Journal of Advanced Zoology
ISSN: 0253-7214
*Volume **43** Issue-**S1 Year 2022** Page **751-757***

Advancement Of Gene Editing In Millets For Improved Food Security: A Review

Vineeta Chattree [1], Shruti Yadav [2]*, Naina Chaudhary[3], Lavanya Arora[4].

[1]Department of Biochemistry, Deshbandhu college, Delhi University, Kalkaji-110019, India.
[2] Department of Biochemistry, Deshbandhu college, Delhi University, Kalkaji-110019, India.*

***Corresponding author:** Shruti Yadav*
**Email: kbshrutiyadav@gmail.com*

CC License
CC-BY-NC-SA 4.0

Abstract:

The editing of gene is a new breeding technology, which can modulate DNA sequences at one or more points in the DNA strand. It empowers us to remodel the expression pattern of genes in prearranged region, which accelerates new insights in functional genomics of an organism. Gene editing, harnesses various mechanisms for regulation of genes, and these are predominantly site- specific recombinase (SSR) or site-specific nuclease (SSN) system. The SSN system induces a single or double strand break in the DNA and operates endogenous repair systems in DNA. The SSR technology intervenes in the eukaryotic genome and functions by the elimination or substitution of gene, depending upon the orientation of specific sites, flanking the desired sequence. The advancement of millets using genetic engineering is trailing behind in the comparison of other crops since these cereals are grown in less-developed countries by and large. Millets possess stronger, weather resistance than other cereals as compared to rice and wheat. This review refers to the current state of affairs of genetic engineering in millets and envisions the future of gene editing to develop nutrient rich, and climate sustainable crops.

Keywords*:* ***Genome Editing, Site-Specific Recombinase, Site-Specific Nuclease, CRISPR-Cas, TALENS, Qualitative trait locus (QTLs), Whole Genome Sequence (WGS).***

Introduction:

The advent of gene editing brings great enthusiasm amongst the Agri-scientist because of its prominence and ease it confers by offering the development of new enriched breed of plants with addition of beneficial qualities or deletion of unwanted features. This method in progress enables us to develop refined varieties with increased output and decreased cost input and also strengthens pest and disease resilience with fortified dietary value (Pixley et al., 2022). In order to cleave the gene sequence SSN technology uses four primary groups that are mega nucleases, ZFNs, TALENS and CRISPR/Cas nuclease system. CRISPR-Cas has become one of the most important methods in recombinant DNA technology because of the convenience of good reproducibility, improved efficiency, streamlined approach and quick cycle of this technique (Ceasar etal.,2019).

In bacteria and archaea CRISPR- Cas has emerged as an adaptive immunity in order to prevent it from the infection of viruses and other foreign toxins. The utilization of genome editing technology include crop improvement, crop protection, production of biofuels, pharmaceuticals, and other high-value secondary metabolites & nutraceuticals. The technology involves genome manipulation, including introduction of foreign gene or genomic sequences. In Indian perspective the technology has been passed through the Biosafety and environmental regulations making the process more inventive and ground- breaking for future prospects. The yield of grains including millet needs to increase from 2.5 to 4.5 times in order to meet the need of anticipated increasing population by 2025.Therefore this review of the genome edited crops will facilitate the biofortification of crops and help in dealing with the food security.

Millets are cereals which are dense in nutrient value and have greater resilience to climate change when correlated to other crops. The areas of Africa and Asia having semi-desert climate consumes these highly variable, mini seeded grains, predominantly cultivated by exhausted farmers. Gene editing has been used, potentially in various food crops, such as wheat and rice. The semi-arid areas are defined by erratic weather patterns, scantly distributed precipitation, deficient soil nutrient, and several other agricultural barriers. Fungal infections also restrict the production of millets, such as in finger millet. Millets are a staple grain that furnishes macronutrient amounts of protein and calories to the huge populations in semi-arid areas. The editing of genes in millets have not been accomplished yet, regardless of the dietary sufficiency. Millets have improved water and nitrogen uptake efficiency due to their usage of the C4 photosynthetic pathway which is very useful in warm weather contrarily to C3 crops (Aubry et al., 2011).

Millets exhibits remarkable resilience to a range of abiotic conditions including heat, salinity, and drought. To adjust with unfavourable ecological factors, they use morpho-physiological and biochemical adaptive techniques (Zohary et al.,2000). Insights for crop development initiatives have been provided by the characterization of genes, alleles, and QTLs (Qualitative trait locus) linked to stress tolerance through genetic and genomic research. Although millets have genetic pool, their genomic resources such as reporter genes and physical/genetic are limited in comparison to most of the cereals. The growing concerns of climate change and rising agricultural expenses due to ever increasing population have positioned millets as viable crops for nutrition and food security (Amadou et al., 2013). This research paper highlights how important it is to look into the genetic variants that naturally occur in millets and how to use genomic methods to improve crops. A renewed emphasis on millets benefits not only their direct cultivation but also other grains and bioenergy grasses because of their common heritage and substantial genetic synteny (Tirthankar et al.,2017).

Genomic Transformation traits in Pearl millet

The perusal of pertinent documents illustrates that particle bombardment is the most frequently used method for the genetic modulation of millets. The pearl millet was transformed for the Glucuronidase (GUS) gene regulated by maize alcohol dehydrogenase promoter using gene gun with embryonic tissue serving as the explants (Taylor et al 1991). Another genetic transformation in pearl millet was done for the Hygromycin phosphotransferase gene which provides resistance to hygromycin regulated by the CaMv35S promoter (Lambe et.al 1995). With the aim of increasing transformation frequency of biolistic system different explants (embryogenic tissue, inflorescence and apical meristem) was used for a reporter gene and the selectable bar gene in the plasmid pAHC25 and p524EGFP driven by CaMV35s promoter and alfalfa mosaic virus. The transformation was increased from 5 to 85 % (Goldman et al 2003). Transgenic pearl millet has also been formed to confer resistance to different fungal diseases, including the downy mildew by using the antifungal protein from Aspergillus giganteas with embryonic explants (Girgi et al 2006). The mapping of drought tolerant traits has also been explored by utilizing the potential germplasm association panel (Sehgal et al.,2015).

Genetic Engineering traits in Finger millets and Foxtail Millets

Finger millet is a self-fertilizing crop with a genome size of 1,593 Mb. It is a common annual herbaceous cereal crop consumed in Asia and Africa (Anil et al., 2022). The amount of protein and mineral nutrients is much higher when correlated to other crops such as wheat, rice and sorghum. It contains calcium (Ca) of remarkably high concentration ranging from 0.01 to 0.06% in whole seeds while most other cereals only have this element in small amounts (Chandra et al., 2016). The seeds are a rich source of nourishment such as fibre, iron, phenylalanine, methionine, leucine, and isoleucine, as well as trypsin inhibitory factors (Rao et al.,2011). The population living in Asia and Africa is estimated to be at risk of calcium scarcity as per reports which becomes critical reason for Vitamin D deficiency in these Country (Anitha et.al., 2021). Briefly cash crops like rice and wheat can contribute to guarantee of food abundance, finger millet has been suggested to pave a way to improve nutritional quotient in economically developing countries. The development of genetic and genomic resources is a critical first step in enhancing crop plants for particular features.

Finger millet production faces significant challenges from both biotic and abiotic stresses. A critical condition affecting harvest of these crop is infections inhibiting nutrient flow and reducing grain formation is caused by the fungal blast leading to yield losses ranging from 28% to as high as 90% per hectare. High seed loss is one of the prime biotic restraints which is induced by this leaf blast infection. This leads to deficiencies in nitrogen, phosphorus, and zinc, as well as drought and salinity which affects growth and yield (Gupta et., 2017).

Genetic transformation in finger millets also exhibits remarkable resistance to fungal blast disease by incorporation of the antifungal protein (PIN) derived from prawn. The PIN gene synthesized chemically was cloned with herbicide gene in PBar35s. Molecular analysis confirmed the success of the gene integration and expression (Latha 2005). Despite having better mineral nutrients and a longer shelf life than other major cereals, finger millet is one of the last cereals to be genome sequenced. This is due to the multiploidy of the gene and the corresponding small plantations in the emergent nations. The genetic information available is in the lowest amounts relative to other important cereals such as rice, maize and barley (Kumar et al., 2016).

Researchers have employed various strategies, including the insertion of genes from other salt-tolerant plants, to enhance the salt tolerance of finger millets (Jayasudha et al., 2014). The transfer of gene (SbVPase) vacuolar H+ pyrophosphate from sorghum has been utilized via agrobacterium driven genetic modification of finger millet. Overexpression of this gene resulted in improved growth performance under salt stress demonstrating the potential of SbVPase in enhancing salt toleration in finger millet (Anjaneyulu etal.,2019). Correspondingly, the introduction of genes from Arabidopsis thaliana(AVP1) and Pennisetum glaucum (PgNHX1) in finger millet also led to increase in the saline tolerance in contrast of the normal plants. These bioengineered finger millet plants exhibited higher levels of saline tolerance, highlighting the effectiveness of these genes in conferring salt tolerance to finger millet (Anil et., al 2022).

Another investigation was focused on overexpressing the serine-rich protein (PcSrp) gene from *Porteresia coarctata* in finger millet under saline conditions. Finger millet having PcSrp represented usual development, blooming and seed lot even undergoing high salinity stress, demonstrating the potential of gene in mitigating the effects of saline stress in finger millet (Mahalakshmi et al., 2006). Moreover, the induction of a bacterial gene mannitol-1-phosphate dehydrogenase (mtlD) into finger millet also resulted in improved growth receiving drought and salinity stress conditions. Transformed finger millet plants expressing the mtlD gene exhibited superior osmotic stress toleration and chlorophyll retaining capacity even in paucity of water when juxtaposed with control plants (Hema et al., 2014).

Agrobacterium mediated transformation was done in foxtail millet to introduce a pollen specific gene Si401 under the guidance of Zm13 promoter. The result repurposed foxtail millet as Si401 expression was found to cause a lot of abnormalities in late stage of Anther development. (Anil et al, Fang et al., 2007). The genome sequence data of foxtail millet has made it easier to create high volumes genome-wide molecular markers, which has provided insights for research on genetic diversity, the creation of genetic maps, and the identification of QTLs associated with nutritional and agronomic traits (Vetriventhan et al., 2020).

CROP	GENE	TRANSFORMATION
Pearl millet	ZmAdh1, Emu, ZmUbi, OsAct, double CaMV 35S, pin2	Embryogenic cell suspension, embryogenic callus, mature embryo
Barnyard millet	ZmUbi, OsAct, RbcS, ppcA-L-Ft	Embryogenic callus and leaf segment
	CaMV 35S	Protoplasts
Finger millet	*SbVPPase,* *PcSRP*	Salt tolerance
	chi11, PIN	Resistance to leaf blast disease
	mtlD *UidA*	Multiple stress tolerance
Foxtail millet	*Col-0*	Increased folate content
	SiARDP, SiLTP	Abiotic stress tolerance
	SiPHT1;1,2 and 3	High affinity Pi transporters
	SiPf40, *DPY1-3FLAG; SiBZR1-GFP; DPY1*	Plant architecture
	SiBOR1	Grain yield

	SiATG8a, SiMYB3; SiMYB3	Tolerance to nitrogen starvation
	SiREM6, *SiGRF,* *SiHAK1*	Salt tolerance
	SiPLDα, *SiBZR1; SiPLT-L1, SiMADS51,* *SiWLIM2b,* *SiMYB56*	Drought tolerance
	SiASR4, *SiBRI1*	Abiotic stress tolerance
	SiACC-R	Herbicide resistance & increased oil content

TABLE: GENETIC TRANSFORMATION TRAIT IN DIFFERENT MILLETS

Wealth of genomic wealth (WGSs) for Future aspects

Nutrient deficiencies are expected to worsen with rising fertilizer demands, particularly nitrogen and phosphorus, posing a significant challenge for resource-poor farmers in low-input agricultural systems. In order to solve these challenges and improve productivity, developing millet varieties tolerant to multiple nutrient stresses through genetic and genomic studies, aided by whole-genome sequencing, presents a promising approach (Ofori et al.,2022). The WGSs offer a wealth of opportunities for further research and application in millet breeding and improvement programs. They can serve as a basis for SNP identification, allele discovery through next-generation sequencing (NGS), and construction of linkage and association maps (Ramesh et al., 2022, Hittalmani et al.,2017). Additionally, most studies have focused on introduction of transgene and characterization of traits under targeted stress conditions. Detailed research such as cellular compartmenting of foreign genes and the addition of finger millet promoters with molecular markers (e.g., GUS, GFP), have yet not been executed in finger millet. The recently released whole-genome sequences (WGS) of finger millet provide a valuable resource for designing such studies. A significant advancement in finger millet research holds great potential for escalating its genetic improvement and cultivation by overall accessibility of WGS. The development of salt-tolerant finger millet varieties through genetic engineering has shown promising results in addressing the challenges posed by salinity stress the identification and isolation of native promoters from finger millet will enable functional analysis by fusing them with reporter genes, facilitating studies similar to those conducted in model plants like rice and Arabidopsis thaliana (Ramesh et al., 2022).

DICUSSION AND CONCLUSION

The major reason for the decrease in utilization of millets is the ignorance of its food value, inconvenience in cooking, underdeveloped processing techniques and government policies not giving any incentives for these crops while subsidizing the price for other fine crops. (FAO,2012). Nutritional benefits from millets are enormous as they contain essential Amino acids, B-vitamins and Minerals such as Calcium, Iron, Zinc, Magnesium and potassium (Kumssa et al., 2015). The intake of millets helps in the lowering of the blood sugar levels, blood pressure regulation and balances the hormone with added advantage of maintaining cardiovascular health. (Chandrasekara etal.,2012). A remarkable work on millets is also done in India by **Raimati Ghiuria**, often referred to as the "Queen of Millets," who has made significant strides in preserving and cultivating rare millet varieties. Her work not only maintains biodiversity but also empowers women in her community through agricultural training. After the unfolding of CRISPR/CAS, genome engineering has been developed to be one of the most accomplished and widely used technique for crop improvement. Apart from yield and stress tolerance, improving the nutrition in millet is one of the most prospective areas where genome editing is being exploited. Millets being the rich source of diverse nutrients can be productively targeted for metabolic engineering to synthesize novel compounds. Number of countries including India has decided to not restrain the transgene-free genome edited crops developed through Site-Directed Nuclease-1 (SDN-1) and Site-Directed Nuclease-2 (SDN-2), which has augmented the genome editing based crop improvement programmes. The only constrain of using the genome editing is the non-availability of transformation and regeneration protocol in several crops and henceforth; to utilize the potential of this technique, it is of utmost importance to develop such protocols in important millets and cereals.

Conflict of Interest : No conflict of interests declared by authors.

REFERENCES:

1. **Amadou, I., Gounga, M. E., and Le, G. W. (2013).** Millets: nutritional composition, some health benefits and processing - a review. Emir. J. Food Agric. 25, 501–508. doi: 10.9755/ejfa. v25i7.12045
2. **Aubry, S., Brown, N. J., and Hibberd, J. M. (2011)**. The role of proteins in C3 plants prior to their recruitment into the C4 pathway. J. Exp. Bot. 62, 3049–3059. doi: 10.1093/xb/err012
3. **Anitha, S., Givens, D.I., Botha, R., Kane Potaka, J., Sulaiman, N.L.B., Tsusaka, T.W., Subramaniam, K., Rajendran, A., Parasannanavar, D.J. and Bhandari, R.K. (2021).** Calcium from Finger Millet—A systematic review and meta-analysis on calcium retention, Bone resorption, and in vitro bioavailability. Sustainability**.** (13): 8677. https://doi.org/10.3390/su13168677
4. **Anjaneyulu, Ediga., Reddy, Palle Surender., Sunita, Merla Srilakshmi |., Kishor, Polavarapu B. Kavi and Meriga, Balaji (2014).** Salt tolerance and activity of antioxidative enzymes of transgenic finger millet overexpressing a vacuolar H (+)-pyrophosphatase gene (SbVPPase) from Sorghum bicolour. Journal of Plant Physiology. 171(10):789-98. doi:10.1016/j.jplph.2014.02.001.
5. **Chandra, D., Chandra, S., Pallavi, and Sharma, A. K. (2016).** Review of finger millet (Eleusine coracana (L.) Gaertn): a power house of health benefiting nutrients. Food Sci. Hum. Welln. 5, 149–155. doi: 10.1016/j.fshw.2016.05.004
6. **Fang FQ, Qian Z, Guang MA and Jing, J.Y. (2007)**. Co-suppression of Si401, a maize pollen specific Zm401 homologous gene, results in aberrant anther development in foxtail millet, Euphytica 163(1):103-111, DOI:10.1007/s10681-007-9610-4
7. **FAO (2012).** http://www.fao.org/3/a-ap106e.pdf
8. **Girgi M, Breese WA, Lörz H, Oldach K.H. (2006).** Rust and downy mildew resistance in pearl millet (Pennisetum glaucum) mediated by heterologous expression of the afp gene from Aspergillus giganteus. Transgenic Res. 2006 Jun;15(3):313-24. doi: 10.1007/s11248-006-0001-8
9. **Girgi, M., O'Kennedy, M.M., Morgenstern, A. (2002).** Transgenic and herbicide resistant pearl millet (Pennisetum glaucum L.) R.Br. via microprojectile bombardment of scutellar tissue. Molecular Breeding 10, 243–252 (2002). https://doi.org/10.1023/A:1020558315265
10. **Goldman, JJ., Hanna, WW., Fleming, G and Ozias-Akins, P. (2003).** Fertile transgenic pearl millet [Pennisetum glaucum (L.) R. Br.] plants recovered through microprojectile bombardment and phosphinothricin selection of apical meristem-, inflorescence-, and immature embryo-derived embryogenic tissues. Plant Cell Rep; 21(10):999-1009. Doi: https://doi.org/10.1007/s00299-003-0615-8
11. **Gupta, S. M., Arora, S., Mirza, N., Pande, A., Lata, C and Puranik, S. (2017).** Finger Millet: a “certain” crop for an “uncertain” future and a solution to food insecurity and hidden hunger under stressful environments. Front. Plant Sci. 8:643. doi: 10.3389/fpls.2017.00643
12. **Hauptmann, RM., Vasil, V., Ozias-Akins, P., Tabaeizadeh, Z., Roger, S.G., Fraley, R.T., Horsch, R.B and Vasil I.K. (1988).** Evaluation of selectable markers for obtaining stable transformants in the gramineae. Plant Physiol; 86(2):602-6. doi: 10.1104/pp.86.2.602.
13. **Hema, R., Vemanna, R. S., Sreeramulu, S., Reddy, C. P., Senthil-Kumar, M., and Udayakumar, M. (2014).** Stable expression of mtlD gene imparts multiple stress tolerance in finger millet. PLoS One 9: e99110. doi: 10.1371/journal.pone.0099110
14. **Hittalmani, S., Mahesh, H. B., Shirke, M. D., Biradar, H., Uday, G., Aruna, Y. R. (2017).** Genome and transcriptome sequence of finger millet (Eleusine coracana (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18:465. doi: 10.1186/s12864-017-3850-z
15. **Jaiswal, V., Gupta, S., Gahlaut, V., Muthamilarasa,M.,Bandyopadhyay, T., Ramchiary, N and Prasad, M. (2019)** Genome-wide association study of major agronomic traits in foxtail millet (Setaria italica L.) using ddRAD sequencing. Sci Rep. https://doi.org/10.1038/s41598-019-41602-6
16. **Jayasudha, B. G., Sushma, A. M., Prashantkumar, H. S., and Sashidhar, V. R. (2014).** An efficient in-vitro Agrobacterium mediated transformation protocol for raising salinity tolerant transgenic finger millet (Eleusine coracana (L.) Gaertn). Plant Arch. 14, 823–829.
17. **Kumar, Anil., Kumar, Rajesh., Pathak, Sanjay., Gupta, Mohan and Sood, Salej (2022)**. The finger Millet genome, Springer Science and Business Media LLC.
18. **Kumar, A., Metwal, M., Kaur, S., Gupta, A. K., Puranik, S., Singh, S. (2016).** Nutraceutical value of finger millet [Eleusine coracana (L.) Gaertn.], and their improvement using omics approaches. Front. Plant Sci. 7:934. doi: 10.3389/fpls.2016.00934

19. **Kumssa, D. B., Joy, E. J. M., Ander, E. L., Watts, M. J., Young, S. D., Walker, S. (2015).** Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Sci. Rep. 5:10974. doi: 10.1038/srep10974
20. **Lambé, P., Mutambel, H.S., Deltour, R. (1995).** Differential long-term expression and methylation of the hygromycin phosphotransferase (hph) and β-glucuronidase (GUS) genes in transgenic pearl millet (Pennisetum glaucum) callus (1998). https://doi.org/10.1016/0168-9452(95)04124-D
21. **Latha, AM., Rao, KV., Reddy, T.P and Reddy, V.D. (2006).** Development of transgenic pearl millet (Pennisetum glaucum (L.) R. Br.) plants resistant to downy mildew. Plant Cell Rep. 2006 Sep;25(9):927-35. doi: 10.1007/s00299-006-0141-6.
22. **Liu, Yinghui., Yu, Jingjuan., Zhao, Qian., Zhu, Dengyun and Ao, Guangming (2005).** Genetic transformation of millet (*TetariaItalica)* by Agrobacterium-mediated, Agric Biotechnol J 13(1):32-37. Doi: https://europepmc.org/article/cba/521723
23. **Mahalakshmi, S., Christophe, G. S., Reddy, T.P., Rao K V and Reddy, V.D. (2006).** Isolation of a cDNA clone (PcSrp) encoding serine-rich-protein from Porteresia coarctata T and its expression in yeast and finger millet (Eleusine coracana L.) affording salt tolerance. Planta.;224(2):347-59. doi: 10.1007/s00425-005-0218-4. Epub 2006 Feb 1. PMID: 16450172.
24. **O'Kennedy, M.M., Burger, J. T and Botha, F. C. (2004).** Pearl millet transformation system using the positive selectable marker gene phosphomannose isomerase. Plant Cell Rep 22:684–690 DOI 10.1007/s00299-003-0746-y
25. **Ofori, K. F., Antoniello, S and English, M. M. (2022).** Improving nutrition through biofortification–a systematic review. Front. Nutr. 9, 1043655. doi: 10.3389/fnut.2022.1043655
26. **O'Kennedy, MM., Grootboom, A and Shewry, P.R. (2006).** Harnessing sorghum and millet biotechnology for food and health. Journal of Cereal Science, Doi: https://doi.org/10.1016/j.jcs.2006.08.001
27. **Pixley, K. V., Falck-Zepeda, J. B., Paarlberg, R. L., Phillips, P. W. B., Slamet-Loedin, I. H and Dhugga, K. S. (2022).** Genome-edited crops for improved food security of smallholder farmers. Nat. Genet. 54, 364–367. doi: 10.1038/s41588-022-01046
28. **Pudake, Ramesh N**., **Solanke, Amol Kumar U., Sevanthi, Amitha Mithra and Kumar, Rajendra P(2022**.) Omics of Climate Resilient in Small Millets, Springer Science and Business Media LLC.
29. **S. Antony Ceasar, T. Maharajan, T. P. Ajeesh Krishna, M. Ramakrishnan, G. Victor Roch, Lakkakula, Satish and Savarimuthu, Ignacimuthu (2018).** Finger Millet [Eleusine coracana (L.) Gaertn.] Improvement: Current Status and Future Interventions of Whole Genome Sequence. doi: 10.3389/fpls.2018.01054
30. **Sehgal, D., Skot, L., Singh, R., Srivastava, R. K., Das, S. P and Taunk, J. (2015).** Exploring potential of pearl -+3. millet germplasm association panel for association mapping of drought tolerance traits. PloS One 10, e0122165. doi: 10.1371/journal.pone.0122165
31. **Rasika, A.**, **Chavan, S.M., Gopi, Sravya and Prathapan, K. (2024).** Review Paper on Millets: Production, Nutrient, Processing and Food Products for Health and Sustainability. International Journal of Environment, Agriculture and Biotechnology. 9, 3.
32. **Taylor, M.G and Vasil, I. K. (1991).** Histology of, and physical factors affecting, transient GUS expression in pearl millet (Pennisetum glaucum (L.) R. Br.) embryos following microprojectile bombardment, 120–125 (1991). https://doi.org/10.1007/BF00232041
33. **Taylor, M.G., Vasil, V. and Vasil, I.K. (1993).** Enhanced GUS gene expression in cereal/grass cell suspensions and immature embryos using the maize uhiquitin-based plasmid pAHC25, 491–495 (1993). https://doi.org/10.1007/BF00236093
34. **Tirthankar, Bandyopadhyay., Mehanathan, Muthamilarasan and Prasad, Manoj (2017).** Millets for Next Generation Climate-Smart Agriculture, Front. Plant Sci., 2017.Sec. Plant Breeding, Doi; https://doi.org/10.3389/fpls.2017.01266
35. **Vetriventhan, M., Azevedo, V.C.R and Upadhyaya, H.D. (2020).** Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63, 217–239. https://doi.org/10.1007/s13237-020-00322-3.
36. **V. Edwin, Hillary and S. Antony Ceasar (2019).** Application of CRISPR/Cas9 Genome Editing System in Cereal Crops. the Open Biotechnology Journal 13(1):173-179 DOI:10.2174/1874070701913010l7
37. **Yamunarani, R., Govind, G., Ramegowda, V., Thammegowda, H. V., and Guligowda, S. A. (2016).** Genetic diversity for grain Zn concentration in finger millet genotypes: Potential for improving human Zn nutrition. Crop J. 4, 229–234. doi: 10.1016/j.cj.2015.12.001

38. **Yadav, R.S., Sehgal, D and Vadez, V.(2011).** Using genetic mapping and genomic approaches in understanding and improving drought tolerance in pearl millet. Journal of Exp Bot. 62, (2):397-408.
39. **Zohary, D., and Hopf, M. (2000).** Domestication of Plants in the Old World: the Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley. Third edition: Oxford University Press.