Annals of Arid Zone 62(1): 77-82, 2023

Characterization of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] Drought Tolerant Lines Developed for A_1 Zone Using SSR Markers

Supriya Ambawat[1]*, R.C. Meena[1], C. Tara Satyavathi[1], Rajbala Meena[1], Vikas Khandelwal[1], Mahendra Sewaliya[1], Manoj Kumar[1], J.P. Bishnoi[1] and Subaran Singh[2]

[1]*ICAR-AICRP on Pearl Millet, Mandor, Agriculture University, Jodhpur 342 304, India*

[2]*College of Horticulture, Maharana Pratap Horticultural University, Karnal 132 001, India*

Received: March 2023

Abstract: Pearl millet [*Pennisetum glaucum* (L.) R. Br] is the sixth most important cereal in the world and is a primary food source of population living in arid and semi-arid tropical regions of Asia and Africa. It is mainly grown over 30 mha worldwide with the majority of the crop grown in Africa and Asia. It is very useful for combating the adverse effects of changing climate and can also ensure livelihood security among farming communities. It has climate-resilient attributes and can grow in less fertile soil even under adverse and harsh climatic conditions. It can show better growth and productivity in low nutrient input conditions and needs lesser synthetic fertilizers and irrigations. Screening and selecting germplasm for drought tolerance and developing drought tolerant hybrids and varieties of pearl millet is very important in the present scenario of global warming and climatic change. In this study, 24 drought tolerant lines of pearl millet developed specifically for A_1 zone were characterized using 15 drought specific SSR primers. All 15 SSRs amplified products of varying sizes ranging from100-550 bp. A total of 38 alleles were obtained in this study and the number of alleles per locus varied from 2 to 4 with an average of 2.53 alleles. Polymorphic Information Content (PIC) varied from 0.41 to 0.69 with an average of 0.51 PIC value. This study can be highly useful for developing high yielding drought tolerant cultivars for low rainfall areas i.e. A_1 zone and increasing pearl millet productivity.

Key words: Abiotic stresses, climate-resilience, drought tolerance, molecular characterization, pearl millet, microsatellites.

Edited by

Praveen Kumar
R.N. Kumawat
R.K. Solanki
N.K. Jat

***Correspondence**

Supriya Ambawat
biotechsupriya@gmail.com

Citation

Ambawat, S., Meena, R.C., Satyavathi, C.T., Meena, R., Khandelwal, V., Sewaliya, M., Kumar, M., Bishnoi, J.P. and Singh, S. 2023. Characterization of pearl millet [Pennisetum glaucum (l.) r. br.] drought tolerant lines developed for A1 zone using SSR markers. Annals of Arid Zone 62(1): 77-82.

doi: 10.59512/aaz.2023.62.1.8

https://epubs.icar.org.in/index.php/AAZ/article/view/134285

Pearl millet is a climate-resilient crop which is most widely grown in the arid and semi-arid tropics of Asia and Africa over 30 mha which receive rainfall from 150-700 mm. It is a highly nutritious cereal crop and rightly termed as nutricereal. It requires low inputs and delivers high cost-effective benefits. It is the 4th most widely cultivated crop in India after rice, wheat and maize. Rajasthan, Haryana, Gujarat, Uttar Pradesh and Maharashtra are the major pearl

millet growing states contributing 90% of total production in India. Out of this, Rajasthan contributes a maximum of around 4.28 mt followed by Uttar Pradesh (1.30), Haryana (1.08), Gujarat (0.96), Maharashtra (0.67) and Tamil Nadu (0.08) (Satyavathi *et al.,* 2021a). It has good adaptability and can survive under harsh and adverse conditions of drought, high temperature, salinity, lodging and poor soils. Further, it also possess a huge potential for high dry matter production under water deficit and high temperature which makes it a crop of choice for cultivation in arid and semi-arid regions of the world (Ambawat *et al.,* 2021). Pearl millet is highly nutritious and very useful for health and has several benefits. It is a rich source of energy (347 Kcal), carbohydrates (61.8 g 100 g^{-1}), proteins (8-19%), fat (3-8%), ash, dietary fibres (11.5 g 100 g^{-1}), antioxidants, vitamins and minerals (2.3 mg 100 g^{-1}) like iron, zinc, potassium, magnesium, manganese, copper, phosphorous (Kumar *et al.,* 2020a; Satyavathi *et al.,* 2021a). Production of pearl millet is likely to become more challenging because of predicted intense drought stress and rise in temperature. Thus, assessment of the effects of global climate changes on agriculture might help to properly anticipate and adapt farming to maximize agricultural production. Pearl millet being a climate-resilient crop is a good alternative to overcome the alarming situation of global warming. It is very crucial for minimizing the adverse effects of changing climate and facilitating income and food security among farming communities.

Pearl millet is being affected by various abiotic stresses like drought, heat, salinity etc. and among them, drought is the major constraint leading to decrease in production and productivity and affecting utilization of its full genetic potential (Mitra 2001; Choudhary *et al.,*2021; Satyavathi *et al.,* 2021b). In India, it is cultivated from near-optimum to highly drought-prone environments leading to distribution of various crop growing regions into three zones *viz.*, A_1, A and B. Zone A_1 is comprised of parts of Rajasthan, Gujarat and Haryana which receive <400 mm annual rainfall. Pearl millet is still a staple cereal in these areas because no other cereal is well adapted or productive under seasonal rainfall of 250 to 300 mm in A_1 zone (Khairwal and Yadav, 2005). Farmers in 55% of the pearl millet area (about 4.5 mha) including A_1 zone are yet to harness the benefits of pearl millet breeding programs in the public and private sectors. Pearl millet is still an important staple crop in A_1 zone and 33 kg per capita per annum consumed in rural Rajasthan and 28 kg per capita per annum in rural Gujarat and the farmer's income is mainly dependent on this crop in these areas (Satyavathi *et al.*, 2019). Hence, it is an area of major priority to increase the productivity of pearl millet and many initiatives were taken by ICAR-AICRP on Pearl millet, Jodhpur to screen germplasm for A_1 zone and develop more hybrids and varieties for this specific zone. But, despite the various breeding efforts, there is narrow cultivar diversity in this drought-prone ecology thus leaving very less cultivar choices for farmers. Under such conditions, prospects for major increase in production based on introducing drought suitable genotypes in addition to other agronomic practices are required to increase its productivity. Moreover, in the present context of "International Year of Millets 2023" it is highly desired to promote the benefits of pearl millet and put efforts to enhance its production, productivity and enrich the gene pool by exploring its ability and underlying mechanisms to survive in harsh conditions.

Conventional approaches are time consuming and require help of advanced biotechnological tools to hasten the pearl millet improvement programs. Thus, genomic studies along with molecular tools have become major approaches these days due to their enormous potential to improve the accuracy and efficiency of conventional breeding (Bollam *et al.,* 2018; Ambawat *et al.,* 2020b). In case of drought tolerance, availability of markers tightly linked to tolerant genes will help in identifying genotypes possessing these genes. Simple sequence repeats (SSRs) are considered as the most efficient and reliable markers for such studies due to their abundance in the genome, even distribution, easy detection, multi allelic nature, genome specificity, high-throughput, high reproducibility and co-dominant behaviour (Ambawat *et al.,* 2020a; Choudhary *et al.,*2021). Therefore, using SSR markers is a very effective technique for molecular characterization among pearl millet genotypes. Hence, the present study was undertaken to categorize and characterize

Table 1. List of pearl millet lines used for molecular characterization

Name of line	Organization
Bikaner -390	SKRAU, Bikaner
Bikaner -406	SKRAU, Bikaner
MIR-1802	SKRAU, Bikaner
MIR-2001	SKRAU, Bikaner
MIR-202	Agriculture University, Jodhpur
RIB-20887	RARI, Jaipur
RIB-20895	RARI, Jaipur
MIR-517	Agriculture University, Jodhpur
MIR-519-1	Agriculture University, Jodhpur
MIR-516	Agriculture University, Jodhpur
MIR-506	Agriculture University, Jodhpur
RIB-494	RARI, Jaipur
RIB-3135-18	RARI, Jaipur
RIB-335/74	RARI, Jaipur
RIB-155076	RARI, Jaipur
BIB-364	SKRAU, Bikaner
BIB-184	SKRAU, Bikaner
BIB-139	SKRAU, Bikaner
BIB-28	SKRAU, Bikaner
MIR-1262	Agriculture University, Jodhpur
MIR-1106	Agriculture University, Jodhpur
RIB-128/134	RARI, Jaipur
MIR-1408	Agriculture University, Jodhpur
RIB-184-190	RARI, Jaipur

the pearl millet lines suitable for drought prone areas of A_1 zone with microsatellites.

Material and methods

Plant Material

Plant material comprised of a total of 24 pearl millet drought tolerant lines developed for A_1 zone under Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India (Table 1). Molecular characterization was performed at PC Unit, ICAR-AICRP on Pearl millet, Jodhpur during 2021-22.

Genomic DNA isolation and quantification

Genomic DNA was isolated from young and fresh leaves of 12 days old plantlets of 24 pearl millet lines (Table 1) using cetyl trimethyl ammonium bromide (CTAB) method with some modifications as described by Ambawat *et al.* (2020c) and analyzed qualitatively on 0.8% agarose gel.

PCR and molecular characterization

DNA was diluted to make available final concentration of 10 ng μl^{-1} and used for PCR. The PCR amplification reactions were carried out in a volume of 10 µl containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit Taq DNA polymerase (GeNei, India) and 10 ng of DNA. Amplifications were carried out in a 96-well thermal cycler (Agilent Technologies). Thermal cycler was programmed to 1 cycle of 5 min at 94°C for initial denaturation. This was followed by 35 cycles of 30s at 94°C for denaturation, 30 s of 58°C for annealing and 1 min at 72°C for primer extension. Finally, 1 cycle of 10 mins at 72°C was used for final extension followed by hold at 4°C (Ambawat *et al.,* 2020a). The PCR products were analyzed on 2.5% agarose gel.

Results and Discussion

Molecular characterization and SSR analysis

A total of 15 SSR primers specifically reported for drought were used for PCR amplification and molecular characterization among 24 pearl millet lines (Jangra *et al.,* 2019; Ambawat *et al.,* 2021). All the 15 SSRs amplified products of varying sizes ranging between 100-550 bp (Fig. 1, 2). A total of 38 alleles were obtained in this study and the number of alleles per locus varied between 2 to 4 with an average of 2.53 alleles. Similar kinds of results were obtained by other investigators (Senthilvel *et al.,* 2008; Shaikh, 2015; Ambawat *et al.,* 2021). But, these values were relatively lower than Kumar *et al.* (2020b) with 4.62 alleles per primer (Mariac *et al.,* 2006) with 2-18 alleles (6.8 alleles per locus) and (Oumar *et al.,* 2008) with (12.5 alleles per locus) which can be attributed to the study of the diverse world collection of germplasm.

Polymorphic information content (PIC) varied from 0.41 to 0.69 with an average of 0.51 PIC value (Table 2). The highest PIC value was observed for the marker CTM 3 (0.69) followed by IPES0236 (0.67), IPES0152 (0.65) and PSMP2077 (0.53) while it was lowest for the markers PSMP2206 (0.41), PSMP2237 (0.42) and PSMP2059 (0.43). Thus, it indicated that CTM3 was the most informative and best marker for characterization followed by IPES0236, IPES0152 andPSMP2077 markers while PSMP2206 was observed to be the least powerful marker. PIC

Table 2. List of SSR primers showing polymorphism

Oligo Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Product range	No. of alleles amplified	PIC value
PSMP2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA	240-250	2	0.42
PSMP2072	GAAATCTACACAAGGGTCTCCA	GTACGGAGCAATGACATCTGAA	200-300	2	0.47
PSMP2066	ATATTAGAGCATTGCATCGC	GCATAGCAGCATACAGCAGCAA	170-180	2	0.50
PSMP2206	AGAAGAAGAGGGGGTAAGAAGGAG	AGCAACATCCGTAGAGGTAGAAG	200-210	2	0.41
PSMP2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	130-250	2	0.43
PSMP2077	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT	150-250	3	0.53
PSMP2078	CATGCCCATGACAGTATCTTAAT	ACTGTTCGGTTCCAAAATACTT	170-300	2	0.50
CTM3	GTCCATCGTCGCCGACGAA	GGATTTGCTAGTTGTGGGCT	250-550	4	0.69
CTM21	ATGCCTCCCACCCCACGTCG	CGTCGCACTAGCCACAGTCA	280-300	3	0.46
IPES0117	TTATTATTCGGTCATCACAGCG	TCCAAAACACAATTCCACCC	100-130	3	0.44
IPES0236	GGCCAGCTCGCCTAGAT	AGATCCACCGCCTAATGAAA	220-260	3	0.67
IPES0218	CCTGGGAACACAAAACCAGA	CCAGGTCCATGTCCTTGACT	250-270	2	0.47
IPES0152	GATACGAAGGGAAGCACAGC	TGTGTGGTAAGCTGCTGGAG	100-120	3	0.65
ICMP3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA	140-250	3	0.48
ICMP3063	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA	160-200	2	0.50

Fig. 1. Agarose gel showing amplification profiles of pearl millet lines using the primer PSMP 3056. Lane M-50 bp ladder, Lane 1-24 pearl millet lines.

values varying from 0.02 to 0.97 were reported by many researchers (Yadav *et al.,* 2013; Tiwari *et al.,* 2015; Mahalingam *et al.,* 2016; Singh *et al.,* 2017; Ambawat *et al.,* 2020a, 2021; Kumar *et al.,* 2020b). An average PIC value of 0.51 observed in this study is similar to other researchers where they reported PIC value in range of 0.53 to 0.58 (Kapila *et al.,* 2008; Kumar *et al.,* 2020b; Ambawat *et al.,* 2021). However, it was lesser (average PIC value of 0.671) than the observation recorded by Tiwari *et al.* (2015) while higher than (0.37 PIC value) reported by Salazar *et al.* (2006) and 0.41 and 0.43 as reported by Yadav *et al.* (2013) and Shrivastava *et al.* (2015), respectively. Markers with PIC values of 0.5 or above are believed to be very useful in distinguishing the genotypes and useful for molecular genetic diversity studies (Akkaya and Buyukunal Bal, 2004). PIC values can range between 0 and 0.5 because of bi-allelic nature of SNPs while it can go above 0.5 in case of SSRs due to their mutli-allelic nature as reported by other investigators (Singh *et al.,* 2017; Jangra *et al.*, 2019; Ambawat

Fig. 2. Agarose gel showing amplification profiles of pearl lines using the primer IPES0152. Lane M-50 bp ladder, Lane 1-24 pearl millet lines.

et al., 2021). Thus, SSR markers are the most preferred and efficient markers due to their ability to detect multiallelic loci, co-dominance, high polymorphism, high reproducibility, easy to use and vast ability to differentiate the genotypes (Verma *et al.,* 2021). Similar kind of results regarding effectiveness of SSR markers in molecular characterization was also reported in other studies (Mariac *et al.*,2006; Kapila *et al.,* 2008; Senthilvel *et al.*,2008; Narshimulu *et al.,* 2011; Radhika Ramya *et al.,* 2017; Singh *et al.,* 2017; Kumar *et al.,* 2020b; Ambawat *et al.,* 2020a, 2021; Choudhary *et al.,* 2021).

Conclusion

A_1 zone covers the major area of pearl millet in India and hence is the area of utmost priority for increasing pearl millet productivity. Lots of breeding efforts were put to deliver products for A_1 zone mainly but they were not much successful as this zone has vast variation in microclimate (day and night temperature and humidity) and soil apart from rainfall which requires proper quantification. Thus, it is the need of the hour to screen drought tolerant germplasm which can survive in harsh conditions of A_1 zone and which can be further used inbreeding programs to develop good drought tolerant cultivars. Such cultivars will be able to sustain under changing climatic scenario. Hence, this study will be useful and can be exploited for developing high yielding cultivars for low rainfall areas *i.e.* A_1 zone and enhance productivity of pearl millet up to a greater extent. The findings of this study can be anticipated to accelerate the efficiency of breeding programs to develop drought tolerant hybrids and varieties and ultimately increasing the pearl millet productivity. They can also prove to be an excellent genomic resource for isolation of candidate genes underlying drought tolerance and can further accelerate genetic improvement of the crop.

Acknowledgement

We sincerely acknowledge Indian Council of Agricultural Research (ICAR), New Delhi for providing financial assistance to ICAR-AICRP on Pearl millet, Jodhpur to carry out this research.

References

Akkaya, M.S. and Buyukunal-Bal, E.B. 2004. Assessment of genetic variation of bread wheat varieties using microsatellite markers. *Euphytica* 35: 179-185.

Ambawat, S., Satyavathi, C.T., Meena, R., Khandelwal, V. and Meena, R.C. 2020a. Molecular characterization and genetic diversity analysis of released hybrids and varieties of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Current Journal of Applied Science and Technology* 39(31): 92-104.

Ambawat, S., Satyavathi, C.T., Meena, R.C., Meena, R., Gurjar, N.R. and Khandelwal, V. 2021. Molecular characterization of drought tolerant genotypes of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for A_1 Zone. *International Journal of Environment and Climate Change* 11(11): 292-301.

Ambawat, S., Singh, S., Satyavathi, C.T., Meena, R., Meena, R.C. and Khandelwal, V. 2020c. Development of a high quality, rapid, efficient and economical DNA extraction protocol from climate resilient pearl millet crop without liquid nitrogen. *International Journal of Environment and Climate Change* 10(12): 85-94.

Ambawat, S., Singh, S., Shobhit, Meena, R.C. and Satyavathi, C.T. 2020b. Biotechnological applications for improvement of the pearl millet crop. In: *Pearl Millet: Properties, Functionality and its Applications*. (Eds. S.K. Gahlawat, S. Punia, A.K. Siroha, S.S. Sandhu and M. Kaur), Taylor & Francis (CRC Press), Boca Raton, Florida. pp. 115-138. doi.10.1201/9780429331732-7

Bollam, S., Pujarula, V., Srivastava, R.K. and Gupta R. 2018. Genomic approaches to enhance stress tolerance for productivity improvements in pearl millet: genomic approaches. In: *Biotechnologies of Crop Improvement* Vol. 3, (Eds. S.S. Gosal and S.H. Wani), pp. 239-264. Springer International Publishing AG, part of Springer Nature

Choudhary, M.L., Tripathi, M.K., Tiwari, S., Pandya, R.K., Gupta, N., Tripathi, N. and Parihar P. 2021. Screening of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplam lines for drought tolerance based on morpho-physiological traits and SSR markers. *Current Journal of Applied Science and Technology* 40(5): 46-63.

Jangra, S., Rani, A., Yadav, R.C., Yadav, N.R. and Yadav, D. 2019. Introgression of terminal drought stress tolerance in advance lines of popular pearl millet hybrid through molecular breeding. *Plant Physiology Reports* 24: 359-369. https://doi.org/10.1007/s40502-019-00464-w

Kapila, R.K., Yadav, R.S., Plaha, P., Rai, K.N., Yadav, O.P., Hash, C.T. *et al.,* 2008. Genetic diversity among pearl millet maintainers using microsatellite markers. *Plant Breeding* 127: 33-37.

Khairwal, I.S. and Yadav, O.P. 2005. Pearl millet (*Pennisetum glaucum*) improvement in India -Retrospect and prospects. *Indian Journal of Agricultural Sciences* 75: 183-191.

Kumar, R.R., Rai, G.K., Goswami, S., Singh, S.P., Satyavathi, C.T. and Praveen, S. 2020a. *Millets the Orphan Crop for Uncertain Future*. Scientific International Pvt. Ltd., New Delhi. 194p.

Kumar, S., Hash, C.T., Singh, G., Basava, R.K. and Srivastava, R.K. 2020b. Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. *Ecological Genetics and Genomics* 14:100051. 28.

Mahalingam, A., Sarawathi, R. and Ramalingam, J. 2016. SSR marker diversity analysis for WA cytoplasm based fertility restorer genes of hybrid rice (*Oryza sativa* L.). *Journal of Innovative Agriculture* 3(1): 19-27.

Mariac, C., Luong, I.V., Kapran, A., Mamadou, F., Sagnard, M., Deu, J. *et al.,* 2006. Diversity of wild and cultivated pearl millet accessions [*Pennisetum glaucum* (L.) R. Br.] in Niger assessed by microsatellite markers. *Theoretical and Applied Genetics* 114: 49-58.

Mitra, J. 2001. Genetics and genetic improvement of drought resistance in crop plants. *Current Science* 80(6): 758-763.

Narshimulu, G., Jamaloddin, M., Vemireddy, L.R., Anuradha, G. and Siddiq, E. 2011. Potentiality of evenly distributed hyper-variable microsatellite markers in marker-assisted breeding of rice. *Plant Breeding* 130: 314-320. DOI: 10.1111/j.1439-0523.2010.01834.x

Oumar, I., Mariac, C., Pham, J.L. and Vigouroux, Y. 2008. Phylogeny and origin of pearl millet [*Pennisetum glaucum* (L.) R. Br.] as revealed by microsatellite loci. *Theoretical and Applied Genetics* 117: 489-497.

Radhika Ramya, A., Ahamed, M.L. and Srivastava, R.K. 2017. Genetic diversity analysis among inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] based on grain yield and yield component characters. *International Journal of Current Microbiology and Applied Sciences* 6(6): 2240-2250.

Salazar, B., Laurentin, H., Davila, M. and Castillo, M. A. 2006. Reliability of the RAPD technique for germplasm analysis of sesame (*Sesamum indicum* L.) from Venezuela. *Interciencia* 31: 456-460.

Satyavathi, C.T., Ambawat, S., Khandelwal, V. and Srivastava, R.K. 2021a. Pearl millet: A climate-resilient nutricereal for mitigating hidden hunger and provide nutritional security. *Frontiers in Plant Science* 12:659938. doi: 10.3389/fpls.2021.659938.

Satyavathi, C.T., Ambawat, S., Sehgal, D., Lata, C., Tiwari, S., Srivastava, R.K., Kumar, S. and Chinnusamy,V. 2021b. Genomic designing for abiotic stress tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. In: *Genomic Designing for Abiotic Stress Resistant Cereal Crops*. (Ed. C. Kole) Springer, Cham. pp. 223-253. https://doi.org/10.1007/978-3-030-75875-2_6

Satyavathi, C.T., Khandelwal, V. and Ambawat, S. 2019. R & D Constraints and opportunities in pearl millet in India. In: One day national workshop on "*Production, Processing, Value Addition of Nutricereals (Millets) in India*" held at CCS HAU, Hisar. 13-16.

Senthilvel, S., Jayashree, B., Mahalakshmi, V., Kumar, P.S., Nakka, S., Nepolean, T. *et al.,* 2008. Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. *BMC Plant Biology*. 8: 119.

Shaikh, T.R. 2015. *Assessment of Hybrid Purity in Sorghum by Using Molecular Markers*. Thesis submitted to Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani-431402 (M.S.) India.

Shrivastava, A., Mishra, D., Koutu, G., Singh, S., Prakash, V. and Sohgaura, N. 2015. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. *The Ecoscan* 9(1-2): 265–269.

Singh, S., Singh, V.V., Supriya, A., Dubey, M. and Singh, D. 2017. Screening and estimation of allelic differentiation in Indian mustard (*Brassica juncea* L.) genotypes based on SSR Markers for background selection. *International Journal of Current Microbiology and Applied Sciences* 6(9): 2506-2516.

Tiwari, K.K., Singh, A., Pattnaik, S., Sandhu, M., Kaur, S., Jain, S. *et al.,,* 2015. Identification of a diverse mini-core panel of Indian rice germplasm based on genotyping using microsatellite markers. *Plant Breeding* 134(2): 164-171.

Verma, R., Tripathi, M.K., Tiwari, S., Pandya, R.K., Tripathi, N. and Parihar, P. 2021. Screening of pearl millet [*Pennisetum glaucum* (L.) R. Br.] genotypes against blast disease on the basis of disease indexing and gene-specific SSR markers. *International Journal of Current Microbiology and Applied Sciences* 10(2): 1108-1117.

Yadav, S., Singh, A.K., Singh, M.R., Goel, N., Vinod, K.K. and Mohapatra, T. 2013. Assessment of genetic diversity in Indian rice germplasm (*Oryza sativa* L.): Use of random versus trait-linked microsatellite markers. *Journal of Genetics* 92: 545-557.

Printed in March 2023