGGGTGAT	CTCAGAAAACGCGCTGCCATT	Mungbean	Tian et al., 2016
CEDG066	AGTAAACAAGAACCCTCCCAAG	GTATTAAATTTGGGGTGGTG	Mungbean	Suman et al., 2019
CEDG071	GGTCCATTGAGACGGATCGAG	TCCCACCTCAGCGGAATCC	Mungbean	Suman et al., 2019
RD29B	AGCTGGAAGAGCCCATCATG	CTCTGTCAAGGGGACTGAGCAA	Soybean	Neves Borges et al., 2012
ERD1	CGTCCAGAAATTTGCTCAACAG	TGGGGTTATAGCCTTGTTGG	Soybean	Neves Borges et al., 2012
FBOX	CTAATGGCACAAATTCAGCTCTC	AGATAGGGAAGATGGTGCAGGT	Soybean	Neves Borges et al., 2012
LEA	CAGTGGCAGTGGGTGGTG	CAGGGTTATGGGACGCAAGG	Mungbean	Neves Borges et al., 2012
CP47	TGGGTGTCTGATCCTTATGGAC	CCTGCCGCAATATGATGAGAAG	Mungbean	Tian et al., 2016
codA gene	CCACCATGATATTCGGCAAC	GTGGAGGCTATTC GGCTA	Mungbean	Tian et al., 2016
HSP20	AGGTGGAAGTGAAGATGGG	GGAAGCCAGAACCTCCTCAT	Mungbean	Baloda et al., 2017
SUT	TGTCTCGTCCACAGCACAC	GGAGGAGCCCAACCAAGTTACAG	Mungbean	Tian et al., 2016
ADH	CGAACCTGAAGCGTGTCC	CGAGTTGCGGAAGATGCC	Mungbean	Tian et al., 2016
FAH	TGACAGCAGCGACAGGTAGG	AGGACCAGGAGCAGCATCAC	Mungbean	Tian et al., 2016
AP2	TTGATGGAAGCTGGGGTGAT	CTCAGAAAACGCGCTGCCATT	Mungbean	Tian et al., 2016
SMP	GTCACCGCCTTATCAGCTTG	CCACACATAACACACGCACT	Mungbean	Tian et al., 2016
VgActin	TTCTTTCTCTCGTTTTTTCATGCT	AAACCGAGGCACTTAATTTCTTG	Mungbean	Tian et al., 2016
ICCM0249	TTTCTTCGCATGGGCTTAAC	GGAGATTGTTGGGTAGGCTC	Chickpea	Nayak et al., 2010
TAA170	TATAGAGTGAGAAGAAGCAAGAGGAG	TATTTGCATCAATGTTCTGTAGTGTTT-3"	Chickpea	Winter et al., 1999
TM-VF	CTTGCACTCAAGCTCCCTTC	CCTTGTCCTCAAGACAGACCA	Vicia faba	Ammar et al., 2017
SODC	ACAAGACCAGAAACACGAACAG	CCACCTCCTCCTCAATCCATAG	Mungbean	Tian et al., 2016

Supplementary Table 2: Continue....

Supple. Table 3: Genotyping data

Amplions	ICOM										TAA	TAA	TAA	MA - VF_1	MA - VF_2	MA - VF_3	MA - VF_4	MA - VF_5	MA - VF_6	MA - VF_7	RD - B2_1	RD - B2_2	RD - B2_3	RD - B2_4	RD - B2_5	RD - B2_6	RD - B2_7	RD - B2_8	RD - B2_9	RD - B2_10	RD - B2_11	RD - B2_12	RD - B2_13	RD - B2_14	RD - B2_15	RD - B2_16	RD - B2_17	RD - B2_18	RD - B2_19	RD - B2_20	RD - B2_21	RD - B2_22	RD - B2_23	RD - B2_24	RD - B2_25	RD - B2_26	RD - B2_27	RD - B2_28	RD - B2_29	RD - B2_30	RD - B2_31	RD - B2_32	RD - B2_33	RD - B2_34	RD - B2_35	RD - B2_36	RD - B2_37	RD - B2_38	RD - B2_39	RD - B2_40	RD - B2_41	RD - B2_42	RD - B2_43	RD - B2_44	RD - B2_45	RD - B2_46	RD - B2_47	RD - B2_48	RD - B2_49	RD - B2_50	RD - B2_51	RD - B2_52	RD - B2_53	RD - B2_54	RD - B2_55	RD - B2_56	RD - B2_57	RD - B2_58	RD - B2_59	RD - B2_60	RD - B2_61	RD - B2_62	RD - B2_63	RD - B2_64	RD - B2_65	RD - B2_66	RD - B2_67	RD - B2_68	RD - B2_69	RD - B2_70	RD - B2_71	RD - B2_72	RD - B2_73	RD - B2_74	RD - B2_75	RD - B2_76	RD - B2_77	RD - B2_78	RD - B2_79	RD - B2_80	RD - B2_81	RD - B2_82	RD - B2_83	RD - B2_84	RD - B2_85	RD - B2_86	RD - B2_87	RD - B2_88	RD - B2_89	RD - B2_90	RD - B2_91	RD - B2_92	RD - B2_93	RD - B2_94	RD - B2_95	RD - B2_96	RD - B2_97	RD - B2_98	RD - B2_99	RD - B2_100	RD - B2_101	RD - B2_102	RD - B2_103	RD - B2_104	RD - B2_105	RD - B2_106	RD - B2_107	RD - B2_108	RD - B2_109	RD - B2_110	RD - B2_111	RD - B2_112	RD - B2_113	RD - B2_114	RD - B2_115	RD - B2_116	RD - B2_117	RD - B2_118	RD - B2_119	RD - B2_120	RD - B2_121	RD - B2_122	RD - B2_123	RD - B2_124	RD - B2_125	RD - B2_126	RD - B2_127	RD - B2_128	RD - B2_129	RD - B2_130	RD - B2_131	RD - B2_132	RD - B2_133	RD - B2_134	RD - B2_135	RD - B2_136	RD - B2_137	RD - B2_138	RD - B2_139	RD - B2_140	RD - B2_141	RD - B2_142	RD - B2_143	RD - B2_144	RD - B2_145	RD - B2_146	RD - B2_147	RD - B2_148	RD - B2_149	RD - B2_150	RD - B2_151	RD - B2_152	RD - B2_153	RD - B2_154	RD - B2_155	RD - B2_156	RD - B2_157	RD - B2_158	RD - B2_159	RD - B2_160	RD - B2_161	RD - B2_162	RD - B2_163	RD - B2_164	RD - B2_165	RD - B2_166	RD - B2_167	RD - B2_168	RD - B2_169	RD - B2_170	RD - B2_171	RD - B2_172	RD - B2_173	RD - B2_174	RD - B2_175	RD - B2_176	RD - B2_177	RD - B2_178	RD - B2_179	RD - B2_180	RD - B2_181	RD - B2_182	RD - B2_183	RD - B2_184	RD - B2_185	RD - B2_186	RD - B2_187	RD - B2_188	RD - B2_189	RD - B2_190	RD - B2_191	RD - B2_192	RD - B2_193	RD - B2_194	RD - B2_195	RD - B2_196	RD - B2_197	RD - B2_198	RD - B2_199	RD - B2_200	RD - B2_201	RD - B2_202	RD - B2_203	RD - B2_204	RD - B2_205	RD - B2_206	RD - B2_207	RD - B2_208	RD - B2_209	RD - B2_210	RD - B2_211	RD - B2_212	RD - B2_213	RD - B2_214	RD - B2_215	RD - B2_216	RD - B2_217	RD - B2_218	RD - B2_219	RD - B2_220	RD - B2_221	RD - B2_222	RD - B2_223	RD - B2_224	RD - B2_225	RD - B2_226	RD - B2_227	RD - B2_228	RD - B2_229	RD - B2_230	RD - B2_231	RD - B2_232	RD - B2_233	RD - B2_234	RD - B2_235	RD - B2_236	RD - B2_237	RD - B2_238	RD - B2_239	RD - B2_240	RD - B2_241	RD - B2_242	RD - B2_243	RD - B2_244	RD - B2_245	RD - B2_246	RD - B2_247	RD - B2_248	RD - B2_249	RD - B2_250	RD - B2_251	RD - B2_252	RD - B2_253	RD - B2_254	RD - B2_255	RD - B2_256	RD - B2_257	RD - B2_258	RD - B2_259	RD - B2_260	RD - B2_261	RD - B2_262	RD - B2_263	RD - B2_264	RD - B2_265	RD - B2_266	RD - B2_267	RD - B2_268	RD - B2_269	RD - B2_270	RD - B2_271	RD - B2_272	RD - B2_273	RD - B2_274	RD - B2_275	RD - B2_276	RD - B2_277	RD - B2_278	RD - B2_279	RD - B2_280	RD - B2_281	RD - B2_282	RD - B2_283	RD - B2_284	RD - B2_285	RD - B2_286	RD - B2_287	RD - B2_288	RD - B2_289	RD - B2_290	RD - B2_291	RD - B2_292	RD - B2_293	RD - B2_294	RD - B2_295	RD - B2_296	RD - B2_297	RD - B2_298	RD - B2_299	RD - B2_300	RD - B2_301	RD - B2_302	RD - B2_303	RD - B2_304	RD - B2_305	RD - B2_306	RD - B2_307	RD - B2_308	RD - B2_309	RD - B2_310	RD - B2_311	RD - B2_312	RD - B2_313	RD - B2_314	RD - B2_315	RD - B2_316	RD - B2_317	RD - B2_318	RD - B2_319	RD - B2_320	RD - B2_321	RD - B2_322	RD - B2_323	RD - B2_324	RD - B2_325	RD - B2_326	RD - B2_327	RD - B2_328	RD - B2_329	RD - B2_330	RD - B2_331	RD - B2_332	RD - B2_333	RD - B2_334	RD - B2_335	RD - B2_336	RD - B2_337	RD - B2_338	RD - B2_339	RD - B2_340	RD - B2_341	RD - B2_342	RD - B2_343	RD - B2_344	RD - B2_345	RD - B2_346	RD - B2_347	RD - B2_348	RD - B2_349	RD - B2_350	RD - B2_351	RD - B2_352	RD - B2_353	RD - B2_354	RD - B2_355	RD - B2_356	RD - B2_357	RD - B2_358	RD - B2_359	RD - B2_360	RD - B2_361	RD - B2_362	RD - B2_363	RD - B2_364	RD - B2_365	RD - B2_366	RD - B2_367	RD - B2_368	RD - B2_369	RD - B2_370	RD - B2_371	RD - B2_372	RD - B2_373	RD - B2_374	RD - B2_375	RD - B2_376	RD - B2_377	RD - B2_378	RD - B2_379	RD - B2_380	RD - B2_381	RD - B2_382	RD - B2_383	RD - B2_384	RD - B2_385	RD - B2_386	RD - B2_387	RD - B2_388	RD - B2_389	RD - B2_390	RD - B2_391	RD - B2_392	RD - B2_393	RD - B2_394	RD - B2_395	RD - B2_396	RD - B2_397	RD - B2_398	RD - B2_399	RD - B2_400	RD - B2_401	RD - B2_402	RD - B2_403	RD - B2_404	RD - B2_405	RD - B2_406	RD - B2_407	RD - B2_408	RD - B2_409	RD - B2_410	RD - B2_411	RD - B2_412	RD - B2_413	RD - B2_414	RD - B2_415	RD - B2_416	RD - B2_417	RD - B2_418	RD - B2_419	RD - B2_420	RD - B2_421	RD - B2_422	RD - B2_423	RD - B2_424	RD - B2_425	RD - B2_426	RD - B2_427	RD - B2_428	RD - B2_429	RD - B2_430	RD - B2_431	RD - B2_432	RD - B2_433	RD - B2_434	RD - B2_435	RD - B2_436	RD - B2_437	RD - B2_438	RD - B2_439	RD - B2_440	RD - B2_441	RD - B2_442	RD - B2_443	RD - B2_444	RD - B2_445	RD - B2_446	RD - B2_447	RD - B2_448	RD - B2_449	RD - B2_450	RD - B2_451	RD - B2_452	RD - B2_453	RD - B2_454	RD - B2_455	RD - B2_456	RD - B2_457	RD - B2_458	RD - B2_459	RD - B2_460	RD - B2_461	RD - B2_462	RD - B2_463	RD - B2_464	RD - B2_465	RD - B2_466	RD - B2_467	RD - B2_468	RD - B2_469	RD - B2_470	RD - B2_471	RD - B2_472	RD - B2_473	RD - B2_474	RD - B2_475	RD - B2_476	RD - B2_477	RD - B2_478	RD - B2_479	RD - B2_480	RD - B2_481	RD - B2_482	RD - B2_483	RD - B2_484	RD - B2_485	RD - B2_486	RD - B2_487	RD - B2_488	RD - B2_489	RD - B2_490	RD - B2_491	RD - B2_492	RD - B2_493	RD - B2_494	RD - B2_495	RD - B2_496	RD - B2_497	RD - B2_498	RD - B2_499	RD - B2_500	RD - B2_501	RD - B2_502	RD - B2_503	RD - B2_504	RD - B2_505	RD - B2_506	RD - B2_507	RD - B2_508	RD - B2_509	RD - B2_510	RD - B2_511	RD - B2_512	RD - B2_513	RD - B2_514	RD - B2_515	RD - B2_516	RD - B2_517	RD - B2_518	RD - B2_519	RD - B2_520	RD - B2_521	RD - B2_522	RD - B2_523	RD - B2_524	RD - B2_525	RD - B2_526	RD - B2_527	RD - B2_528	RD - B2_529	RD - B2_530	RD - B2_531	RD - B2_532	RD - B2_533	RD - B2_534	RD - B2_535	RD - B2_536	RD - B2_537	RD - B2_538	RD - B2_539	RD - B2_540	RD - B2_541	RD - B2_542	RD - B2_543	RD - B2_544	RD - B2_545	RD - B2_546	RD - B2_547	RD - B2_548	RD - B2_549	RD - B2_550	RD - B2_551	RD - B2_552	RD - B2_553	RD - B2_554	RD - B2_555	RD - B2_556	RD - B2_557	RD - B2_558	RD - B2_559	RD - B2_560	RD - B2_561	RD - B2_562	RD - B2_563	RD - B2_564	RD - B2_565	RD - B2_566	RD - B2_567	RD - B2_568	RD - B2_569	RD - B2_570	RD - B2_571	RD - B2_572	RD - B2_573	RD - B2_574	RD - B2_575	RD - B2_576	RD - B2_577	RD - B2_578	RD - B2_579	RD - B2_580	RD - B2_581	RD - B2_582	RD - B2_583	RD - B2_584	RD - B2_585	RD - B2_586	RD - B2_587	RD - B2_588	RD - B2_589	RD - B2_590	RD - B2_591	RD - B2_592	RD - B2_593	RD - B2_594	RD - B2_595	RD - B2_596	RD - B2_597	RD - B2_598	RD - B2_599	RD - B2_600	RD - B2_601	RD - B2_602	RD - B2_603	RD - B2_604	RD - B2_605	RD - B2_606	RD - B2_607	RD - B2_608	RD - B2_609	RD - B2_610	RD - B2_611	RD - B2_612	RD - B2_613	RD - B2_614	RD - B2_615	RD - B2_616	RD - B2_617	RD - B2_618	RD - B2_619	RD - B2_620	RD - B2_621	RD - B2_622	RD - B2_623	RD - B2_624	RD - B2_625	RD - B2_626	RD - B2_627	RD - B2_628	RD - B2_629	RD - B2_630	RD - B2_631	RD - B2_632	RD - B2_633	RD - B2_634	RD - B2_635	RD - B2_636	RD - B2_637	RD - B2_638	RD - B2_639	RD - B2_640	RD - B2_641	RD - B2_642	RD - B2_643	RD - B2_644	RD - B2_645	RD - B2_646	RD - B2_647	RD - B2_648	RD - B2_649	RD - B2_650	RD - B2_651	RD - B2_652	RD - B2_653	RD - B2_654	RD - B2_655	RD - B2_656	RD - B2_657	RD - B2_658	RD - B2_659	RD - B2_660	RD - B2_661	RD - B2_662	RD - B2_663	RD - B2_664	RD - B2_665	RD - B2_666	RD - B2_667	RD - B2_668	RD - B2_669	RD - B2_670	RD - B2_671	RD - B2_672	RD - B2_673	RD - B2_674	RD - B2_675	RD - B2_676	RD - B2_677	RD - B2_678	RD - B2_679	RD - B2_680	RD - B2_681	RD - B2_682	RD - B2_683	RD - B2_684	RD - B2_685	RD - B2_686	RD - B2_687	RD - B2_688	RD - B2_689	RD - B2_690	RD - B2_691	RD - B2_692	RD - B2_693	RD - B2_694	RD - B2_695	RD - B2_696	RD - B2_697	RD - B2_698	RD - B2_699	RD - B2_700	RD - B2_701	RD - B2_702	RD - B2_703	RD - B2_704	RD - B2_705	RD - B2_706	RD - B2_707	RD - B2_708	RD - B2_709	RD - B2_710	RD - B2_711	RD - B2_712	RD - B2_713	RD - B2_714	RD - B2_715	RD - B2_716	RD - B2_717	RD - B2_718	RD - B2_719	RD - B2_720	RD - B2_721	RD - B2_722	RD - B2_723	RD - B2_724	RD - B2_725	RD - B2_726	RD - B2_727	RD - B2_728	RD - B2_729	RD - B2_730	RD - B2_731	RD - B2_732	RD - B2_733	RD - B2_734	RD - B2_735	RD - B2_736	RD - B2_737	RD - B2_738	RD - B2_739	RD - B2_740	RD - B2_741	RD - B2_742	RD - B2_743	RD - B2_744	RD - B2_745	RD - B2_746	RD - B2_747	RD - B2_748	RD - B2_749	RD - B2_750	RD - B2_751	RD - B2_752	RD - B2_753	RD - B2_754	RD - B2_755	RD - B2_756	RD - B2_757	RD - B2_758	RD - B2_759	RD - B2_760	RD - B2_761	RD - B2_762	RD - B2_763	RD - B2_764	RD - B2_765	RD - B2_766	RD - B2_767	RD - B2_768	RD - B2_769	RD - B2_770	RD - B2_771	RD - B2
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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 Dhauri 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	Dhauri	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			
Dhauri	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. glabrescens	0.52	0.36	0.32	0.43	0.39	0.50	0.35	0.41	0.35	1.0

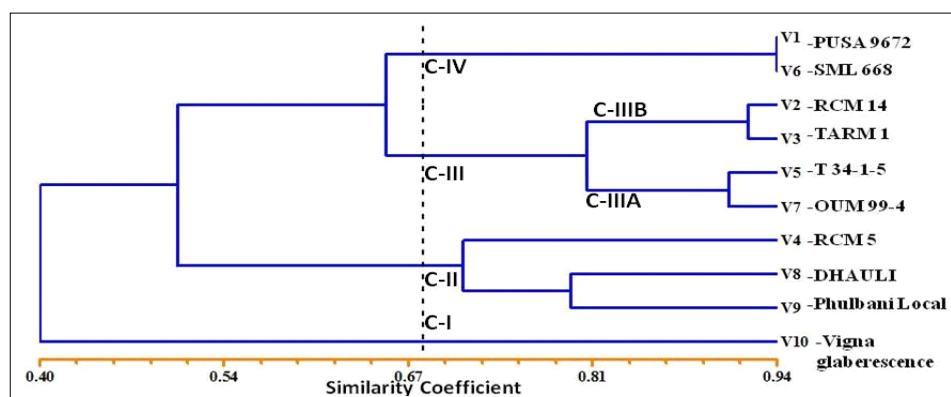


Fig 2: UPGMA based clustering of a set of 10 mungbean test genotypes using SSR data.

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

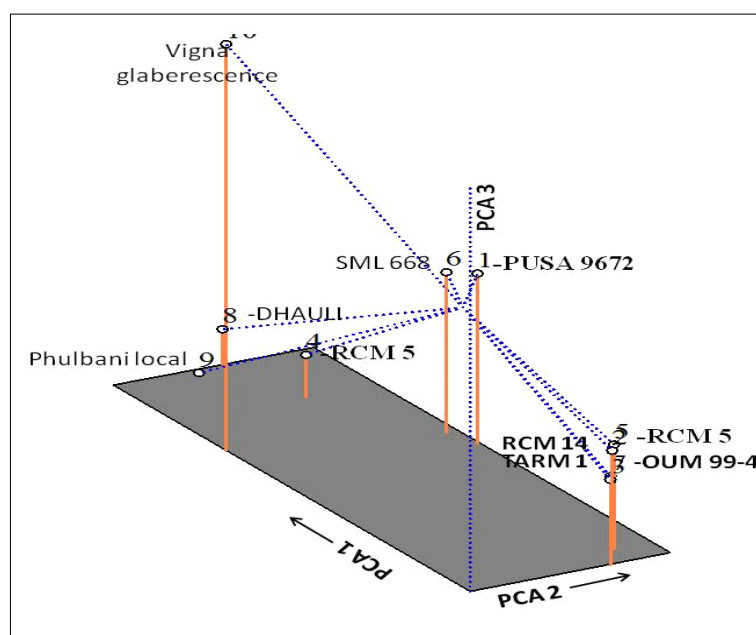


Fig 3: 3D -PCA plot of ten mung bean test genotypes using SSR data.

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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