INTRODUCTION

Pearl millet is one of the sixth most and economically significant small seeded millet crop in the world. It contributes to 50% of world millet production. Pearl millet has its origin in Sahel of West Africa, where it was domesticated about 3000 years BP (Clotault et al., 2010). It belongs to the family Poaceae with 2n=2x=14 chromosomes and has a genome size of 1.76 Giga bases (Varshney et al., 2017). It is the staple food of Africa and North-west India, feeding about 90 million poor people across the world. India is the largest producer of pearl millet in the world that was grown in 6.93 million ha owing to average production of 8.61 million tonnes and productivity of 1243 kg/ha during 2018-2019 (AICRP, 2020). Pearl millet is considered to be a high energy cereal, rich in protein (8-19%), low starch content, high fiber content, rich in vitamins A and B, high calcium, iron, zinc with minor amounts of nutrients such as potassium, phosphorus, magnesium, copper, and manganese (Pattanashetti et al., 2016). In addition, it can be used as animal feed, brewery, and as roofing material.
material. Pearl millet is a plant with climate-smart vegetative, reproductive, and morphological features that makes the crop the ideal choice for the future (Taylor, 2016).

The genetic diversity of pearl millet is so wide, that it can be exploited and efficiently utilized for the development of new and economically superior varieties and hybrids. The present-day pearl millet varieties/hybrids development focused on meeting the nutritional quality requirement like Fe and Zn along with higher yield. There are about 66,682 accessions of pearl millet wild and cultivated germplasm across 65 countries in about 97 gene banks. ICRISAT gene bank in Hyderabad, India has the largest collection of accessions (https://www.genesys-pgr.org/c/pearlmillet). SSR markers are the most preferred marker type for fingerprinting, since they are very effective in distinguishing the germplasm. SSR markers have more advantages over the other markers due to its simplicity and higher reproducibility. SSR markers produces polymorphic genetic informations where the hyper-degenerate nature of the SSR marker produces very high allelic variations even among the closely related species (Vieira et al., 2016). Many SSR markers have been previously developed and utilized for varietal/hybrid identification and marker-assisted breeding in many crops such as rice, maize, and sorghum whose genome resources are available. These markers are used in the study of genetic distance, evolutionary studies, construction of linkage maps and marker assisted selection, and defining cultivar specific fingerprints (Vieira et al., 2016).

Many pearl millet varieties and hybrids are released for commercial cultivation from the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University for the past 50 years. This study focuses on the most popular varieties and hybrids available in Tamil Nadu that has to be fingerprinted for varietal identification and germplasm registration with NBGCR. The EST-based markers that focused on fingerprinting belongs to PSMP markers series (Qi et al., 2001 and Qi et al., 2004), ICMP markers (Senthilvel et al., 2008) and IPES series of markers (Rajaram et al., 2013).

MATERIALS AND METHODS

Plant Materials

The Pearl millet cultivars viz., CO (Cu) 9, CO 10, Pearl Millet Hybrid CO 9 along with its parents A (male sterile) line ICMA 93111A and R (pollinator) line PT 6029-30 were raised in the Department of Millets, Centre for Plant Breeding & Genetics, TNAU, Coimbatore during Kharif, 2019 in the yield trials. The TNAU pearl millet varieties used in the study are popular in Tamil Nadu due to its promising yield potential. The CO (Cu) 9 variety have special characters such as short duration (80-85 days), high yield, and resistance to downy mildew. The composite variety CO 10 is developed by mixing and random mating of five elite inbred lines PT6029, PT6033, PT6034, PT6039, and PT6047 that is having a higher yield along with downy mildew disease resistance. Pearl Millet Hybrid CO 9 is a high yielding, early maturing hybrid developed from a cross between Cytoplasmic Male Sterile (CMS) line ICMA93111 (A line) and a pollinator line PT 6029-30 (R line). For which seedlings are planted on the side of the ridge and half way from the bottom. Depth of planting is 3-5 cm with the spacing of 45 x 15 cm. Randomized Block Design (RBD) is followed with three replications.

Observation of Morphological Characteristics

The five randomly selected plants were subjected to morphological characterization to study the phenotypic performance under the plot size of 10 cents. The DUS characters for the 11 quantitative traits including plant height (cm), number of productive tillers, leaf sheath length (cm), leaf blade length (cm), leaf blade width (cm), days to 50% flowering, number of nodes (No’s), spike exertion, spike length (cm), spike girth (cm), and 1000 seed weight (g) and 7 qualitative traits including anther color, node pigmentation, spike shape, node pubescence, spike density, seed color, seed shape were recorded.

Genomic DNA Isolation and PCR Analysis

Total genomic DNA was extracted from three-week-old leaves using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray & Thompson, 1980). The isolated DNA samples were quantified with the micro-volume Spectrophotometer. Based on the quantity the DNA present in samples, it was diluted to a working concentration of 25ng/µL for the Polymerase Chain Reaction (PCR) amplification. The thirty-six primers were randomly selected based on the markers reported (Qi et al., 2001 & Qi et al., 2004; Senthilvel et al., 2008; Rajaram et al., 2013). The PCR (Eppendorf, Hamburg, Germany) reactions were performed to a total volume of 12-µL reaction mixtures containing 2 µL of Genomic DNA as a template, 7 µL of 1X Master Mix diluted from smart Prime MasterMix-Red (2X), 1µL of the primer pairs (Forward and Reverse) and 2 µL of Milli-Q water. The PCR profile consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of amplification at 94°C for 30 seconds of subsequent denaturation, 50-60°C for 30 seconds for annealing of primers to the template, and 72°C for 30 seconds for an extension. A final extension step at 72°C for 7 min was followed by termination of the cycle. The PCR amplicons were ran in 3% agarose gel prepared using a 1x TBE buffer at 100 V for 3 h. The resolved amplified products were visualized using a gel documentation system (Bio-Rad, CA, USA).

RESULTS AND DISCUSSION

The Pearl millet germplasm is characterized using morphological traits (Nehra et al., 2016) for varietal and hybrid identification (Sumathi et al., 2012). Most of the recent molecular markers studies in pearl millet focusing on allele richness and genetic diversity among the wild and cultivated plants. The hybrid purity tests and phylogenetic relationship analysis with the utilization of molecular markers such as RAPD (Randomly Amplified Polymorphic DNA) markers (Govindaraj et al., 2009) and SSR markers (Kapila et al., 2008; Wagmode, 2016; Chandra-Shekara et al., 2017) are getting important because
Morphological Descriptors

The information outlined in Table 1 can effectively be used to find out distinct features of pearl millet cultivars. Plant height ranged from 80 cm to 220 cm for the cultivars under study. Among the cultivars, CO (Cu) 9 variety recorded the highest plant height (186-220 cm) followed by pearl millet hybrid CO 9 (160-180 cm) that is on par with CO 10 composite variety. Pearl millet hybrid CO 9 recorded the highest value for more than four quantitative characters. For instance, Number of productive tillers (4-6), Leaf blade length (60-68 cm), Leaf blade width (4.0-4.5 cm), number of nodes (8-10), and 1000 seed weight (13-14 g), which is on par and comparable with the CO 10 composites and higher than that of the variety CO (Cu) 9. The highest leaf-sheath range was recorded in CO 10 composites (13.5 to 14.5 cm) and that is comparable with the pearl millet hybrid CO 9 (12.5-13.5 cm). For the traits spike length and girth, the highest value was recorded in the variety CO (Cu) 9 (33-39 cm and 3-4 cm) followed by CO 10 composite (25-34 cm and 3.1-3.6 cm) and pearl millet hybrid CO 9 (25-35 cm and 3.1-3.6 cm). Eight qualitative traits were recorded and it showed that another color in pearl millet hybrid CO 9 which is distinct from the CO 10 (Yellow). Node pubescence is absent in the case of pearl millet hybrid CO 9 and is occasionally present in the composite variety CO 10, whereas glabrous in CO (Cu) 9 variety. Red color node pigmentation was observed in pearl millet hybrid CO 9 and its parents viz., ICMA 93111A (Male), and PT 6029-30 (Female), and the composite CO 10 reflects green color node pigmentation. Spike shape and density are candle and compact in case of pearl millet hybrid CO 9 which is spindle/occasionally cylindrical and compact/rarely semi-compact for the composite CO 10 and CO (Cu) 9 it is a candle to cylindrical and the density is compact. Seed color is grayish-yellow for pearl millet hybrid CO 9 and CO 10 whereas, grayish seed with yellow base is recorded in CO (Cu) 9. Pearl millet hybrid CO 9 had a globular seed shape and it is recorded elliptical for CO 10, ICMA 93111A (Male), and PT 6029-30 (Female) (Table 1). Results were in accordance with (Sumathi et al., 2012; Singh et al., 2016). The Spike of the composites CO 10 (Figure 1A) and Pearl millet hybrid CO 9 (Figure 1B) (Sumathi et al., 2017; Subbulakshmi et al., 2018) is shown in Figure 1.

Fingerprinting of Pearl Millet Cultivars using SSR Markers

Most of the SSR markers were developed from the genomic and EST regions of the pearl millet genome. The Pearl millet SSR markers are derived from the conserved regions of the genome and hence easily differentiating the varieties/hybrids. A total of 36 SSR markers covering various chromosomes were taken for the initial polymorphism survey. All the 36 SSR primers (Table 2) showed proper amplification for the varieties viz., CO (Cu) 9, CO 10, Pearl Millet Hybrid CO 9, and its parents A‘ line ICMA 93111A and R’line PT 6029-30. Among the SSR makers, only two markers ICMP3021 and PSMP2089 distinguished the variety CO 9 from other pearl millet varieties. The markers ICMP3021 (Figure 2A) and PSMP2089 (Figure 2B) have

![Image](image_url)
distinguished CO 9 variety by producing alleles at 200bp and 150bp, respectively. Whereas, other cultivars showed the alleles at 190bp and 130bp.

The composite variety CO 10 was discriminated from other lines using three markers viz., ICMP3018 (Figure 3A), PSMP2219, and PSMP2220 by the presence of an additional allele indicating its composite nature. In the composite CO 10, the marker ICMP3018 produces an additional allele at 210bp, the marker PSMP 2219 (Figure 3B) at 305bp, and the marker PSMP2220 (Figure 3C) at 128bp. None of the SSR makers helped for distinguishing TNAU Pearl millet Hybrid CO 9 from its parental

Table 2: Details of markers used for the polymorphic study

| S. No | Marker name | Forward sequence | Reverse sequence | Annealing Temp.(˚C) | Status of the allele | Allele size (bp) |
|-------|-------------|------------------|------------------|---------------------|---------------------|-----------------|
| 1     | ICMP3014    | TGCTTCACAGCCCTCCATA | CCACCATGCACACCGCAATAA | 55 | M           | 220 |
| 2     | ICMP3018    | AGACGAGGAAACGCTTGGAA | AGACGCGCATCTGACATA | 55 | P           | 240 |
| 3     | ICMP3021    | GCCGCGGACGAAGATTGAGA | AGCAAAACGACAAACAGA | 55 | P           | 200 |
| 4     | ICMP3032    | CGAAGGAGGTCAAGATCGA | ACACGACTCGACTGACCAC | 58 | M           | 200 |
| 5     | ICMP3033    | GCCAAGGAGGTCAAGATCGA | ACACGACTCGACTGACCAC | 55 | M           | 190 |
| 6     | ICMP3035    | GCCAAGGAGGTCAAGATCGA | ACACGACTCGACTGACCAC | 56 | M           | 190 |
| 7     | ICMP3036    | TGCTTCACAGCCCTCCATA | CCACCATGCACACCGCAATAA | 52 | M           | 214 |
| 8     | ICMP3037    | CCACGAGGAGGAAACGCAAC | ACACCGTGAAACACACAC | 50 | M           | 176 |
| 9     | ICMP3038    | TGCTTCACAGCCCTCCATA | CCACCATGCACACCGCAATAA | 53 | M           | 133 |
| 10    | ICMP3039    | GCGGCGTACAGCAAAACGTA | TCGTTCACATGTTTCCACAC | 51 | M           | 115 |
| 11    | ICMP3040    | GCCGTTCACAGCAAAACGTA | TCGTTCACATGTTTCCACAC | 52 | M           | 115 |
| 12    | ICMP3041    | GCCGTTCACAGCAAAACGTA | TCGTTCACATGTTTCCACAC | 50 | M           | 160 |
| 13    | ICMP3042    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 51 | M           | 233 |
| 14    | ICMP3043    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 53 | M           | 264 |
| 15    | ICMP3044    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 52 | M           | 122 |
| 16    | ICMP3045    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 55 | M           | 126 |
| 17    | ICMP3046    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 56 | M           | 150 |
| 18    | ICMP3047    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 52 | M           | 176 |
| 19    | ICMP3048    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 53 | M           | 200 |
| 20    | ICMP3049    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 55 | M           | 200 |
| 21    | ICMP3050    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 56 | M           | 200 |
| 22    | ICMP3051    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 57 | M           | 200 |
| 23    | ICMP3052    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 58 | M           | 200 |
| 24    | ICMP3053    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 59 | M           | 200 |
| 25    | ICMP3054    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 60 | M           | 200 |
| 26    | ICMP3055    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 61 | M           | 200 |
| 27    | ICMP3056    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 62 | M           | 200 |
| 28    | ICMP3057    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 63 | M           | 200 |
| 29    | ICMP3058    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 64 | M           | 200 |
| 30    | ICMP3059    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 65 | M           | 200 |
| 31    | ICMP3060    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 66 | M           | 200 |
| 32    | ICMP3061    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 67 | M           | 200 |
| 33    | ICMP3062    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 68 | M           | 200 |
| 34    | ICMP3063    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 69 | M           | 200 |
| 35    | ICMP3064    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 70 | M           | 200 |
| 36    | ICMP3065    | TTGCCGAACCTGCAACACGAC | TCGTTCACATGTTTCCACAC | 71 | M           | 200 |

(M) Monomorphic, (P) Polymorphic
lines. Hence, additional SSR makers have to be screened to distinguish the parental lines and hybrids.

CONCLUSION

In this study, morphological descriptors recorded using DUS characteristics and molecular screening for fingerprinting the pearl millet varieties, hybrids, and their respective parents were done. Nowadays, it is mandatory to fingerprint the recently released varieties/hybrids for the registration by NPBGR and also for the Protection of Plant Varieties and Farmers Rights Authority (PPVFWRA). Thus, fingerprinting of varieties/hybrids using molecular markers and DUS characteristics paves a way for registration as well as for varietal identification.

ACKNOWLEDGMENT

The Core projects for research activities at Colleges and Research stations of TNAU- Phase-I (CPMB/CBE/PBT2012/CP004) were funded by the Agricultural Department (AU), Government of Tamil Nadu (B-Agriculture Plan-27-University Research Scheme-NV-Core projects TNAU Phase-I) is kindly acknowledged.

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