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Assessment of Genetic Diversity for *Cercospora* Leaf Spot (CLS) Resistance in Mung Bean [*Vigna radiata* (L.) Wilczek] using SSR Markers

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ABSTRACT

Background: CLS causes severe yield loss in mung bean. To sustain mung bean health, it is vital to include alleles that may be useful in resisting CLS. Therefore, in the present study, 90 mung bean genotypes were included for assessing genetic diversity using 66 SSR markers for CLS resistance.

Methods: The mung bean crop was regularly monitored for the presence of pathogen and development of CLS disease in natural field conditions during *pre rabi* 2018 and *pre rabi* 2019. CLS screening assessments of germplasms were carried out using a 1-5 rating scale. Total genomic DNA was isolated from the leaf samples of all the genotypes and SSR genotyping was performed. The genotyping data were analysed by using GenAlEx 6.51b2 and TASSEL 5.0 software programme for genetic diversity parameters.

Result: A moderate molecular diversity was observed in the panel population as a wide variation in alleles showed a range of 80 bp to 300 bp with the average PIC value of 0.40. The maximum percentage of polymorphic loci was 75.76% in the resistance genotypes followed by 56.06% in moderately resistant genotypes. The percentages of Shannon information among and within the population were found to be 44% and 56%, respectively. The archaeopterx tree differentiated the panel population into two major clusters, *i.e.,* cluster I and cluster II, which were again sub divided into different sub-clusters and sub-sub clusters. These findings indicate that, the mung bean germplasm panel used in the present study could enrich the local gene pool and provide information for CLS resistance breeding.

Key words: *Cercospora* leaf spot resistance, Genetic diversity analysis, Mung bean, SSR.

INTRODUCTION

Mung bean [*Vigna radiata* (L.) Wilczek] is a popular commercial species of the genus *Vigna* and its production is steadily increasing in South and Southeast Asia. It is a popular legume because of its short-life cycle (Chen *et al.,* 2021). Mung bean has valuable nutritional and health benefits due to its high digestibility, Vitamin B and protein content (Chand *et al.,* 2015). However, mung bean often succumbs to the spread of *Cercospora canescens*, which is the causal organism of *Cercospora* leaf spot disease, but requires special attention as it can affect plant growth and reduce seed yield (Priyadarshini *et al.,* 2020). The incidence of mung bean CLS is widespread in India during the *kharif* season in warm and humid climatic conditions, causing considerable losses to mung bean growers. In the warm and wet seasons, yield losses of up to 47% under the northeast plain zone in India (Kumari *et al.,* 2021), 61% in Australia and Okara, Pakistan (Rasool *et al.,* 2021), 23-75% in Thailand (Somta *et al.,* 2015) and 37.4% in South Korea (Bushra *et al*. 2013) have been documented world wide.

Despite the efforts of plant breeders during the past few decades, the CLS resistance varieties or lines of mung bean has not been developed substantially due to a lack of sufficient genetic diversity for desirable traits in the germplasm used for breeding (Kundu *et al.,* 2022). It is imperative to further explore the genetic diversity available in this crop for better utilization of genetic resources for CLS resistance (Sahoo and Sharma, 2018). Various molecular markers linked to fungal disease resistance were also identified in mung bean (Lakhanpaul *et al.,* 2000), black gram (Souframanien and Gopalakrishna, 2004) and cowpea (Bangar *et al.,* 2021), including simple sequence repeat (SSR) markers for CLS disease resistance (Chankaew *et al.,*

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2011). However, limited work has been done so far for genetic diversity assessment in mung beans for CLS resistance. Therefore, the present study evaluated the genetic diversity among the mung bean germplasm using SSR markers under CLS stress.

MATERIALS AND METHODS

Experimental details

CLS disease reaction of a ninety mung bean genotypes, including four suitable susceptible checks were evaluated under natural field condition with augmented block design without replication in the EB-2, at College of Agriculture, OUAT, Bhubaneswar, during the *pre rabi* 2018 and the *pre rabi* 2019, respectively. Standard package and practices were followed for growing the mung bean crops except the use of fungicides. All the necessary measures were taken to ensure maximum CLS disease pressure, including maintaining optimum humidity and planting susceptible checks all along the borders and after every twenty test genotypes. At 20, 25 and 30 days after sowing (DAS), prepared spore suspension was artificially inoculated by spraying using a manual sprayer to all the genotypes. After inoculation, the crop was regularly monitored for the development of CLS disease. Infection on the leaves of each plant was then scored for CLS reaction at 40 DAS on a rating scale of 1-5 (Chankaew *et al.,* 2011), where, 1: resistance (R); 2: moderately resistance (MR); 3: moderately susceptible (MS); 4: susceptible (S) and 5: highly susceptible (HS).

SSR marker genotyping

Plant genomic DNA was extracted from young leaf tissue for each of the ninety mung bean genotypes by following a standard protocol (Doyle and Doyle, 1987), with little modification. The quality of DNA extracted was checked by electrophoreting the samples using 0.8% agarose gel and quantified using Nanodrop® ND-1000 Spectrophotometer. Polymerase chain reactions for SSR analysis were carried out under standard conditions for all the sixty-six primer pairs (Wang *et al.,* 2004; Somta *et al.,* 2009; Isemura *et al.,* 2012) using 1 U of Taq polymerase with 1 × PCR buffer (100 mm Tris-HCl at pH 9.0, 500 mm KCl and 15 mm $MgCl_2$), 2.5 mm dNTP, 3 mm $MgCl_2$, 20 pM of each primer and 50 ng of DNA template with a final reaction volume of 25 µL. The PCR reactions were denatured at 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, required annealing temperature for 1 minute and extension at 72°C for 2 minutes. The final extension was done at 72°C for 7 minutes. The amplicons were run on a 3% agarose gel with a 100 bp DNA ladder and then visualized through a UV GELDOC unit to assess the results.

Genotyping data Scoring and Statistical analysis

The PCR fragments were scored for presence and absence (Fig 1). Spurious and missing data were repeated for verification. The genetic diversity parameters *i.e.,* polymorphism information content (PIC) (Powell *et al.,* 1996), the allele frequency distribution (Scitable-Nature, 2022), the percentage of polymorphic loci per population (Glsturgeon, 2022), the Shannon diversity index (Shrivastava *et al.,* 2014), allelic patterns across the population (Bangar *et al.,* 2021), the Jaccard's similarity coefficient (Jaccard, 1908) and archaeopterx tree were analyzed using GenAlE × 6.51b2 and TASSEL 5.0, software program.

RESULTS AND DISCUSSION

Polymorphism information content (PIC) values

PIC measures the ability of a marker to detect polymorphisms and therefore has enormous importance in selecting markers for genetic studies (Serrote *et al.,* 2020). A moderate molecular diversity was observed in the panel population. The marker CEDG118 estimated the maximum genetic diversity (PIC value = 0.74). Wide variation in alleles showed a range of 80 bp to 300 bp. The average PIC value

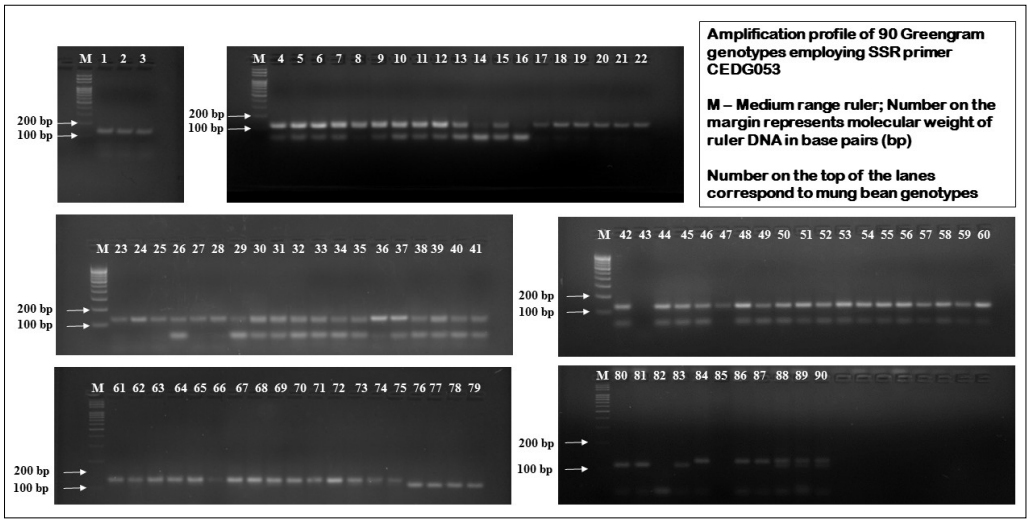

Fig 1: Genotyping pattern of 90 mungbean genotypes using CEDG053 in 3% agarose gel.

was obtained to be 0.40 for all the employed SSR markers. However, PIC values for co-dominant markers generally range from 0 (monomorphic) to 1 (very highly informative) (Serrote *et al.,* 2020). Results obtained from the present study revealed that, the PIC values of the markers were found between 0 to 1, which was similar to previous research finding in mung bean (Chen *et al.,* 2021; Dash *et al.,* 2022; Sahoo *et al.,* 2020a). However, the present investigation on trait-based genetic diversity is almost similar to the earlier findings (Sushmita *et al.,* 2021; Sahoo *et al.,* 2020b; Sahoo *et al.,* 2018) of moderate genetic diversity reported in mung bean for the single trait.

Allele frequency distribution

In the present investigation, the allelic frequency distribution across the codominant loci varied between 0 to 1 for all the population types, *i.e.* highly susceptible, moderately resistance, moderately susceptible, resistance and susceptible (Fig 2). However, Similar results were also observed in some previous studies (Moe *et al.,* 2012; Sahoo *et al.,* 2019) in mung bean germplasm, which suggests that, determining the allele frequency of candidate gene could have practical applications for research design, data interpretation, identifying genetic associations with particular diseases, estimating the number of individuals with disease susceptibility in a population and performing evolutionary studies.

Table 1: Percentage of polymorphic loci across populations under CLS.

Population type as per CLS reaction	Percentage (%) of polymorphic loci
Highly susceptible	6.06%
Moderately resistance	56.06%
Moderately susceptible	18.18%
Resistance	75.76%
Susceptible	39.39%
Mean	39.09%
SE	12.57%

Percentage of polymorphic loci

In the present study, the maximum percentage of polymorphic loci was found to be 75.76% in the resistance genotypes followed by 56.06% in moderately resistance genotypes. The least percentage of polymorphic loci was 6.06% in the highly susceptible genotypes. The mean and SE for the percentage of polymorphic loci were found to be 39.09% and 52.57%, respectively (Table 1). These findings are similar to the previous findings suggesting a range of 25-85% in mung bean by ISSR markers (Singh *et al.,* 2013) and 95.6% in mung bean and also in black gram population panel by using ISSR markers (Tantasawat *et al.,* 2010). However, the high degree of polymorphism is not due to a single species or population within a species, but rather polymorphic loci are spread evenly across all species and individual populations (Sahoo *et al.,* 2021a).

Shannon information diversity by population

In the present study, the Shannon information diversity statistics showed that the Shannon information was 0.269 among the population and 0.336 within the population (Table 2). The diversity estimate among the population was 1.308 and within the population 1.399, with the estimated probability among the population 0.001 and within the population 1, respectively. The percentages of Shannon information among and within the population were 44.452% and 55.548%, respectively. The scaled diversity was found to

Table 2: Summary of Shannon diversity statistics.

Source of information	Degrees of freedom	Log-like. Chi-Sq	Shannon Info.	% of total	Diversity estimate	Scaled diversity	Scaled overlap	Estimated probability
Among populations	4	48.331	0.269	44.452	1.308	0.317	0.683	0.001
Within populations	85	60.396	0.336	55.548	1.399	0.298	0.702	1.000
Total	89	108.727	0.604	100.000	1.829	0.458	0.542	

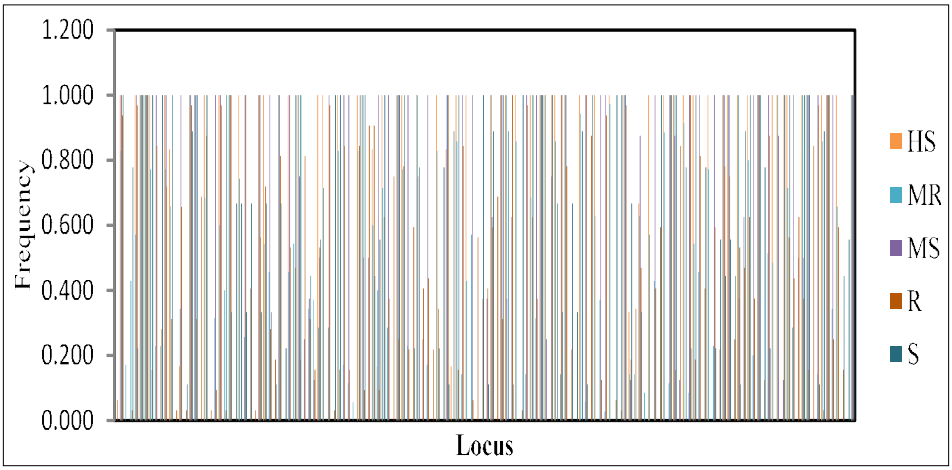

Fig 2: Allele frequency distribution across all the locus.

be 0.347 and 0.595 and the scaled overlap was found to be 0.653 and 0.405 among and within the population respectively. These findings are similar to the previous studies (Chen *et al.,* 2015; Sahoo *et al.,* 2021b). However, the index was proven negatively biased at small sample sizes. Modifications to the original Shannon's formula have been proposed to obtain an unbiased estimator (Sahoo *et al.,* 2022b; Samal *et al.,* 2021).

Allelic patterns distribution across the population

In the present study, the analysis of mean allelic patterns showed that the mean of expected heterozygosity was highest in the resistance population type (0.255), followed by the moderately resistance population type (0.210) (Fig 3). Results obtained from the analysis of allelic patterns for codominant data were similar to the results obtained in the previous studies on mung bean using SSR markers (Sarıkamış *et al.,* 2009; Sahoo *et al.,* 2022a) and in alfalfa using SSR markers (Diwan *et al.,* 2000). However, genetic variation in a population is derived from a wide assortment of genes and alleles. The persistence of populations over time through changing environments depend on their adaptability to the shifting external conditions (Scitable-Nature, 2022).

Cluster diagram and distribution of genotypes in different clusters

The archaeopterx tree construction revealed two major clusters for all the ninety genotypes, *i.e.,* I and II (Table 3), containing equal number (45 number) of accessions. Cluster I was again sub-divided into two major sub-clusters, *i.e.,* IA and IB respectively, which were again subdivided into different sub-sub clusters. Cluster II was also sub-divided into two sub-clusters, *i.e.,* IIA and IIB, respectively, which were again

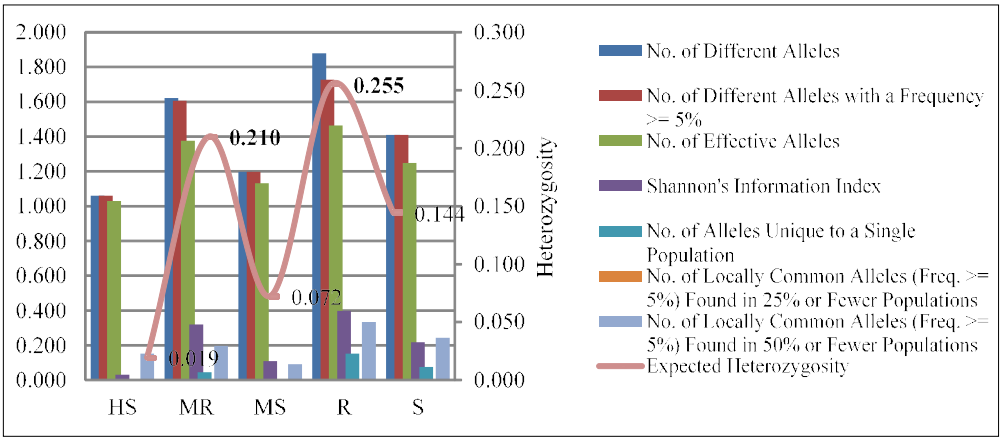

Fig 3: Allelic patterns across populations.

Table 3: Clustering of genotypes into two major clusters as per the Archaeopterx tree.

Major clusters	Total no. of accessions	Sub-clusters	Sub-Sub-clusters	Name of the accessions
Cluster I	45	IA	IA.1	PUSA – M – 1771, RMB – 12 – 07, AVMU-1681, COGG-13-39, IPM-409-4, BGS-9
			IA.2	KVK PURI – 4, PUSA – 105, SML – 1901, NBPGR – 150, CPR BAM GP -289, MH -421, TARM-2, PANT MUNG-2
		IB	IB.1	KPS-2, IPM-312-9, AVMU-1699, ML-1907, IPM-410-3, RMG-1092, KPS-1, ASKA MUNG-BL, BM-2012-9
			IB.2	HUM-8 (C1), PM-1522 (C2), PUSA-0672 (C3), KAMDEV (C4), NDMK-15-513, AVMU-1687, AVMU-1683, EC-693367, AVMU-1680, PAU-911, TMB-134, AVMU-1698, ASHA MUNG, AVMU-1682, ML-818, GANGA-1, AVMU-1678, AVMU-16100, AVMU-1684, PUSA-9531, SML-1815, IPM-410-9
Cluster II	45	IIA	IIA.1	SML-1808, VC-6372, NARENDRA MUNG-1, MH-13-23, NVL-722
			IIA.2	SVM-6133, LGG-460, IPM-205-7, IPM-409-4, PUSA-BM-1, JHAIN-MUNG-GREEN, RMG-62, COGG-13-19, AVMU-1691, PUSA-1841, PM-14-11, PUSA-672
		IIB	IIB.1	VC-6368-46-40-1, PANT MUNG-8, VGG-16-036, IGKM-6-26-5, EC-693369, MDGVV-18, ML-2479, VGG-15-30, RMG-1087, NBPGR-150, KM-2355, VC-3960-88, PDM-11
			IIB.2	V-1000319-AG, NMK-15-12, PUSA-9072, RMG-364, VGG-15-29, HUM-6, PUSA-1672, IPM-312-20, KNM-1502, NVL-855, DGG-8, HVM-2, BANSA-PAL-GR, RMB-12-07, CPR BAM GP -349

subdivided into different sub-sub clusters (Table 3). Therefore, it is concluded that the panel population used for the study possesses considerable genetic variation for CLS resistance. Earlier researchers had also confirmed about the existence of genetic variation for CLS resistance in mung bean (Datta *et al*., 2012; Kundu *et al*., 2022). On the whole, the clustering results revealed by SSR closely reflected the previously understood relationship among these mung bean accessions.

CONCLUSION

The results obtained from the present investigation indicate that, SSR markers used in the present studies could be effectively used for molecular studies in mung bean, because these were highly influential in estimating mung bean's genetic diversity. However, sufficient variability exists in the genotypes of current mung bean panel population, which could be useful in selecting suitable parents for breeding purposes and genetic map development for CLS disease resistance.

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Conflict of interest: None.

REFERENCES

Bangar, P., Tyagi, N., Tiwari, B., Kumar, S., Barman, P., Kumari, R. and Chaudhury, A. (2021). Identification and characterization of SNPs in released, landrace and wild accessions of mung bean [*Vigna radiate* (L.) Wilczek] using whole genome re-sequencing. Journal of Crop Science and Biotechnology. 24: 153-165.

Bushra, M., Yun, X.X., Pan, S.Y., Hydamaka, A. and Feng, W.L. (2013). Effect of oxidation and esterification on functional properties of mung bean [*Vigna radiata* (L.) Wilczek] starch. The Journal European Food Research and Technology. 236: 119-128.

Chand, R., Pal, C., Singh, V., Kumar, M., Singh, V.K. and Chowdappa, P. (2015). Draft genome sequence of *Cercospora canescens*: A leaf spot causing pathogen. Current Science. 109: 2103-2110.

Chankaew, S., Somta, P., Sorajjapinun, W. and Srinives, P. (2011). Quantitative trait loci mapping of *Cercospora* leaf spot resistance in mung bean, [*Vigna radiata* (L.) Wilczek]. Molecular Breeding. 28: 255-264.

Chen, H., Wang, L., Wang, S., Liu, C., Blair, M. W. and Cheng, X. (2015). Transcriptome sequencing of mung bean [*Vigna radiate* (L.)] genes and the identification of EST-SSR markers. PloS One. 10: e0120273.

Chen, Y., Hu, W., Li, P., Liu, Y., Chen, X., Xie, H. and Zhang, Y. (2021). Phytoremediation of hexavalent chromium by mung bean through bio-accumulation and bio-stabilization in a short duration. International Journal of Environmental Science and Technology. 18: 3023-3034.

Dash, S.S.S., Lenka, D., Sahoo, J.P., Tripathy, S.K., Samal, K.C., Lenka, D. and Panda, R.K. (2022). Biochemical characterization of maize (*Zea mays* L.) hybrids under excessive soil moisture stress. Cereal Research Communications. Available at: https://doi.org/10.1007/s42976-021-00241-2. Accessed on: 10th August 2022.

Datta, S., Gangwar, S., Kumar, S., Gupta, S., Rai, R., Kaashyap, M. and Nadarajan, N. (2012). Genetic diversity in selected Indian mung bean [*Vigna radiata* (L.) Wilczek] cultivars using RAPD markers. American Journal of Plant Sciences. 3: 1085-1091.

Diwan, N., Bouton, J.H., Kochert, G.A.R.Y. and Cregan, P.B. (2000). Mapping of simple sequence repeat (SSR) DNA markers in diploid and tetraploid alfalfa. Theoretical and Applied Genetics. 101: 165-172.

Doyle J.J and Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19: 11-15.

Glsturgeon. (2022). Measures of Genetic Diversity. Available at: https://www.glsturgeon.com/wp-content/uploads/2012/08/measures-of-diversity.pdf. Accessed on: 11th June 2022.

Isemura, T., Kaga, A., Tabata, S., Somta, P., Srinives, P., Shimizu, T. and Tomooka, N. (2012). Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). Plos One. 7: e41304-e41304.

Jaccard, P. (1908). Nouvelles Recherches Sur La Distribution Florale. Bulletin de la Société vaudoise des Sciences Naturelles. 44: 223-270.

Kumari, G., Lavanya, G.R., Shanmugavadivel, P.S., Singh, Y., Singh, P., Patidar, B. and Pratap, A. (2021). Genetic diversity and population genetic structure analysis of an extensive collection of wild and cultivated *Vigna* accessions. Molecular Genetics and Genomics. 296: 1337-1353.

Kundu, A., Ganguli, S. and Pal, A. (2022). Genomic Designing towards Biotic Stress Resistance in Mung bean and Urd bean. In Genomic Designing for Biotic Stress Resistant Pulse Crops (pp. 381-414). Springer, Cham.

Lakhanpaul, S., Chadha, S. and Bhat, K.V. (2000). Random amplified polymorphic DNA (RAPD) analysis in Indian mung bean [*Vigna radiata* (L.) Wilczek] cultivars. Genetica. 109: 227-234.

Moe, K.T., Gwag, J.G. and Park, Y.J. (2012). Efficiency of Power core in core set development using amplified fragment length polymorphic markers in mung bean. Plant breeding. 131: 110-117.

Powell, W., Machray, G.C. and Provan, J. (1996). Polymorphism revealed by simple sequence repeats. Trends in Plant Science. 1: 215-222.

Priyadarshini, L., Samal, K.C., Sahoo, J.P. and Mohapatra, U. (2020). Morphological, biochemical and molecular characterization of some promising potato [*Solanum tuberosum* (L.)] cultivars of Odisha. Journal of Pharmacognosy and Phytochemistry. 9: 1657-1664.

Rasool, B., Ramzani, P.M.A., Zubair, M., Khan, M.A., Lewiñska, K., Turan, V. and Iqbal, M. (2021). Impacts of oxalic acid-activated phosphate rock and root-induced changes on Pb bioavailability in the rhizosphere and its distribution in mung bean plant. Environmental Pollution. 280: 116903.

Sahoo, J.P. and Sharma, V. (2018). Impact of LOD score and recombination frequencies on the microsatellite marker based linkage map for drought tolerance in *kharif* rice of Assam. International Journal of Current Microbiology and Applied Sciences. 7: 3299-3304.

Sahoo, J.P., Behera, L., Praveena, J., Sawant, S., Mishra, A., Sharma, S.S. and Samal, K.C. (2021a). The golden spice turmeric (*Curcuma longa*) and its feasible benefits in prospering human health-A review. American Journal of Plant Sciences. 12: 455-475.

Sahoo, J.P., Behera, L., Sharma, S.S., Praveena, J., Nayak, S.K. and Samal, K.C. (2020a). Omics studies and systems biology perspective towards abiotic stress response in plants. American Journal of Plant Sciences. 11: 2172-2194.

Sahoo, J.P., Mishra, A.P., Samal, K.C. and Dash, A.K. (2021b). Insights into the antibiotic resistance in Biofilms-A Review. Environment Conservation Journal. 22: 59-67.

Sahoo, J.P., Mohapatra, U. and Mishra, P. (2020b). An outlook on metabolic pathway engineering in crop plants. Archives of Agriculture and Environmental Science. 5: 431-434.

Sahoo, J.P., Mohapatra, U., Saha, D., Mohanty, I.C. and Samal, K.C. (2022a). Linkage disequilibrium mapping: A journey from traditional breeding to molecular breeding in crop plants. Indian Journal of Traditional Knowledge. 21: 434-442.

Sahoo, J.P., Sharma, V., Verma, R.K., Chetia, S.K., Baruah, A.R., Modi, M.K. and Yadav, V.K. (2019). Linkage analysis for drought tolerance in *kharif* rice of Assam using microsatellite markers. Indian Journal of Traditional Knowledge. 18: 371-375.

Sahoo, J.P., Singh, S.K. and Saha, D. (2018). A review on linkage mapping for drought stress tolerance in rice. Journal of Pharmacognosy and Phytochemistry. 7: 2149-2157.

Sahoo, J.P., Samal, K.C., Tripathy, S.K., Lenka, D., Mishra, P., Behera, L., Acharya, L.K., Sunani, S.K. and Behera, B. (2022b). Understanding the genetics of *Cercospora* leaf spot (CLS) resistance in mung bean (*Vigna radiata* L. Wilczek). Tropical Plant Pathology. Available at: https://doi.org/10.1007/s40858-022-00525-w. Accessed on: 10th August 2022.

Samal, K.C., Sahoo, J.P., Behera, L. and Dash, T. (2021). Understanding the Blast (Basic local alignment search tool) program and a step-by-step guide for its use in life science research. Bhartiya Krishi Anusandhan Patrika. 36: 55-61.

Sarıkamış, G., Yaşar, F., Bakır, M., Kazan, K. and Ergül, A. (2009). Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey. Genetics and Molecular Research. 8: 880-887.

Scitable-Nature. (2022). Scitable by Nature Education: A Collaborative Learning Space for Science. Available at: https://www. nature.com/scitable/. Accessed on: 12th August 2022.

Serrote, C.M.L., Reiniger, L.R.S., Silva, K.B., Santos, D., Rabaiolli, S.M. and Stefanel, C.M. (2020). Determining the polymorphism information content of a molecular marker. Gene. 726: 144175.

Shrivastava, D., Verma, P. and Bhatia, S. (2014). Expanding the repertoire of microsatellite markers for polymorphism studies in Indian accessions of mung bean (*Vigna radiata* L. Wilczek). Molecular Biology Reports. 41: 5669-5680.

Singh, R., van Heusden, A.W. and Yadav, R.C. (2013). A comparative genetic diversity analysis in mungbean [*Vigna radiata* (L.)] using inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP). African Journal of Biotechnology. 12: 6574-6582.

Somta, P., Chankaew, S., Kongjaimun, A. and Srinives, P. (2015). QTLs controlling seed weight and days to flowering in mung bean [*Vigna radiate* (L.) wilczek], their conservation in azuki bean [*V. angularis* (Ohwi) Ohwi and Ohashi] and rice bean [*V. umbellata* (Thunb.) Ohwi and Ohashi]. Agrivita. Journal of Agricultural Science. 37: 159-168.

Somta, P., Seehalak, W. and Srinives, P. (2009). Development, characterization and cross-species amplification of mungbean (*Vigna radiata*) genic microsatellite markers. Conservation Genetics. 10: 1939-1943.

Souframanien, J. and Gopalakrishna, T. (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theoretical and Applied Genetics. 109: 1687-1693.

Sushmita, V.T., Arya, M., Jambhulkar, P.P., Manjunatha, N., Singh, A. and Chaturvedi, S.K. (2021). Identification of donors and molecular characterization of *Corynespora cassiicola* causing fungal leaf spot of mung bean and Urd bean. International Journal of Agriculture. Environment and Biotechnology. 14: 265-276.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Thongpae, T., Petkhum, C., Seehalak, W. and Machikowa, T. (2010). Variety identification and genetic relationships of mung bean and black gram in Thailand based on morphological characters and ISSR analysis. African Journal of Biotechnology. 9: 4452-4464.

Wang, X.W., Kaga, A., Tomooka, N. and Vaughan, D.A. (2004). The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi]. Theoretical and Applied Genetics. 109: 352-360.