DNA fingerprinting of foxtail millet (*Setaria italica* L.)
variety ATL 1 using SSR and RAPD markers along with
morphological descriptors

Senthil Natesan¹*, Gowtham Murugesan¹, Nandhini Murugan¹,
Sarankumar Chandran¹ and Nirmalakumari Angamuthu²

¹Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and
Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India
²Centre of Excellence in Millets, Athiyanthal, Tamil Nadu Agricultural University, Thiruvannamalai-606603,
Tamil Nadu, India

*Corresponding Author: senthil_natesan@tnau.ac.in

[Accepted: 20 October 2020]

**Abstract:** Foxtail millet (*Setaria italica*) is a cultivated nutritional cereal, which originated in
South Asia and is considered one of the oldest cultivated millets in India. DNA fingerprinting is
mandatory for registration of newly developed varieties with National Bureau of Plant Genetic
Resources (NBPGR) and Protection of Plant Varieties and Farmers’ Rights Authority
(PPV&FRA). Due to the limited availability of genomic information in foxtail millet, the use of
DNA based markers in fingerprinting of crop varieties is also limited. Hence in the present
investigation, available RAPD and SSR markers of cereals are used for fingerprinting the foxtail
millet varieties. The newly released variety ATL 1 is differentiated from popular variety CO (Te) 7
using SSR and RAPD markers. About 66 maize SSR primers, 16 sorghum SSR primers, and 10
RAPD primers were used in the study. Out of 66 maize SSR markers used for study, one showed
polymorphism. The marker umc1704 showed polymorphism between CO (Te) 7 and ATL 1 by the
presence of 670 bp allele CO (Te) 7. The RAPD primers OPB4, OPA5, OPA11 and OPB1 also
helped for differentiation of the two varieties. The identified makers will help for genetic purity
testing of CO (Te) 7 and ATL 1 in the seed chain.

**Keywords:** DNA fingerprinting - SSR - RAPD primer - Foxtail millet.

[**Cite as:** Natesan S, Murugesan G, Murugan N, Chandran S & Angamuthu N (2020) DNA fingerprinting of
foxtail millet (*Setaria italica* L.) variety ATL 1 using SSR and RAPD markers along with morphological
descriptors. Tropical Plant Research 7(3): 587–593]

**INTRODUCTION**

Foxtail millet (*Setaria italica* L.), is one among the cereal annual grass grown for human food (Heuze
*et al*. 2015) belongs to the Panicoideae of Poaceae family. Foxtail millet is considered to be a dry farming
cereals that forms the oldest cultivated food grain known to humans (Lu *et al*. 2009). It is an important
nutritional cereal in China, India and part of Asia because of its drought tolerance and well adapted to
arid and semiarid regions. The diploid nature of foxtail millet nowadays is considered as an ideal plant
for the genetics of drought tolerance and nutritional research (Andrew *et al*. 1998). Currently, is
becoming more popular among the urban population of India because of changing diet habit of the
people. Foxtail millet has potential for abiotic stress tolerance and its genetic relatedness to many
bioenergy sorts of grasses-like switchgrass, Napier grass and Pearl millet and hence use of genomic
information from other cereals will help for detailed study (Muthamilarasan & Prasad 2015). Foxtail
millet contains significant levels of protein, fibre, mineral, and phytochemicals. Anti-nutrients like phytic
acid and tannin present in this millet are often reduced to negligible levels through modified method of
processing. The millet is additionally reported to have hypolipidemic, low-glycemic index, and high
antioxidant characteristics. (Sharma & Niranjan 2018). Application of molecular marker techniques to
identify differences in cultivars is routinely followed in many kinds of cereal except the small millets like foxtail millet. The DNA fingerprints are accurate as it differentiates the individuals based on marker information which is further used for estimating genetic diversity, marker-assisted selection in plant breeding (Weising et al. 2005). Finally, DNA fingerprinting plays a vital role in protecting the novelty of a newly evolved plant variety which is submitted to NBPGR and PPV&FRA for notification and its registration. Simple Sequence Repeats are the tandem repeat of around six nucleotides in both the coding as well as non-coding regions. The SSRs have become a marker of choice in genotyping because of their abundance, high level of allelic variation, co-dominant inheritance and analytical simplicity (Miah et al. 2013). Moreover, microsatellite markers might be effectively applied to differentiate phylogenetically related species consistent with their conserved sequences, which could be useful to study the genetic constituents of the related species. RAPD marker provides amplicon from one individual and not to the another as it is a dominant marker and tedious to identify the amplified segment is from the heterozygous loci or homozygous loci (Williams et al. 1990). RAPD markers are well suited for DNA fingerprinting where the information on genomics about the crops like foxtail millet is limited. RAPD markers can also provide an efficient assay for polymorphism, which would allow rapid identification of DNA fingerprint for varietal identification. Hence the present investigation was focused on the development of varietal-specific fingerprints of popular foxtail millet variety along with the newly released variety for varietal identification and germplasm registration.

MATERIALS AND METHODS

Plant materials

The plant materials used for the study include foxtail millet cultivars viz., ATL 1 and CO (Te) 7. The variety CO (Te) 7 was a cross between CO 5 and ISe 248 which was a non-lodging and high yielding variety (Nirmalakumari et al. 2005). The tenai (Vernacular name in tamil) culture ATL 1 is a cross derivative of PS 4 x Ise 198 and evolved by Centre of Excellence in Millets, Athiyanthal. This variety is going to replace already existing variety, CO (Te) 7 with high yielding ability and resistance to biotic and abiotic stresses for tenai growing regions of Tamil Nadu. The culture ATL 1 having bold grains, high nutrients, good grain qualities for value addition and nutritive and palatable fodder. The two foxtail millet varieties viz., ATL 1 and CO (Te) 7 are evaluated at Centre of Excellence in Millets at Athiyanthal for various morphological descriptors and DUS characters during Rabi 2020 season. The DNA extracted from two foxtail millet genotypes were used for fingerprinting with maize and sorghum SSR primers followed by RAPD primers.

DNA extraction and quantification

Total genomic DNA was isolated from foxtail millet cultures by using the modified CTAB method. The leaf sample of 1 g (from 15 days old crop leaf) was ground in CTAB buffer (120mM Tris, pH-8.0, 1.3M NaCl, 25mM EDTA pH-8.0, 0.2% β-mercaptoethanol, pinch of PVP, 2% CTAB), incubated at 65°C for 45 minutes. The extract was purified with 24:1 mixture of chloroform-isoamyl alcohol before precipitation of DNA with a double volume of isopropanol. The precipitate was separated out and dissolved with RNAse then followed by addition of 70% ethanol, sodium acetate and dissolved with TE buffer.

DNase product (2 μl) from each sample was tested for its quality through electrophoresis using 0.8% agarose gel having ethidium bromide in a 1x TAE buffer (40 mMTris, 20 mM acetic acid, and 1 mM EDTA.) at 100 V for about 30 minutes. Finally, the DNA segments on the gel were subjected to a Gel Doc unit and documented. Also, the quantity of the DNA was checked by spectrophotometer. The quantity of DNA was found to be high for all the genotypes. The ratio of 260/280 showed all the DNA samples scored between 1.8 and 2.0 which indicates the absence of other contaminants such as protein and RNAse.

Amplification of foxtail millet cultures was performed using 66 maize SSR primers, 16 sorghum SSR primers and 10 RAPD primers. Maize SSR markers were obtained from the database Maize GDB (https://www.maizegdb.org/datacentre/ssr). RAPD primer sequence obtained from Eurofins genomics India (Table 1).

PCR amplification with SSR and RAPD markers

The polymerase chain reactions (PCR) were performed using Eppendorf, Mastercycler Gradient, Germany. SSR markers in 10 μl reaction volume containing 7 μl of 1x master mix, 0.5 μM of both forward as well as reverse primers, 1 μl of sterilized water and about 200 ng (1 μl) of template DNA. The reaction conditions for maize genomic SSRs were initial denaturation for 7 min at 94°C and 35 cycles of final denaturation for the 30s
duration at 94°C, 30 s of annealing temperature at 55°C, an extension of 45s at 72°C, with a final extension of 7 min at 72°C, hold at 10°C.

**Table 1. List of the Primers.**

| MAIZE SSR | SORGHUM SSR | RAPD PRIMERS |
|-----------|-------------|--------------|
| umc 1703  | bnlg 2077   | xtxp 316     |
| umc 1594  | umc 1408    | xtxp 043     |
| umc 1690  | umc 1568    | xtxp 248     |
| umc 1028  | umc 2230    | xtxp 075     |
| umc 2204  | umc 1505    | xtxp 006     |
| umc 1552  | umc 1678    | xtxp 145     |
| umc 1970  | phi 295450  | xtxp 331     |
| umc 2101  | umc 1223    | xtxp 024     |
| umc 1166  | umc 1257    | xtxp 297     |
| umc 1474  | umc 1446    | xtxp 027     |
| umc 0381  | umc 1137    | xtxp 021     |
| umc 1178  | bnlg 198    | xtxp 343     |
| phi 193125| phi 213914  | xtxp 042     |
| bnlg 1209 | bnlg 420    | xtxp 018     |
| bnlg 589  | bnlg 1396   | xtxp 017     |
| bnlg 371  | umc 2214    | xtxp 008     |
| bnlg 198  | umc 2324    | umc 2257     |
| bnlg 469  | umc 2071    | phi 087      |
| umc 2204  | umc 1144    | umc 2049     |
| umc 1552  | umc 1060    | umc 2321     |
| umc 1970  | umc 1076    | umc 1127     |
| umc 1142  | umc 2170    | umc 1594     |

The polymerase chain reactions (PCR) were performed using RAPD markers in 10 µl reaction volume containing 7 µl of 1x master mix, 1 µM of primer, 1 µl of sterilized water and about 200 ng (1µl) of template DNA. The reaction conditions for RAPDs were initial denaturation for 7 min at 94°C and 35 cycles of final denaturation at 94°C for the 30s duration. 30 s of annealing temperature at 37°C, an extension of 45s at 72°C, with a final extension of 7 min at 72°C, hold at 10°C.

**PCR product analysis and documentation**

The total volume of the amplified product (10 µl) of each sample was subjected to electrophoresis on 3% agarose gel containing ethidium bromide in 1x TAE buffer at 120 V for 2 h. Finally, the DNA bands were observed on a Gel Doc system and the photographs were captured. The SSR profiles were analysed based on pattern bands. Only clear and unambiguous SSR bands were scored based on base pair (bp) size in each genotype. The amplified product (10 µl) from all the samples were separated through electrophoresis on 1.5% agarose gel having ethidium bromide intact in 1x TAE buffer at 120 V for 1.5 h. Finally, the DNA bands were observed on a Gel Doc system and documented. Scores were done based on the presence or absence of the amplicon.

**Morphological descriptors and DUS characters**

The crop was raised during Rabi 2020 at Centre of Excellence in Millets, Athiyanthal in the Advanced Yield Trail (ART). The following morphological and the quantitative traits were recorded viz., Days to 50% flowering (day), Plant height (cm), Number of basal tillers, Flag leaf length (cm), Flag leaf width (cm), Peduncle length (cm), Panicle length (cm), Panicle exertion, Days to maturity (day), Grain yield per plant (g), Fodder yield per plant (g), Thousand-grain weight (g), Plant pigmentation at flowering, Leaf colour, Blade pubescence, Sheath pubescence, Degree of lodging at maturity, Midrib colour, Inflorescence lobes, Inflorescence bristles, Inflorescence shape, Inflorescence compactness, Fruit colour, Grain shape, Apical sterility in panicle. The mean data is compared between the accessions (Table 2) of the varieties under study are also conducted.

**RESULTS AND DISCUSSION**

Amplification of Foxtail millet DNA was done using 66 Maize SSR primers, 16 sorghum SSR primers, and 10 RAPD primers. All the primers were initially standardized for the unique amplicon using the gradient PCR. Most of the SSR primers showed unique amplicon between 100 to 300 bp sizes. The RAPD markers showed multiple alleles. The polymorphic primer was always used for the differentiation of cultivars.
SSR marker studies

The SSR primers derived from the maize and sorghum database were used for the amplification of DNA from CO (Te) 7 and ATL 1 varieties. The amplified products having polymorphic nature were scored. Out of 66 maize SSR markers used for the study, only one SSR marker umc1704 showed polymorphism. The other 65 maize SSR markers showed monomorphic bands. The marker umc1704 showed polymorphism for the varieties CO (Te) 7 and ATL 1 by the presence of 670 bp and 680 bp alleles respectively (Fig. 1). The marker umc1704 showed polymorphism for the varieties, CO (Te) 7 and ATL 1 at around 670 bp which was used to differentiate these varieties. The similar results are in accordance with Jia et al. (2007), Chandrashekara et al. (2007), Panwar et al. (2010).

Sixteen sorghum SSR markers were used for polymorphism survey using varieties CO (Te) 7 and ATL 1. But none of the markers showed the polymorphism between the varieties.

![Figure 1. DNA fingerprinting of foxtail millet varieties using SSR markers.](image1)

[Note: Polymorphic primer (umc 1704); Forward primer- TTCACCGGGTAGTCCTTCTTACTG; Reverse primer- AAGTACGCTGTACGCAGCAG; Annealing temperature- 55°C; Cycles amplification- 35; Amplicon size- ATL 1 (680 bp); CO (Te) 7 (670 bp)]

![Figure 2. DNA Finger printing of foxtail millet varieties using RAPD markers.](image2)

[1- OPB4 (GGACTGGAGT); 2- OPB1(GTTTCGCTCC); 3- OPA5 (AGGGGTCTTG); 4- OPA 11(CAATCGCCGT); Annealing temperature- 37°C; Cycles amplification- 35]

RAPD marker studies

A panel of RAPD marker survey was followed to identify the polymorphic fragments for the fingerprinting purpose. In this study, the RAPD marker showed clear discrimination between varieties. Ten RAPD primers were used for DNA fingerprinting (Table 1) of foxtail millet varieties. Out of ten primers used, four primers
namely OPB4, OPB1, OPA5 and OPA11 produced the most distinguished and scorable alleles. For the RAPD primers viz., OPB4 and OPB1 the alleles are present at 500–600 bp and 600–700 bp in ATL 1 respectively which were absent in CO (Te) 7. Similarly, for other primers like OPA5 and OPA11, the alleles were observed at 400–500 bp and 300–400 bp for ATL 1 (Fig. 2). It was found that there was no amplicon for CO (Te) 7 variety at the same location. The DNA fingerprint generated by each RAPD primer was scored for the presence of specific alleles. RAPD analysis was first developed to detect polymorphism between organisms, despite the time absence of genomic sequence information in many crops. The RAPD markers also earlier used to develop genetic markers and to construct a genetic map. The different number of bands was observed in PCR cultures also earlier used to develop genetic markers and to construct a genetic map for different primers which might be due to the sequence of primer and availability of complementary sequence in the genome or template quality. Similar results were accorded by Sastry et al. (1995), Gupta et al. (2010), Kumari & Pande (2010).

Morphological traits differentiation between ATL 1 and CO (Te) 7

The morphological traits differentiation between the foxtail millet cultures viz., ATL 1 and CO (Te) 7 was discussed in table 2. The pre-release culture flowered within 50 days after sowings which is earlier than the CO (Te) 7 variety which flowered in 55 days after sowing. The height of the crop was found to be 110–120 cm. (Fig. 3) Grain yield per plant of the pre-release culture was 14.5 g which was more than CO (Te) 7 variety which yielded 12.0 g. Per plant grain yield of ATL 1 accounted 20.8% increase over the check variety CO (Te) 7. When comparing the plant height, CO (Te) 7 will be growing taller to 120 cm than the pre-release culture which grows upto 115 cm only. Apart from the height, while comparing the maturity period, the pre-release culture will attain the maturity in 83 days earlier than the CO (Te) 7 which attains maturity in 88 days. In comparison with CO (Te) 7, the variety ATL 1 (3.6 g) has 3.7% increase in thousand-grain weight over CO (Te) 7.

Table 2. Descriptors of foxtail millet culture ATL 1 and CO (Te) 7.

| S.N. | Character                                      | ATL 1 | Mean | Range       | CO (Te) 7 * | Range       | Mean |
|------|-----------------------------------------------|-------|------|-------------|------------|-------------|------|
| 1    | Days to 50% flowering (day)                   | 48.0–53.0 | 50.0 | 53.0–58.0   | 55.0       |             |      |
| 2    | Plant height (cm)                             | 110.0–120.0 | 115.0 | 115.0–130.0 | 120.0      |             |      |
| 3    | No. of basal tillers                          | 5.0–8.0 | 6.0   | 6.0–9.0     | 7.0        |             |      |
| 4    | Flag leaf length (cm)                         | 32.0–42.5 | 37.8 | 30.5–40.6   | 35.9        |             |      |
| 5    | Flag leaf width (cm)                          | 1.3–2.9 | 2.4   | 0.8–2.4     | 1.6        |             |      |
| 6    | Peduncle length (cm)                          | 15.6–26.2 | 21.3 | 13.5–21.2   | 18.5       |             |      |
| 7    | Panicle length (cm)                           | 28.3–32.8 | 28.5 | 25.6–33.9   | 29.0        |             |      |
| 8    | Panicle exertion                              | Full exertion |        |             |            | Full exertion |      |
| 9    | Days to maturity (day)                        | 80.0–85.0 | 83.0 | 85–90       | 88.0        |             |      |
| 10   | Grain yield per plant (g)                     | 12.2–19.5 | 14.5 | 10.0–16.0   | 12.0        |             |      |
| 11   | Fodder yield per plant (g)                    | 15.9–23.3 | 19.3 | 14.2–20.1   | 15.6        |             |      |
| 12   | Thousand grain weight (g)                     | 3.1–3.9 | 3.6   | 2.8–3.0     | 3.2        |             |      |
| 13   | Plant pigmentation at flowering               | Green  |       |             | Green to purple |       |      |
| 14   | Leaf colour                                   | Green  |       |             | Green to purple |       |      |
| 15   | Blade pubescence                              | Intermediate |     | Intermediate | Glabrous  | Glabrous |      |
| 16   | Sheath pubescence                             | Glabrous |       |             | Intermediate |         |      |
| 17   | Degree of lodging at maturity                 | Non-lodging |     |             | Non-lodging |         |      |
| 18   | Senescence                                    | Green at maturity | | Yellow at maturity | |      |
| 19   | Midrib colour                                 | White  |       |             | Green      |             |      |
| 20   | Inflorescence lobes                           | Medium |       |             | Short primaries |     |      |
| 21   | Inflorescence bristles                        | Short  |       |             | Very short |          |      |
| 22   | Lobe compactness                              | Compact |       |             | Intermediate |       |      |
| 23   | Inflorescence shape                           | Oblong  |       |             | Cylindrical |         |      |
| 24   | Inflorescence compactness                     | Compact |       |             | Compact     |         |      |
| 25   | Fruit colour                                  | Brownish Yellow |     |             | Yellow     |         |      |
| 26   | Grain shape                                   | Elliptical |     |             | Oval       |         |      |
| 27   | Apical sterility in panicle                   | Absent |       |             | Present    |         |      |

7 (3.2 g) (Fig. 4). Fodder yield per plant of pre-release culture is 19.3 g whereas CO (Te) 7 has 3.2 g test weight, which is more than CO (Te) 7 which yields only 15.6 g. During maturity, the pre-release culture will be green in colour and CO (Te) 7 will be in yellow during maturation. This pre-release culture variety can also be used as fodder since it is green in colour even during maturity. The grain shape varies from each other, ATL 1 is elliptical and the check variety is oval. Apical sterility is absent in ATL 1 and present in CO (Te) 7 variety. Fruit colour of ATL 1 is brownish-yellow in colour and CO (Te) 7 variety is yellow. The count of basal tillers was
found to be 5 to 8 per plant. The plant maintains green pigmentation at flowering, and it retains green colour even at senescence stage. The inflorescence lobes were compact and medium, bristles were short and oblong. The apical sterility of the panicle is absent, which was present in CO (Te) 7.

Figure 3. Quantitative characters differentiating the newly released variety (ATL 1) and the existing variety (CO (Te) 7.

Figure 4. Morphological representation: A–C, The newly released variety ATL 1; D–F, The existing variety CO (Te) 7.

Earlier report of Foxtail millet says that the plant can be harvested at 75–90 days after planting (DAP) (Cash et al. 2002). It can produce good quality hay when gathered into windrows and left until fall/winter grazing (Koch 2002). Plant height is about 120 to 200 cm. It has dense inflorescence and panicle is hairy with 10 to 30 cm long which gives the shape of fox’s tail. Its grain diameter is about 2 mm. A grain of foxtail millet is about 2 mm in diameter. Foxtail millet, when grown for hay or silage, takes 65 to 70 days. When grown for the grain, the harvest is after 75 to 90 days (Nirmalakumari et al. 2005).

CONCLUSION

The DNA based fingerprinting was generated for the most common cultivated variety CO (Te) 7 and newly released high yielding variety, ATL 1 using SSR and RAPD markers. Hence marker-based cultivar
identification is demonstrated in foxtail millet which will be useful and highly practical, reliable, and efficient. The marker umc1704 showed polymorphism for the varieties, CO (Te) 7 and ATL 1 at around 670 bp. The RAPD studies also helped for developing markers using the primers OPB4, OPAA5, OPAA11 and OPB1 which can be used for screening new cultivars for fingerprinting purpose.

ACKNOWLEDGEMENTS

The Core projects for research activities at Colleges and Research stations of TNAU-Phase-I (CPMB/CBE/PBT2012/CP004) 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 and the financial aid supported by the TNAU research sub project CPBG/ATL/PBG/SMM/2014/002 - Genetic improvement of small millets for drought resistance and evolving high yielding varieties suitable for the Northeastern zone of Tamil Nadu. (Project period Aug 2014-July 2019) supported by TNAU is also acknowledged.

REFERENCES

Andrew N, Elizabeth A, Katrlien M & Jeffrey L (2009) Foxtail millet: a sequence-driven grass model system. *Plant Physiology* 149: 137–141.

Cash D, Johnson D & Wichman D (2002) Growing millet in Montana. MSU Ext. Serv. Available from: http://www.co.yellowstone.mt.gov/extension/ag/pubs/millet.pdf (accessed 30 Jul. 2014). Chandrashekara AC, Prasanna BM, Bhat SR & Singh BB (2007) Genetic diversity analysis of elite pearl millet inbred lines using RAPD and SSR markers. *Journal of Plant Biochemistry and Biotechnology* 16: 23–28.

Gupta R, Verma K, Joshi, DC, Yadav D & Singh M (2010) Assessment of genetic relatedness among three varieties of finger millet with variable seed coat color using RAPD and ISSR markers. *Journal of Genetic Engineering and Biotechnology* 2: 1–9.

Heuze V, Tran G, Sauvant D, Bastianelli D & Lebas F (2015) *Foxtail millet (Setaria italica), grain*. Feedipedia.

Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, Song Y & Li Y (2009) Development and genetic mapping of SSR markers in foxtail millet (*Setaria italica* (L.) P. Beauv.). *Theoretical and Applied Genetics* 118: 821–829.

Koch DW (2002) *Foxtail millet: Management for supplemental and emergency forage*. Cooperative Extension Service.University of Wyoming. Laramie, Wyoming.

Kumari K & Pande A (2010) Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers. *African Journal of Biotechnology* 9: 4542–4549.

Lu H, Zhang J, Wu N, Liu Kb, Zu D & Li Q (2009) Phytoliths analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). *Plos One* 4: E4448.

Miah RMY, Ismail MR, Puteh AB, Rahim HA, Islam K & Latif MA (2013) A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences* 14: 22499–22528.

Muthamilarasan M & Prasad M (2015) Advances in Setaria genomics for genetic improvement of cereals and bioenergy grasses. *Theoretical and Applied Genetics* 128: 1–14.

Nirmalakumari A, Senthil N, JohnJoel A, Kumaravadivel N, Mohanasundaram K & Raveendran TS (2005) CO(7e) 7 - A high yielding Tenai variety. *Madras Agriculture Journal* 92(7–9): 381–386.

Panwar P, Nath M, Yadav VK & Kumar A (2010) Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L.). *Journal of Genetics* 89: 121–133.

Sastry JG, Ramakrishna W, Sivaramakrishnan S, Thakur RP, Gupta VS & Ranjekar PK (1995) DNA fingerprinting detects genetic variability in the pear millet downy mildew pathogen (*Sclerospora graminicola*). *Theoretical and Applied Genetics* 91: 856–861.

Sharma N & Niranjan K (2018) Foxtail millet: Properties, processing, health benefits, and uses. *Food Reviews International* 34: 329–363.

Weissing K, Nybom H, Pfenninger M, Wolff K & Kahl G (2005) *DNA fingerprinting in plants: principles, methods, and applications*, 2nd edition. CRC press. Boca Raton, Florida.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA & Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18: 6531–6535.