ASSESSMENT OF GENETIC AND MOLECULAR DIVERSITY FOR DIFFERENT TRAITS IN FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv)

By

Miss. Shingane Smita Narendra

(Reg.No. 08/14)

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

CYTOGENETICS AND PLANT BREEDING

DEPARTMENT OF AGRICULTURAL BOTANY
POST GRADUATE INSTITUTE
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2012

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma

Place: M.P.K.V., Rahuri

Date: - / /2012

(S. N. Shingane)

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CERTIFICATE

This is to certify that the thesis entitled, **"ASSESSMENT OF GENETIC AND MOLECULAR DIVERSITY FOR DIFFERENT TRAITS IN FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv)** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **CYTOGENETICS AND PLANT BREEDING**, embodies the results of a *bona fide* research carried out by **Miss. SHINGANE SMITA NARENDRA,** under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

Place : MPKV, Rahuri.
Dated : / /2012

(J.V. PATIL)
Research Guide

Dr. R. S. Patil,
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Place : MPKV, Rahuri
Dated : / /2012 (R. S. Patil)

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Place : MPKV, Rahuri
Date : / /2012 (Ms. Smita Shingane)

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LIST OF ABBREVIATIONS

%	:	Per cent
µM	:	Micro molar
µg	:	Microgram
µl	:	Microlitre
σ^2g	:	Genotypic variance
σ^2ge	:	Variance due to genotype × environment interaction
σ^2p	:	Phenotypic variance
/	:	Per
^0C	:	Degree Celsius
AFLP	:	Amplified Fragment Length Polymorphism
AICRP	:	All India Coordinated Research Project
b.s.	:	Broad sense
C:I	:	Chloroform : Isoamyl alcohol
C.D.	:	Critical difference
cm	:	Centimeter (s)
d.f.	:	Degree of freedom
DNA	:	Deoxyribonucleic acid
dNTPs	:	Deoxy nucleoside triphosphates
E1	:	Environment 1 (Season 2009)
E2	:	Environment 2 (Season 2010)
e.g.	:	Exampligratia
EDTA	:	Ethylenediamine tetraacetic acid
et al.	:	And others
Fig.	:	Figure
GCV	:	Genotypic coefficient of variation
g	:	Gram (s)
g/l	:	Gram per litre
HCL	:	Hydrochloric acid

hr	:	Hour
ISSR	:	Inter simple sequence repeat
i.e.	:	That is
kg	:	Kilogram (s)
KOFM	:	Kolhapur Foxtail Millet
M	:	Molar
$MgCl_2$	:	Magnesium chloride
M	:	Meter
mg	:	Milligram (s)
mg/l	:	Milligram per litre
ml	:	Millilitre (s)
mm	:	Millimeter
MSS	:	Mean sum of squares
No.	:	Number
ng	:	Nanogram
PC	:	Polymerase chain reaction
PCV	:	Phenotypic coefficient of variation
PIC	:	Polymorphic Information Content
pH	:	Potential of hydrogen
ppm	:	Parts per million
P	:	Pooled (across seasons 2009 and 2010)
PVP	:	Polyvinyl pyrrolidone
RAPD	:	Random Amplified Polymorphic DNA
RNase	:	Ribonuclease
rpm	:	Revolution per minute
S.E.	:	Standard error
SDS	:	Sodium dodecyl sulphate
SSR	:	Simple sequence repeats
T: E	:	Tris EDTA
TBE	:	Tris Borate EDTA

U	:	Unit
UPGMA	:	Unweighted Pair Group Method using Arithmetic Mean
UV	:	Ultravoilet
viz.	:	*Videlicet* (Namely)

ABSTRACT

ASSESSMENT OF GENETIC AND MOLECULAR DIVERSITY FOR DIFFERENT TRAITS IN FOXTAIL MILLET (*Setaria italica*. (L.) P. Beauv.)

by

Miss. Shingane Smita Narendra

A candidate for the degree
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Mahatma Phule Krishi Vidyapeeth
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2012

Research Guide	:	Dr. J. V. Patil
Department	:	Agricultural Botany (Cytogenetics and Plant breeding)

The experiment was conducted during the year 2009-10 at Post Graduate Farm and at State Level Biotechnology Centre, M.P.K.V., Rahuri with a view to assess the genetic and molecular diversity in foxtail millet by variability parameters, D^2 statistics, RAPD and ISSR analysis.

Forty-four genotypes were grown in randomized block design with three replications. Observations were recorded on 12 quantitative characters. The variability studied among 44 genotypes indicated the presence of good amount of variation for all the characters studied. Variability observed for grain yield per plant ranged between 6.00 g to 20.32 g. Likewise, other characters such as days to panicle initiation (45.50-71.17days), days to 50 per cent flowering (57.00-79.00days), days to maturity (90.00-123.00days), number of productive tillers per plant (0.35-3.92), plant height (109.00-184.00cm), number of panicles

per plant (1.02-5.70), panicle length (8.27-22.42 cm), 1000 grain weight (1.07-3.48 g), grain yield per plant (6.87-23.87 g), straw yield per plant (13.80-42.35 g), protein content (7.08-13.75%) and iron content (0.03-0.11%) showed wide range of variation.

The estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) had high magnitude for number of productive tillers per plant, followed by number of panicles per plant, grain yield per plant, straw yield per plant and iron content in Environment 1, Environment 2 and on pooled basis.

High heritability coupled with high genetic advance as per cent of mean was observed for all the characters except plant height indicating that the variations were attributable to high level of heritable variation, and selection would be effective for improvement of these traits.

The grain yield per plant was significantly and positively correlated with number of productive tillers per plant, panicle length, number of panicles per plant, 1000-grain weight and straw yield per plant. It could be inferred that selection for high yield would be effective through selection for these traits.

The direct effect of 1000-grain weight on grain yield per plant (g) was positive and high in both the environments separately and on pooled basis, which indicated the true relationship of this trait with grain yield and direct selection through this trait will be effective. The indirect effect of number of panicles, panicle length (cm), number of productive tillers and straw yield through 1000-grain weight was positive and moderate to high. It can be inferred that the direct selection of 1000-grain weight in foxtail millet lead to simultaneous indirect selection of number of panicles, panicle length (cm), number of productive tillers, straw yield and grain yield per plant.

D^2 statistics showed that there was considerable divergence among the genotypes with D^2 values ranging from 1.90 to 610.09 for characters studied. The genotypes under study were grouped into six clusters. Cluster I emerged as the largest cluster with 37 genotypes, followed by cluster-II, V with 2 and all other were solitary. Maximum intra cluster distance was observed for cluster V, followed by cluster I and II. Whereas, maximum inter cluster distance was observed between cluster II and IV and indicating wide divergence between these clusters. The 1000-grain weight followed by straw yield per plant and number of productive tillers per plant were major contributors towards divergence.

Out of the 29 RAPD and 20 ISSR primers used, 19 RAPD primers and 12 ISSR primers amplified. A total of 212 scoreable amplification products (135 RAPD and 77 ISSR) were generated. Average number of alleles generated by RAPD was 7.1 and ISSR was 6.41. Per cent polymorphism shown by RAPD primers varied from 62.50 per cent to 100 per cent and ISSR from 66.66 percent to 100 per cent. The average PIC value for RAPD and ISSR was 0.74 and 0.73, respectively. The genetic similarity matrices based on the Jaccard's coefficient ranged from 0.374 to 0.964 and 0.35 to 0.98 for RAPD and ISSR, respectively. The UPGMA based dendrogram revealed three major groups, with 22 genotypes in one cluster and 20 genotypes in second cluster and two genotypes in third cluster. Grouping of genotypes under study based on morphological diversity and molecular diversity was concurrent for some clusters. The genotypes KOFM 1, KOFM 14, KOFM 36, KOFM 89, KOFM 90, KOFM 94 and KOFM 95 were found superior to create more variability in foxtail millet.

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1. INTRODUCTION

Foxtail millet (*Setaria italica* (L.) P. Beauv) is also known as Italian millet, *Kangu, Kangani, Kalakangani, Koni, Rala and Kaon* in different parts of India. It is one of the oldest crops cultivated for food grain, hay and pasture. The most recent archaeological evidence demonstrated that the foxtail millet is the most ancient crop as its domestication in China dates back to 8,700 years ago (Lu *et al.*, 2009). According to Vavilov (1926), the principal centre of diversity for foxtail millet is East Asia, including China and Japan. It is an important grain crop in temperate, subtropical, tropical Asia and in parts of southern Europe. China, India and Japan are the major foxtail millet growing countries in the world. In India, the cultivation of foxtail millet is confined to Andhra Pradesh, Karnataka and Tamil Nadu and some parts of Maharashtra.

Foxtail millet is well recognized as a short duration, rainy season crop. It belongs to genus *Setaria*, tribe *paniceae* and family *Poaceae* or *Graminae* in the grass family. There are about 125 species widely distributed in warm and temperate parts of the world. Foxtail millet (*Setaria italica* (L.) P. Beauv.) is an autogamous, diploid (2n = 18), C4 panicoid crop species with a relatively small genome size of ~515 Mb (Li and Brutnell, 2011) with haploid Chromosome number (n = 9). It is essentially grain crop of 90-100 days duration. Taxonomically, foxtail millet consists of two subspecies, *S. italica* subsp. *italica* and subsp. *viridis*. The geographical origin of foxtail millet based on cytological studies indicated that wild ancestor of foxtail millet is *S. viridis* (Kihara and Kishimoto, 1942; Li *et al.*, 1945). Based on the comparative morphology of the foxtail millet accessions, foxtail millet is

classified into a European complex (Race *Moharia*) and a far Eastern complex (Race *maxima*) (Prasada Rao *et al.*, 1986). Race *Moharia* includes cultivars with relatively small inflorescences, while race *maxima* include pendulous inflorescences. Cultivars from India are morphologically different from those of Europe and the Far East and are recognized as race *indica* (Prasada Rao *et al.*, 1986).

The plant is an erect leafy stem that grows 60-75 cm tall and bends quite a bit at maturity due to heavy weight of ear head. The leaves are flat, linear or lanceolate tapering to a setaceous point having 30-40 cm long and 1.25 cm wide green in colour. Panicles are erect, dense, cylindrical and bristly having 2-4 spikelets in each involucre. The spike is 5-32 cm long and 2-4 cm in diameter. Spikelets are two flowered, protected by two glumes and are generally in clusters of 40-50. There are 1-4 bristles at the base of each spike. Morphology and anthesis behaviour make foxtail millet one of the most difficult species to cross pollinate. Foxtail millet and the weedy green foxtail are morphologically and genetically allied. Foxtail millet also crosses naturally (de Wet *et al.*, 1979) and experimentally with green foxtail (Li *et al.*,1945) to produce fertile hybrids (Prasada Rao *et al.*, 1986) and both have same number of chromosomes (2n=18). Foxtail millet is largely a self pollinated crop with cross pollination averaging about 4 per cent (Li *et al.*, 1935).

With the rapid development of maize and other crops, foxtail millet has gradually become a minor crop in the last 80 years but it is still widely cultivated in Asia, Europe, North America, Australia and North Africa as grain food or forage (Austin, 2006). It is not correct to consider foxtail millet as a low yielding crop, the actual

problem being that growing conditions in many areas are poor and grown as rainfed beside lack of improved cultivars. The yield level of 1,500-2,250 Kg ha-1 has been reported from China (Jiaju, 1986). At present, in India the crop is cultivated on a very limited area of around 5 lakh hectares in sporadic patches in the states of Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Rajasthan, Madhya Pradesh, Uttar Pradesh and North Eastern states with annual production of 2.9 lakh tonnes and productivity of 600 Kg ha^{-1}. The foxtail millet grain is (per 100g) rich in protein (11.2g) and iron (2.8mg) as compared to rice (7.9 g protein and 1.8 mg Fe) and rich in fat 4.0g per 100g which is superior to rice and wheat (http://www.fao.org/docrep/t0818e/T0818E0a.htm).The grain is good source of β-carotene, which is the precursor of Vitamin A (Murugan and Nirmalakumari, 2006). Foxtail millet is mixed with legumes to make porridge and also mixed with soybean to make mixed flour. Foxtail millet has low glycemic index (GI), used for preparation of low GI biscuits and burfi, a sweet product, and it is an ideal food for people suffering from diabetes (Thathola *et al.*, 2010; Anju and Sarita, 2010). Foxtail millet is also fermented to make vinegar, yellow wine, maltose, beer and other related products. It is also used for feeding cage birds and by-product of the foxtail millet is used as animal feed.

In view of the several merits, this crop deserves increased attention in research. Foxtail millet has received little research attention in the past years and continued to be a neglected and underutilized crop (Upadhyaya *et al.*, 2008). This is due to the poor seedling establishment, need for hand weeding and lack of breeding efforts for improvement are major reasons for its reduced use

(Ahanchede *et al.*, 2004). The potentiality of foxtail millet is not yet exploited properly in India (Channappagaudar *et al.*, 2008).

Hence, the greater use of diverse germplasm in breeding and improved crop management is suggested to improve the productivity of this crop. Progress in any crop improvement programme depends mainly on the variability existing in the base population. Genetic variability studies provide basic information regarding the genetic properties of the population based on which breeding methods are formulated for further improvement of the crop. Heritability gives the information on magnitude of inheritance of quantitative traits. Genetic advance will be helpful in formulating suitable selection procedures. Correlation studies provide an opportunity to study the magnitude and direction of association of yield with its components and also among various components. Correlation in conjunction with path analysis would help in identifying suitable selection criteria for improving the yield.

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. Analysis of genetic relationships in crop species is an important component of crop improvement program, since it provides information about genetic diversity of the crop species which is a basic tool for crop improvement. Various types of data have been used to analyse the phenotypic and genetic diversity including taxonomical, morphological, agronomical and molecular data. Since each method provides different types of information, the choice of method depends on the need of the researchers.

Genetic diversity is the variability available among the different genotypes or species. A method suggested by Mahalanobis

(1936) known as 'Mahalanobis D^2 statistics', is widely used to know genetic diversity in the available germplasm. This method measures the forces of differentiation at intra-cluster and inter cluster levels and thus helps in selection of genetically divergent parents for their exploitation in hybridization programme. The D^2 statistics also measures the degree of diversification and determines the relative proportion of each component character to the total divergence.

Morphological characterization does not reliably portray the genetic relationships among the genotypes because of environmental interactions, unknown genetic control of the traits and inadequate sampling of the genome in terms of phenotype. Thus, for genetic diversity assessment, molecular markers offer considerable advantages over the morphological markers.

DNA-based markers are now extensively used to characterize and evaluate genetic diversity germplasm in large number of crop species including foxtail millet (Cooke, 1995). An array of DNA markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been deployed to study genetic diversity in various crops. RAPD is quite efficient in bringing out genetic diversity at DNA level (Jaya Prakash *et al.*, 2006). Also the Inter Simple Sequence Repeats (ISSR)–PCR is a technique which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. ISSR markers are highly polymorphic and are used in studies of genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Reddy *et al.*, 2002).

Good amount of variability has been reported in foxtail millet for the various characters such as height, flowering, maturity, tillering, branching, panicle characters, seed colour and irrigation response but it has not been fully exploited in breeding programmes. Limited molecular diversity analyses have been reported in foxtail millet. In a genetic diversity study using RFLPs, Fukunaga *et al.* (2002) found that foxtail millet landraces have differentiated genetically between different regions and that Chinese landraces were highly variable. This is in contrast to the results obtained by de Wet *et al.* (1979) and Jusuf and Pernes (1985), who reported that Chinese cultivars were uniform for storage protein and enzyme alleles. A few of the earlier workers had used RAPDs for the analysis of genetic diversity in foxtail millet (Li *et al.* 1998; Schontz and Rether 1999). Since co-dominant marker systems such as SSRs were not available in foxtail millet, the available random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers techniques can serve as markers of choice. It is therefore, felt necessary to study the genetic variability at field condition and at molecular level by using RAPD and ISSR markers in different genotypes of foxtail millet and suggest a sound breeding strategy based on the studies (Godwin *et al.*, 1997).

Hence, the present study was undertaken with following objectives:

1. To study the genetic variability and divergence in the genotypes for different traits in foxtail millet.
2. To study the molecular diversity present in the genotypes by using RAPD and ISSR markers.

2. REVIEW OF LITERATURE

The review of literature on genetic variability, correlation, and path analysis, morphological and molecular diversity has been collected, out of which the most relevant literature in context of topic is as follows.

2.1 Genetic Variability

Range is a crude measure of variability and it provides a spread of variability for a particular character. Presence of wide range of variation in foxtail millet was reported in various studies (Reddy *et al.* 2006; Upadhyaya *et al.* 2008; Nirmalakumari and Vetriventhan, 2010) for various morphological and agronomical traits.

The relative values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) give an idea about the magnitude of variability present in the population. Heritability is a quantitative measure which provides information about the correspondence between genotypic variance and phenotypic variance, i.e., the ratio of variance due to hereditary differences (σ^2g) to the total phenotypic variance (σ^2p) an expressed as percent. Since heritability is also influenced by environment, the information on heritability alone may not help in pin pointing characters enforcing selection. The heritability estimates along with the predicted genetic gain will be more reliable for formulating suitable breeding methods (Johnson *et al.*, 1955). Heritability gives the information on the magnitude of inheritance of quantitative traits, while genetic advance will be helpful in formulating suitable selection procedures. The earlier studies on phenotypic and

genotypic coefficients of variation, heritability and genetic advance as per cent in foxtail millet are as follows.

Charles and Smith (1939) partitioned genetic variance from total variance by use of estimates of environmental variance from non segregating population. This work made possible to use genotypic coefficient of variation (GCV) as a relative magnitude of genetic diversity present in the material and helps to compare the genetic variability present for different traits. The statistical method to calculate the genetic component of variance was given by Panse and Sukhatme (1985).

Harinarayan and Seetharam (1981) noted that the variability available in foxtail millet for panicle shape, size, arrangement of spikelets, tillering, seed size and colour was very high, offering great scope for exploitation.

Seetharam *et al.* (1983) noted that the variability available for protein content in foxtail millet ranged from 7.16 to 15.73 per cent and identified the sources with high protein for both direct exploitation and use in breeding.

Islam *et al.* (1989) studied five hundred germplasm accessions of foxtail millet for variability and correlation among the yield contributing characters. High coefficient of variation was observed for number of tillers per plant (52.16%), grain yield per plant (32.00%) and panicle length (17.93%). whereas low coefficients of variation were obtained for days to maturity (4.20%) and plant height (11.97%).

Twenty eight genotypes of foxtail millet were studied for dry matter production, harvest index and grain yield with other seven characters. High PCV and GCV were obtained for root weight. Low

heritability and low genetic advance observed for all other characters indicated that these characters may be partially governed by additive genes (Chidambaram and Palanisamy, 1995).

Rathod *et al.* (1995) studied the extent of genetic variability for morphological traits and yield in foxtail millet using 12 genotypes. Maximum and minimum phenotypic and genotypic variance observed for plant height and 1000 grain weight, respectively. The GCV and PCV were highest for grain yield/plant. Total tillers, productive tillers and panicle length recorded the high estimates of heritability associated with high genetic advances.

Kumar and Parmeswaran (1998) studied the characterization of storage protein from selected varieties of foxtail millet. Protein content of grain of foxtail millet was 91.7, 105.2 and 112.0 g/kg in Cv. CO-6, TNAU-172 and TNAU-173, respectively.

Gowda *et al.* (2000) studied the possibilities of combining high protein content with high yield in finger millet. In which they studied mean protein content, grain yield and yield components of parents and progenies of crosses. The parents differed distinctly for all characters such as protein content (%), grain yield/plant (g), productive tillers, fingers/ear, ear length and plant height (cm). The parent (WR-13) was a high protein genotype than the other two parents (GE-1409, GE-1546). But it was poor yielder although excelled in number of productive tillers compared to other two parents. The mean protein content, yield and yield components both in F_2 and F_3 generations were in between the parents. The maximum and minimum values in the segregating populations exceeded parental limits on either side. This offered scope for selection.

Selvarani and Gomathinayagam (2000b) studied the genetic variability in 50 genotypes of foxtail millet. The high level of GCV and PCV were estimated for number of productive tillers and grain yield per plant. Low GCV and PCV levels were obtained for days to 50 per cent flowering, plant height and days to maturity. The differences between GCV and PCV for these traits were low. High heritability estimates were observed in all traits, indicating that the characteristics were stable. The genetic advance was high for the number of productive tillers, grain yield/plant and plant height. High heritability and low genetic advance were recorded for days to 50 per cent flowering and days to maturity.

Lakshmana and Guggeri (2001) studied the genetic variability in foxtail millet for seven quantitative traits (days to 50 per cent flowering, plant height, days to maturity, number of productive tillers/plant, earhead length, grain yield and fodder yield) in 34 genotypes. High PCV and GCV were observed for grain yield, fodder yield, earhead length and productive tillers/plant. High heritability coupled with moderate genetic advance was observed for grain yield, earhead length and plant height, indicating the occurrence of additive gene effects for these characters. Earliness showed low estimates of genetic advance coupled with higher heritability, indicating the presence of non additive gene interaction.

Basheeruddin and Sahib (2004) evaluated 15 genotypes of foxtail millet for yield and its components (days to 50 per cent flowering, plant height, productive tillers/plant and days to maturity). Plant height had the highest mean (132.6), genetic variability (233.74) and phenotypic variability (277.68). Heritability

was highest for days to 50 per cent flowering (96.19%) while grain yield had the highest genotypic (31.13) and phenotypic (49.02) coefficient of variability and genetic advance as per cent of mean (40.60).

Tyagi and Rawat (2004) developed on induced mutant cultivar of foxtail millet i.e. PS4. It was high in protein content (13.15%) than its parents SIA 2616 (12.25%) and the control SIA 326 (12.22%).

Kalinova and Moudry (2006) studied the content and quality of protein in prosomillet varieties. In which they evaluated eight varieties of prosomillet for protein content by Kjehladl method. They found that protein in prosomillet was 11.6 per cent of dry matter and was similar to wheat.

Zheng-li *et al.* (2006) developed the new foxtail millet germplasm with super early maturity and high iron content (Super early maturation No. 2). The iron content of the millet of Super early maturation No. 2 was 54.10 mg/kg which was 62 per cent higher than the average iron content of foxtail millet varieties of China.

Channappagaudar *et al.* (2008) studied the physiological basis of yield variation in 20 genotypes of foxtail millet. In which they found that plant height, photosynthetic rate and number of tillers had positive correlation with total dry matter and grain yield.

Nirmalakumari and Vetriventham (2010) evaluated 741 germplasm accessions of foxtail millet to determine the genetic variability of yield and its components. Data were recorded on various morphological traits such as days to 50 per cent flowering, plant height (cm), total number of tillers, number of productive

tillers, panicle length (cm), days to maturity and grain yield per plant. They observed considerable diversity among all accessions studied for all the seven characters. Highest heritability, GCV and genetic advance as per cent of mean was recorded in grain yield per plant and lowest were recorded in days to 50 per cent flowering.

The earlier studies on phenotypic and genotypic coefficients of variation, heritability and genetic advance as per cent of mean in foxtail millet are summarized in Table 2.1.

Table 2.1. The earlier studies on phenotypic and genotypic coefficients of variation, heritability and GA in foxtail millet

Trait	PCV	GCV	Heritability	GA (% of mean)	Authors
Days to panicle initiations	-	High	High	High	Cill and Randhawa (1975)
Days to 50 per cent flowering	Moderate	Moderate	High	Moderate	Nirmalakumari and Vetriventhan (2010); Basheeruddin and Sahib (2004)
	Low	Low	High	High	Nirmalakumari *et al.* (2008)
	Low	Low	Medium	Low	Lakshmanan and Guggeri (2001)
	Low	Low	High	Low	Selvarani and Gomathinayagam (2000b)
	-	-	High	moderate	Chidambaram and Palanisamy (1995)
	-	-	High	Low	Islam *et al.* (1990)
	Low	Low	High	Moderate	Reddy and Jhansilakshmi (1991a)
Days to maturity	-	High	High	Low	Cill and Randhawa (1975), Dasthagiraiah and Reddy (1995)
	Low	Low	-	-	Nirmalakumari and Vetriventhan (2010)
	-	-	High	Moderate	Chidambaram and Palanisamy (1995)
Plant Height (cm)	Moderate	Moderate	High	High	Nirmalakumari and Vetriventhan (2010); Basheeruddin and Sahib (2004); Selvarani and Gomathinayagam

					(2000b); Reddy and Jhansilakshmi (1991a)
	Low	Low	High	Moderate	Lakshmanan and Guggeri (2001)
	-	-	High	Moderate	Chidambaram and Palanisamy (1995)
	-	-	High	High	Islam *et al.* (1990)
	High	High	High	Moderate	Cill and Randhawa (1975)
	-	Low	Low	-	Dasthagiraiah and Reddy (1995)
Number of productive tillers /plant	High	High	-	-	Nirmalakumari and Vetriventhan (2010)
	-	-	High	Moderate	Chidambaram and Palanisamy (1995)
	-	-	High	High	Islam *et al.* (1990); Rathod *et al.* (1995)
	-	-	Low	-	Dasthagiraiah and Reddy (1995)
Panicle length (cm)	Moderate	Moderate	Medium	High	Reddy and Jhansilakshmi (1991a)
	Moderate	Moderate	High	High	Nirmalakumari and Vetriventhan (2010); Nirmalakumari *et al.* (2008); Lakshmanan and Guggeri (2001)
	-	-	High		Rathod *et al.* (1995)
	Moderate	Low	Medium	Low	Cill and Randhawa (1975)
	-	-	High	High	Islam *et al.* (1990)
1000-grain weight (g)	-	Low	High	High	Cill and Randhawa (1975)
Grain yield/plant (g)	Moderate	High	Medium	High	Reddy and Jhansilakshmi (1991a)
	High	High	High	High	Nirmalakumari and Vetriventhan (2010); Selvarani and Gomathinayagam (2000b)
	Moderate	Moderate	High	High	Nirmalakumari *et al.* (2008); Lakshmanan and Guggeri (2001)
	-	-	Low	Llow	Chidambaram and Palanisamy (1995), Dasthagiraiah and Reddy (1995)
	-	-	High	High	Islam *et al.* (1990);
	High	High	-	-	Rathod *et al.* (1995)
	High	High	Medium	-	Basheeruddin and Sahib (2004); Cill and Randhawa (1975)
Straw yield /plant(g)	-	-	Moderate	Moderate	Chidambaram and Palanisamy (1995)

2.2 Correlation

Estimation of correlation coefficient provides a measure of association between characters. The correlation coefficient provides the basic information to the breeders to identify characters that have little or no importance in the selection programme.

Correlation studies are helpful in determining the components of complex traits like yield. But they do not provide an exact picture of the relative importance of direct and indirect influences of each of the component characters towards the yield.

Islam *et al.* (1989) studied five hundred accessions of foxtail millet for correlation among the yield contributing characters. Grain yield per plant showed significant positive correlation with plant height, panicle length and days to maturity. Days to maturity however showed positive association with plant height, panicle length and number of tillers per plant.

Chidambaram and Palanisamy (1995) reported the positive association of grain yield with total dry matter, earhead weight and straw weight but not with Harvest index (HI) suggested that HI alone will have no value for the improvement of grain yield. Though the grain yield is positively associated with dry matter and earhead weight, selection for improvement of grain yield either through earhead weight or by direct selection for grain yield will not be of much use since the two traits had low heritability and genetic advance.

Radhod *et al.* (1996) Studied character correlation and selection indices in foxtail millet. They evaluated eleven diverse indigenous genotypes and a local control for variability and associations among 12 yield components. Total tillers, productive

tillers/plant, harvest index and biological yield were the most important yield components including yield having significant positive effects at the genotypic and phenotypic levels.

Santhakumar (1999) studied correlation between 200 genotypes of foxtail millet for seven yield components. Grain yield was positively and significantly correlated with plant height, panicle length and fodder yield.

Murugan and Nirmalakumari (2006) evaluated 75 foxtail millet genotypes to study the correlations among the characters. They observed that correlation between straw yield per plant and HI were the major determining characters for grain yield. Higher values of straw yield attributed that the selection for dual purpose cultivar would be more effective for genetic improvement of foxtail millet.

Channappagaudar *et al.* (2008) reported the trait correlations in foxtail millet and noticed that plant height, photosynthetic rate and number of tillers had positive correlation with total dry matter and grain yield.

Kadam *et al.* (2009) studied correlation in finger millet and reported highly significant association with yield and almost all the growth and yield contributing characters except flag leaf blade width and exertion.

Nirmalakumari and Vetriventhan (2010) evaluated 741 germplasm accessions of foxtail millet to study the correlation of yield and its components. All the seven characters viz., days to 50 per cent flowering, plant height (cm), total number of tillers, number of productive tillers, panicle length and days to maturity exhibited highly significant positive correlation with grain yield.

Salini *et al.* (2010) evaluated 364 germplasm accessions of proso millet to study the correlation of yield and its components. Days to 50 per cent flowering, plant height (cm), total number of tillers/plant, number of productive tillers/plant, panicle length (cm), and 100 grain weight (g) exhibited highly significant positive correlations with grain yield.

Summary of earlier studies about correlation in foxtail millet are presented in Table 2.2.

Table 2.2. Earlier studies about correlation on different yield and yield contributing traits in foxtail millet

Traits	Positive correlation with	Negative correlation with	References
Days to panicle initiation	Maturity, Plant height, panicle length, 1000-grain weight	-	Cill and Randhawa (1975)
Days to 50 per cent flowering	Days to maturity, plant height, panicle length and grain yield	-	Nirmalakumari and Vetriventhan (2010)
	Plant height and panicle length	Basal tillers	Upadhyaya *et al.* (2008)
	Plant height and straw yield	-	Basheeruddin and Sahib (2004)
	Days to maturity, plant height and straw yield	-	Santhakumar (1999)
	Days to maturity	-	Chidambaram and Palanisamy (1995)
	Days to maturity and plant height	Grain yield/plant	Reddy and Jhansi Lakshmi (1991b)

	Plant height, days to maturity and tiller number	-	Islam *et al.* (1990)
	Days to maturity, plant height, panicle length and 1000-grain weight	Tiller number and grain yield	Cill and Randhawa (1975)
Days to maturity		Grain yield	Cill and Randhawa (1975)
Plant height	Days to flowering, days to maturity, panicle length and grain yield	-	Nirmalakumari and Vetriventhan (2010)
	Days to flowering and panicle length	-	
	Grain yield	-	Channappagoudar *et al.* (2008); Murugan and Nirmalakumari (2006); Ling *et al.* (2008)
	Days to 50 per cent flowering, days to maturity and straw yield	-	Basheeruddin and Sahib (2004)
	Panicle length, straw yield and grain yield	-	Santhakumar (1999)
	Total dry matter production and straw yield	-	Chidambaram and Palanisamy (1995)
	Days to flowering, days to maturity and panicle length	Basal tillers	Reddy and Jhansi Lakshmi (1991b)
	-	Productive tillers, days to maturity and panicle length	Reddy and Jhansi Lakshmi (1991a)

	Days to flowering and panicle length	1000-grain weight	Islam *et al.* (1990)
	Panicle length, days to maturity, yield/plant	-	Islam *et al.* (1989) Cill and Randhawa (1975)
	1000-grain weight and days to flowering	Grain yield, tiller number	Cill and Randhawa (1975)
Productive tillers per plant	Grain yield	-	Nirmalakumari and Vetriventhan (2010); Channappagoudar *et al.* (2008)
	-	Days to flowering, plant height and panicle length	Upadhyaya *et al.* (2008)
	Days to flowering, days to maturity, 1000-grain weight and grain yield	-	Islam *et al.* (1990)
	Grain yield per plant	Days to flowering and panicle length	Reddy and Jhansi Lakshmi (1991b)
	Days to maturity	-	Islam *et al.* (1989)
	Grain yield	Panicle length	Navale and Harinarayana (1987)
	Grain yield	Plant height, days to flowering, Panicle length, 1000-grain weight	Cill and Randhawa (1975)

Panicle length	Days to flowering, days to maturity, plant height and grain yield	Productive tillers	Nirmalakumari and Vetriventhan (2010)
	Days to flowering, plant height, inflorescence width and weight of five panicles	-	Upadhyaya *et al.* (2008)
	Grain yield	-	Murugan and Nirmalakumari (2006); Ling *et al.* (2008)
	Grain yield, fodder yield and plant height	-	Santhakumar (1999)
	Plant height	-	Reddy and Jhansi Lakshmi (1991b)
	-	Plant height and productive tillers	Reddy and Jhansi Lakshmi (1991a)
	Plant height, days to maturity, 1000-grain weight and grain yield	-	Islam *et al.* (1990)
	Plant height, days to maturity and yield/plant	-	Islam *et al.* (1989)
	1000-grain weight, days to flowering and plant height	-	Cill and Randhawa (1975)
Straw yield	Grain yield	-	Chidambaram and Palanisamy (1995)

2.3 Path analysis

Path coefficient analysis suggested by Dewey and Lu (1959) proves helpful in partitioning the correlation coefficients into the measure of direct and indirect variables on the dependent variable

if the correlation is due to direct effect it reflects true relationship and for improving the yield such characters can be selected.

Hawlader and Hamid (1988) studied path coefficient analysis in thirty local and exotic genotypes of foxtail millet to delineate the nature and extent of direct and indirect effects of yield components on grain yield. Of the six yield components studied, 1000 grain weight had the highest direct contribution (0.639) to grain yield, followed by days to flower (0.605), productive tillers (0.313) and ear length (0.290). Plant height had a negligible direct effect (-0.004). The indirect effects of days to maturity through days to flower and 1000-grain weight were highly positive. Residual path made a low contribution in the determination of grain yield. The results clearly indicated that 1000-grain weight and days to flower were the two major component characters that directly contributed to grain yield and most important in breeding for yield improvement in foxtail millet

Lal *et al.* (1996) studied 40 genotypes of Ragi for determining the path coefficient analysis on the basis of grain yield/plant, harvest index, biological yield/plant, 1000-grain weight, finger length, fingers/ear, tillers/plant and leaf area. Path analysis revealed positive and direct effect of biological yield, harvest index and maturity duration on grain yield.

Mishra (1996) observed highest direct effect of plant height along with its significant positive association towards yield.

Rao and Agrawal (2000) evaluated 28 diverse genotypes of Barnyard millet for path coefficient analysis among yield and its components. Path analysis indicated that highest direct effects of ear length followed by fodder yield and days to flower on grain yield,

whereas tillers/plant had highest effects on fodder yield followed by days to maturity and plant height. Considering the path analysis, ear length and fodder yield had a great influence on grain yield.

Murugan and Nirmalakumari (2006) evaluated 75 genotypes of foxtail millet and found that straw yield/plant and harvest index were major determining characters for grain yield among foxtail millet genotypes. Higher values of straw yield attributed that selection for dual purpose cultivar would be more effective for genetic improvement of foxtail milet cultivars. High residual effect on the contrary suggested that further investigation would be required involving more number of characters to explain the variability of grain yield efficiently.

Kadam *et al.* (2009) studied finger millet for Plant height, days to 50 per cent flowering, flag leaf blade length, inflorescence length, yield, inflorescence width, flag leaf sheath length and 1000-grain weight. Path analysis showed that indirect effect of yield had masked the direct or indirect effects in almost all the characters except inflorescence width.

Satish *et al.* (2009) studied path coefficient analysis of grain yield components in finger millet. The characters like ear weight per plant and straw yield/plant had high positive effect on grain yield. Two character viz., plant height and days to maturity had negative direct effect on grain yield. Selection based on ear weight/plant and straw yield/plant will be effective for grain yield improvement.

Nirmalakumari and Vetriventhan (2010) evaluated 741 germplasm accessions of foxtail millet. The path analysis revealed that direct effect of days to 50 per cent flowering on grain yield was positive and negligible. Direct effect of number of productive tillers

on grain yield was positive and high, Panicle length showed moderate positive direct effect on grain yield and negligible indirect effects through other trait studied.

Nirmalakumari *et al.* (2010) evaluated 109 little millet germplasm accessions and observed that high positive direct effect and indirect effects of other characters through days to 50 per cent flowering indicating importance these characters in selection.

Salini *et al.* (2010) evaluated 364 prosomillet germplasm accessions for yield and its components. Positive effect of plant height, number of productive tillers and 100-grain weight indicated that direct selection for these characters would improve the grain yield in prosomillet.

Studies on the extent of direct and indirect influence of different yield attributing characters on grain yield reported by earlier workers are presented in Table 2.3.

Table 2.3. Earlier studies related to path coefficient analysis in foxtail millet

Authors	Contribution
Dezfouli and Mehrani (2010)	The number of tillers, stem diameter and days to 50 per cent flowering were positive and directly affected seed yield, while spike length effected seed yield negatively (-0.323). The number of seeds per spike, number of leaves and number of tillers as well as days to 50 per cent flowering had positive direct effect on fodder yield.
Nirmalakumari and Vetriventhan (2010)	The direct effect of days to 50 per cent flowering on grain yield was positive and negligible and

	number of productive tillers on grain yield was positive and high. Panicle length showed moderate positive direct effect on grain yield. Greater yield advantage could be achieved by using germplasm with more productive tillers, medium panicle length and medium duration.
Murugan and Nirmalakumari (2006)	Correlation and path coefficient analysis revealed that straw yield per plant and harvest index were the major determining characters for grain yield among foxtail millet genotypes.
Maloo and Philip (2001)	The maximum direct effects of biological yield and harvest index on seed yield and other characters like weight of panicles, seed yield per panicle, flag leaf area, 1000 seed weight, seed protein content, seed oil content and days to flowering showed positive direct contribution in atleast one crop season. Weight of panicle and seed yield per panicle showed positive and high direct effect in only one environment. Plant height, panicle length, flag leaf area and 1000-seed weight had low or negative correlation.
Santhakumar (1999)	Direct effect of plant height and panicle length was low, and fodder yield recorded moderate direct effect on grain yield.
Rathod *et al.* (1996)	Total tillers, productive tillers per plant, harvest index and biological yield were the most important yield components influencing yield, having significant positive effects at the genotypic and phenotypic levels.
Reddy and Jhansilakshmi	Direct effect of plant height, inflorescence

(1991b)	length and bristle length were high positive direct effect on grain yield whereas harvest index and biological yield had very high positive direct effect.
Reddy and Jhansilakshmi (1991a)	Path coefficient analysis indicated higher direct contribution (effects) of harvest index and biological yield towards grain yield. Plant height, inflorescence length was found to have high direct effect whereas weight of main ear showed high negative effect towards grain yield. Harvest index and biological yield could be relied on in improving grain yield potential in foxtail millet.

2.4 Genetic diversity by D^2

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. Analysis of genetic relationships in crop species is an important component of crop improvement program, since it provides information about genetic diversity of the crop species which is a basic tool for crop improvement. Accurate assessment of the levels and patterns of genetic diversity is invaluable in crop breeding which facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purpose (Mohammadi and Prasanna, 2003). Various types of data have been used to analyze the genetic diversity in crops, including pedigree, morphological, biochemicals obtained by analysis of isoenzymes, seed proteins and molecular

marker data. Since each method provides different types of information, the choice of method depends on the need of the researchers.

A method suggested by Mahalanobis (1936) known as "Mahalanobis D^2 statistics" is widely used to know genetic diversity in the available germplasm. This technique measures the forces of differentiation at intra-cluster and inter-cluster levels and thus helps in the selection of genetically divergent parents for their exploitation in hybridization programme. The D^2 statistics also measures the degree of diversification and determines the relative proportion of each component character to the total divergence.

Nagrajan and Prasad (1980) studied the genetic diversity in 50 genotypes of foxtail millet. A wide genetic diversity was revealed by the D^2 analysis where in the 50 genotypes partitioned into as many 15 clusters. The geographic distribution was not related to genetic diversity. Genotypes chosen from same eco-geographic region were found scattered in different clusters. Based on average inter-cluster distances, three clusters were found to be highly divergent from the others.

Sheriff (1992) studied the divergence among 20 varieties of finger millet grown across two environments using Mahalanobis D^2 statistics. Varieties were grouped into four clusters under rainfed and 11 under irrigated condition. Days to flowering, days to maturity, plant height, ear length, ear weight and grain weight contributed maximum to genetic diversity in both the environment.

Shriff and Shivashankar (1992) studied genetic divergence in foxtail millet by analysing 225 genotypes by multivariate analysis using Mahalanobis D^2 statistics and canonical analysis. Both

analyses suggested the existence of considerable divergence among the material. D^2 statistic resulted in 33 clusters. Genetic divergence has not been found to be related with geographical diversity.

Li *et al.* (1996) analyzed phenotypic diversity of 2381 foxtail millet landraces of Chinese origin for seven qualitative traits and four quantitative traits. Hierachical analysis of variance indicated that most of the variation was due to differences among characteristics. Only the diversity indices for leaf colour of seedlings, starch composition and 1000-grain weight showed significant differences among regions.

Maloo and Bhattachargee (1999) studied the genetic divergence in forty genetically and geographically diverse varieties of foxtail millet. The D^2 values ranged from 4.30 to 6058.21 and genotypes were grouped into four clusters cluster-I (28 varieties), II (9 varieties), III (2 varieties) and IV (1 variety). Characters contributing largely to the divergence were test weight, seed protein content, harvest index and seed yield/plant.

Selvarani and Gomathinayagam (2000a) evaluated 50 genotypes of foxtail millet for their genetic divergence by D^2 analysis for a set of divergence characters including seed yield and four other metric traits. The genotypes were grouped into six clusters. Cluster-V, VI and VII were identified as genetically more divergent based on inter cluster values.

Murugan and Nirmalakumari (2006) studied genetic divergence in foxtail millet comprising eleven characters viz., days to 50 per cent flowering, plant height (cm), number of productive tillers, ear length (cm), ear weight (g), days to maturity, grain yield/plant (g), 1000 grain weight (g), straw yield/plant (g), harvest

index (%) and β-carotene content (mg/100 g). The 75 genotypes evaluated were grouped into nine clusters. Cluster-I had a maximum of 61 genotypes followed by cluster-III with five genotypes. While cluster-IV exhibited three genotypes and remaining cluster possessed one genotype each. The results revealed that genotypes from wide range of eco-geographical areas assembled together to contribute a major cluster.

Reddy *et al.* (2006) collected and characterized the world's foxtail millet germplasm for morphological traits. In which they found that diversity for days to 50 per cent flowering was greater in germplasm accessions originating from Sri Lanka (55-135 days) while it was narrowest in Russian germplasm (30-50 days). Plant height varied from 20 cm to 215 cm, with accessions from China tending to be dwarf (20 cm) and those from India were taller (215 cm). Based on plant colour, accessions were classified into three classes: green (74.6%), pigmented (23.6%) and deep purple (1.8%).

2.5 Molecular diversity

Several DNA markers systems are now commonly used in diversity studies of plants. The most commonly used marker systems are Random Amplified polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) (Zietkiewicz *et al.*, 1994).

2.5.1 RAPD

M'Ribu and Hilu (1994) detected interspecific and intraspecific variation in *Panicum* millets through RAPD. Polymorphism in RAPD markers was observed across and within species. The four species were distinct in RAPD pattern and were separated at low correlation values even with small samples involving single genotype per species.

M'Ribu and Hilu (1996) studied the genetic diversity in *Paspalum scorbiculatum* L. by using RAPD among collection of kodo millet. A high level of polymorphism in RAPD markers was observed among the individual accessions, demonstrating the high genetic diversity of the crop. The markers obtained from the RAPD method were analysed with the cluster analysis, principle coordinates and minimum spanning tree methods. Three major groups were resolved, one representing the African accessions and two for the Indian accessions. The accessions of North African Kodomillet and *P. polystachyum* (considered conspecific with *P. scorbiculatum*) were quite distinct.

Li *et al*. (1998) studied the intraspecific and interspecific variation in *Setaria* revealed by RAPD primer using 20 accessons of foxtail millet. A total of 148 scoreable RAPD markers were generated with the 19 random 10-mer primers with 72.80% variability. The number of DNA bands generated by each primer varied from 3 to 12.

Schontz and Rether (1999) studied the genetic variability in foxtail millet by using RAPD. The DNA of 37 lines of foxtail millet used for the detection of polymorphism from each other by one or two G-C inversions were used. Twenty five bands were polymorphic and allowed the identification of 33 different genotypes. A factorial analysis of correspondence was performed on the presence/absence data, through which three genetic groups could be identified. These genetic groups were closely related to geographic origin of different lines : one Central European and two Asiatic groups. The lines originating from Western Europe were very variable and were dispersed in the three genetic groups.

Fakrudin *et al.* (2004) studied the genetic diversity of finger millet germplasm though RAPD analysis among 12 selected finger millet accessions, representing different geographic origins and pedigree back-grounds. A total of 37 RAPD primers, detected 254 unambiguous, repeatable fragments with an average of 6.86 amplified fragments per primer. A large number of fragments (218), representing 85.82 per cent of the total, were polymorphic. Cluster analysis based on unweighted pair group method using arithmetic average clearly indicated grouping of finger millet accessions in concordance with geographic origin and pedigree history.

Kalyan Babu *et al.* (2006) assessed the genetic diversity among finger millet accessions using 50 RAPD markers. Out of total 529 loci generated, 479 loci (91%) were polymorphic and informative to differentiate the accessions. Cluster analysis grouped the 32 finger millet accessions into two major clusters. Among the 32 genotypes, GEC 182 and CO-12 were distantly related with a low similarity index of 0.315. These two accessions also differed considerably in days to flowering and grain weight, GEC 182 is early flowering and had bold grains, while CO-12 is late flowering and had smaller grains.

Gupta *et al.* (2010) studied the genetic relatedness of three varieties of finger millet with varying seed coat colour by using 10 RAPD markers. The RAPD profiling of these varieties generated 86 loci with 49 polymorphic and 37 monomorphic loci. The RAPD marker with 8.5 loci per primer was found to be better than ISSR marker (5.7 loci/primer).

Kumari and Pande (2010) studied the genetic diversity in finger millet using RAPD markers in 12 germplasms of finger millet

including two of the same variety (VL-149) but from different regions. Three replica of each germplasm were amplified using 17 random primers. A total of 113 distinct fragments ranging from 117 bp to 2621 bp were amplified. Of these 70 (61.9%) were found to be polymorphic.

Panwar *et al.* (2010) investigated genetic relationships among finger millet genotypes by using 18 RAPD primers revealed 49.4 per cent polymorphism. Mean polymorphic information content (PIC) for this marker system (0.351 for RAPD) suggested that this marker system was effective in determining polymorphism with pairwise similarity index value of 0.505. The dendrogram developed by RAPD analysis revealed that genotypes are grouped in different clusters.

Ratna kumari *et al.* (2011) analysed the set of 125 foxtail millet accessions selected from 11 different agro ecological regions of India using random amplified polymorphic DNA (RAPD) marker technique. A total of 115 RAPD scoreable markers were generated with 16 RAPD primers. A total of 115 scoreable amplification products) were generated. The number of amplicons generated by each primer varied from four (OPA-09) to twelve (OPD-02) for RAPDs. The average number of amplicons detected was 7.3 per primer. Out of146 bands generated 119 (81.5%) were polymorphic and27 (18.5%) were monomorphic.

2.5.2 ISSR

Inter Simple Sequence Repeat (ISSR) PCR is a technique, which involves the use of microsatelite sequences as primers in polymerase chain reaction to generate multilocation markers. ISSR markers are highly polymorphic and are used in studies on genetic

diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Reddy *et al.*, 2002).

ISSR-PCR is technique that overcomes most of limitations of RAPD, AFLP, SSR or microsatellite (Zietkiewicz *et al.*, 1994; Gupta *et al.*, 1994; Wu *et al.*, 1994; Meyar *et al.*, 1993). In this method SSRs are used as primers to amplify mainly the inter-SSR regions (Reddy *et al.*, 2002). ISSR have been successfully used to estimate the extent of genetic diversity at inter and intra specific level in wide range of crop species which include rice (Joshi *et al.*, 2000), wheat (Nagaoka and Ogihara, 1997), finger millet (Salimath *et al.*, 1995), Vigna sp. (Ajibade *et al.*, 2000) and sweet potato (Huang and Sun, 2000).

Salimath *et al.* (1995) assessed the genome origin and genetic diversity in the genus *Eleusine* by using ISSR to analyse 22 accessions belonging to 5 species of *Eleusine*. Six ISSR primers showed 26 per cent polymorphism in 17 accessions of finger millet from Africa and Asia. This result indicated that very low level of DNA sequence variability in finger millet, but did allow each line to be distinguished. The 16 per cent intraspecific polymorphism exhibited by two analysed accessions of *E. floccifolia* suggested a much higher level of diversity in this species than in *E. coracana. Eleusine floccifolia* and *E. compressa* were found to be the most divergent among the species examined.

Gupta *et al.* (2010) studied the genetic relatedness of these varieties of finger millet with varying seed coat colour by using 10 ISSR markers. The molecular characterization of these varieties using 10 ISSR markers generated 57 loci with 18 polymorphic and 39 monomorphic loci. The ISSR marker based analysis revealed

maximum similarity among PRM-701 and PRM-801 which has been further confirmed by morphological, physiological and enzymatic characterization

Ratna kumari *et al.* (2011) analysed the set of 125 foxtail millet accessions selected from 11 different agro ecological regions of India using inter simple sequence repeat (ISSR) marker techniques. A total of 31 ISSR scoreable markers were generated with four ISSR primers. A total of 31 scoreable amplification products were generated. The number of amplicons generated by each primer varied from three (UBC885) to ten (UBC888) for ISSRs. The average number of amplicons detected was 7.3 per primer. Out of146 bands generated 119 (81.5%) were polymorphic and 27 (18.5%) were monomorphic.

3. MATERIAL AND METHODS

The present investigation on "Assessment of genetic and molecular diversity for different traits in foxtail millet (*Setaria italica* (L.) P. Beauv)" was carried out at State Level Biotechnology Research Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri.

The details of experimental material used, the experimental approaches and statistical procedure followed during the present experiment are given as under following headings:

3.1 Material

Forty-four genotypes for the present investigation were collected from the Millet Breeder, AICRP on small millet, NARP, Shenda Park, Kolhapur. The list of genotypes used is given with their pedigree in Table 3.1.

Table 3.1 List of genotypes with their pedigree

Sr. No.	Genotype	Pedigree
1	KOFM 1	Local collection
2	KOFM 2	Local collection
3	KOFM 6	Local collection
4	KOFM 14	Local collection
5	KOFM 17	Local collection
6	KOFM 18	Local collection
7	KOFM 24	SIA 3043

8	KOFM 25	Sel from SIA 326
9	KOFM 28	SIA 3039
10	KOFM 29	SIA 3044
11	KOFM 33	GPUS 27
12	KOFM 36	GPUS 30
13	KOFM 37	CO 5 × TNAU 200
14	KOFM 41	SIA 3035
15	KOFM 42	Local collection
16	KOFM 44	Local collection
17	KOFM 46	Local collection
18	KOFM 48	Local collection
19	KOFM 51	Local collection
20	KOFM 52	Local collection
21	KOFM 53	Local collection
22	KOFM 54	Local collection
23	KOFM 55	Local collection
24	KOFM 58	Local collection
25	KOFM 59	Local collection
26	KOFM 61	Local collection
27	KOFM 62	Local collection
28	KOFM 64	Local collection

29	KOFM 65	Local collection
30	KOFM 66	Local collection
31	KOFM 70	Local collection
32	KOFM 73	Local collection
33	KOFM 77	Local collection
34	KOFM 79	Local collection
35	KOFM 80	Local collection
36	PS 4	Mutant of 543/ Sie 2616
37	GPUS 28	UAS Bangalore, India
38	SIA 326	Pureline selection – Mandya
39	KOFM 88	Sie 1472 UK
40	KOFM 89	Sie 1537 India
41	KOFM 90	Sie 1539 India
42	KOFM 93	Sie 1541 3 India
43	KOFM 94	Sie 1598 India
44	KOFM 95	Sie 1599 India

3.2 Methods

3.2.1 Experimental design

The experiment was conducted in RBD with three replications with spacing 30 cm × 10 cm. All the standard cultural practices such

as fertilizer application, interculturing, weeding etc., were followed to raise good crop.

3.2.2 Observations recorded

Following observations were recorded on ten randomly selected plants from each genotype in each replication and averages were worked out.

3.2.2.1 Days to panicle initiation (No.)

Number of days required for panicle initiation of each observational plant was recorded from the date of sowing.

3.2.2.2 Days to 50 per cent flowering (No.)

Number of days required for about 50 per cent flowering of each observational plant was recorded from the date of sowing.

3.2.2.3 Days to maturity (No.)

Number of days required from sowing till the physiological maturity of the earhead on the observational plant was considered as days to maturity.

3.2.2.4 Number of productive tillers per plant (No.)

Productive tillers were recorded at ground level from each observational plant in numbers.

3.2.2.5 Plant height (cm)

Plant height was recorded from ground level to tip of plant in centimeter at maturity on selected observational plants.

3.2.2.6 Number of panicles per plant (No.)

Number of panicles was computed by adding 1to the number of productive tillers per plant.

3.2.2.7 Panicle length (cm)

Length of panicle was measured from each observational plant in centimeter.

3.2.2.8 1000-grain weight (g)

Weight of 1000 seeds was measured from each replication in grams.

3.2.2.9 Grain yield per plant (g)

Grain yield per plant was measured in gram by taking the total seed weight per plant after sun drying.

3.2.2.10 Straw yield per plant (g)

Straw yield per plant was measured in gram by taking the straw per plant after sun drying.

3.2.2.11 Protein content (%)

The protein content was determined by Micro-kjeldahl method (A.O.A.C., 1990), procedure given in appendix-I.

3.2.2.12 Iron content (%)

The procedure for determination of iron is same as protein content up to digestion and reading were taken using Atomic Absorption Spectrophotometer (AAS).

3.3 Statistical analysis

Experiment was conducted during *Kharif* 2009 at Post Graduate Farm, MPKV, Rahuri and in *Kharif* 2010 at Pulses Research Unit, MPKV, Rahuri. In both the environments, the experiment was conducted in Randomized Block Design (RBD) with three replications.The mean value of ten randomly selected observational plants for 12 different traits were used for statistical analysis. The following statistical measures/parameters were calculated for presentation of data on different quantitative attributes.

3.3.1 Analysis of variance (ANOVA)

The analysis of variance was done as suggested by Panse and Sukhatme (1985) in the following form

Source of variation	**DF**	**MSS**	**Expected mean square**
Replication	(r-1)	MSr	$\sigma^2e + t\sigma^2r$
Treatment	(t-1)	MSt	$\sigma^2e + r\sigma^2r$
Error	(r-1) (t-1)	MSe	σ^2e
Total	(rt-1)		

Where,

r = Number of replications

t = Number of treatments

3.3.2 Estimation of mean and range

The mean value for each character was worked out by using following formula

$$\bar{X} = (1/n) \sum_{i=1}^{n} Xi$$

Where,

$\sum Xi$ = Sum total of the each character

n= Number of observations

The lowest and the highest values from mean of each character were recorded as range.

3.3.3 Estimation of components of variation

The phenotypic and genotypic variances were calculated by utilizing the respective mean square values (Johnson *et al.* 1955).

i) Environmental Variance ($\sigma^2 e$) = MSe

ii) Genotypic Variance ($\sigma^2 g$) = (MSt - MSe) / r

iii) Phenotypic Variance ($\sigma^2 p$) = $\sigma^2 g + \sigma^2 e$

Where,

MSt = Genotypic mean sum of squares

MSe = Error mean sum of squares

r = Number of replications

3.3.4 Estimation of coefficient of variation

The genotypic and phenotypic coefficients of variation were calculated by following Burton (1952)

i) Phenotypic coefficient of variation (PCV)

$$PCV = \sqrt{\sigma^2 p / \bar{X} \times 100}$$

Where,

$\sigma^2 p$ = Phenotypic variance

$\bar{X}$ = General mean of the character

ii) Genotypic coefficient of variation (GCV)

$$GCV = \sqrt{\sigma^2 g / \bar{X} \times 100}$$

Where,

$\sigma^2 g$ = Genotypic variance

$\bar{X}$ = General mean of the character

The coefficients of variation were categorized as suggested by Sivasubramanian and Madhavamenon (1973)

Percent variability	Category
< 10 %	Low
11 – 20 %	Moderate
>20 %	High

3.3.5 Estimation of heritability percentage

Heritability percentage in broad sense was estimated for various characters as per the formulae suggested by Hanson *et al.* (1956).

$$h^2 \text{ (b.s.)} = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where,

$\sigma^2 g$ = Genotypic Variance

$\sigma^2 p$ = Phenotypic Variance

The heritability values were categorized as given by Lush (1940)

Heritability in per cent	Category
< 30	Low
31 – 61	Medium
> 61	High

3.3.6 Estimation of Genetic Advance

The genetic advance was calculated in per cent by the formulae suggested by Johnson *et al.* (1955).

$$G\ A = \sigma^2 g \,/\, \sigma^2 p \times \sigma p \times K$$

or

$$G\ A = K \times h^2 \text{ (bs)} \times \sigma^2 p$$

G A as percentage of mean = $\frac{GA}{\bar{X}} \times 100$

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

σp = Phenotypic standard deviation

K = Selection differential at 5 per cent selection intensity

h^2 (b. s.) = Heritability (broad sense)

$\bar{X}$ = Mean of the character.

The range of genetic advance as per cent of mean is classified as suggested by Johnson *et al.* (1955).

Low	:	less than 10%
Moderate	:	10-20 %
High	:	More than 20 %

3.3.7 Correlation

Analysis of covariance was carried out by taking two characters at a time. The genotypic covariance was calculated as per (Johnson *et al.* 1995) as below:

Environmental covariance ($COV.e_{1.2}$) =EMP

Genotypic covariance ($COV.g_{1.2}$) = $\frac{GMP-EMP}{r}$

Phenotypic covariance ($COV.p_{1.2}$) = ($COV.g_{1.2}$) + ($COV.e_{1.2}$)

Appropriate variances and co variances were used for calculating phenotypic and genotypic correlation coefficients (Johnson *et al.* 1955).

The phenotypic correlation coefficient (rp) was calculated as:

$$r\ p_{1.}p_2 = \frac{COV.\ p_{1.}p_2}{\sqrt{(\sigma^2\ p_1).(\sigma^2\ p_2)}}$$

Where,

$r\ p_{1.}p_2$ = Phenotypic correlation coefficient between character 1 and 2

$COV.\ p_{1.2}$ = Phenotypic covariance between character 1 and 2

$\sigma^2\ p_1$ & $\sigma^2\ p_2$ = Phenotypic variance of character 1 and 2, respectively.

The genotypic correlation coefficient (rg) was calculated as:

$$r\ g_{1.2} = \frac{COV.\ g_{1.2}}{\sqrt{(\sigma^2\ g_1).(\sigma^2\ g_2)}}$$

Where,

$rg_{1.2}$ = Genotypic correlation coefficient between character 1 and 2

$COV.\ g_{1.2}$ = Genotypic covariance between character 1 and 2

$\sigma^2 g_1$ & $\sigma^2 g_2$ = Genotypic variance of character 1 and 2 respectively.

The significance of correlation was tested by 't' test.

3.3.8 Path coefficient analysis

To establish a cause and effect relationship the first step used was to partition genotypic and phenotypic correlation coefficient into direct and indirect effects by path analysis as suggested by Dewey and Lu (1959) and developed by Wright (1921).

The second step in path analysis is to prepare path diagram based on cause and effect relationship. In the present study, path diagram was prepared by taking yield as the effect *i.e.* function of various components like X_1, X_2, X_3 and these component showed following type of association with each other.

In path diagram the yield is the result of X_1, X_2, X_3 and some other undefined factors designated by R.

Path coefficients were obtained by solving a set of simultaneous equations of the form.

$$r_{ny} = P_{ny} + r_{n2} P_{2y} + r_{n3}P_{3y} + \ldots\ldots\ldots\ldots$$

Where,

r_{ny} = represents the correlation between one component and yield

P_{ny} = represents path coefficient between that character and yield

r_{n2} = represents correlation between that character and each of other components in turn

$$\begin{bmatrix} r_{1y} \\ r_{2y} \\ r_{ny} \end{bmatrix} = \begin{bmatrix} r_{11} & r_{12} & r_{13} \ldots\ldots\ldots\ldots & r_{1n} \\ r_{21} & r_{22} & r_{23} \ldots\ldots\ldots\ldots & r_{2n} \\ r_{n1} & r_{n2} & r_{n3} \ldots\ldots\ldots\ldots & r_{nn} \end{bmatrix}$$

Matrix – A Matrix – B

Where, $r_{12} = r_{21}$ and so on and r_{1y}, correlation between one component character and yield. The B-matrix was inverted (B^{-1}) and path coefficient (P_{ij}) were obtained as:

$$(P_{ij}) = A \times (B^{-1})$$

The indirect effects of a particular character through other characters were obtained by multiplication of direct paths and particular correlation between these characters separately.

$$\text{Indirect effects} = r_{ij} \times p_{iy}$$

Where,

i = 1 to 10

j = 1 to 10

P_{iy} = $P_{1y}, P_{2y}, \ldots\ldots\ldots\ldots\ldots\ldots, P_{ny}$

Path coefficient (P_{ij}), correlation coefficient (r_{ij}) and residual factors (R) were diagrammatically presented. The residual factor *i.e.* variation in yield unaccounted for by these associations was calculated with the following formula:

$$\text{Residual factor (R)} = (1 - R^2)$$

Where,

$$R^2 = P_{1y}\, r_{1y} + P_{2y}\, r_{2y} + \ldots\ldots\ldots\ldots\ldots + P_{ny}\, r_{ny}$$

Where,

$P_{1y}, P_{2y}, \ldots\ldots\ldots\ldots\ldots, P_{ny}$ = Path values

$r_{1y}, r_{2y}, \ldots\ldots\ldots\ldots\ldots, r_{ny}$ = Correlation coefficient

The direct and indirect effects were classified based on the scale given by Lenka and Misra (1973).

Value of direct or indirect effects	Rate or scale
More than 1.00	Very high
0.30 to 0.99	High
0.20 to 0.29	Moderate
0.10 to 0.19	Low
0.00 to 0.09	Negligible

3.4 Mahalanobis generalized distance (D^2)

The generalized distance between two population is defined by Mahalanobis (1936) as $D^2 = \lambda ij didj$

Where,

λij = Reciprocal matrix to the common dispersion matrix

d_i = difference between the mean values of two populations for i^{th} character

d_j = difference between the mean values of two populations for j^{th} character

3.4.1 Determination of gene constellation

Tocher's method as described by Rao (1952) was followed for cluster formation. No formal rules can be laid down for finding the clusters because a cluster is not well defined term. The only criteria appeared to be that any two groups belonging to the same cluster should at least on an average show a smaller D^2 than those belonging

to the two different clusters. A simple method suggested by K.D. Tocher is to start with the two closely associated groups and find a third group which has the smaller D^2 from the two. Similarly, the fourth is chosen to have the smaller D^2 from the first three and so on. If at any stage the average D^2 of a group form those already listed appears to be high. Then this group does not fit in the former groups and is therefore, taken outside the former cluster. The group of first cluster are then omitted and the rest are treated similarly. It is also useful to calculate the change in average D^2 within a cluster due to inclusion of an additional group. If the changes are appreciable, then newly added group has to be considered as outside the cluster.

3.4.2 Average intra and inter cluster D^2 and D Values

3.4.2.1 Average intra cluster D^2

$$D^2 = \Sigma Di^2 / n$$

Where,

'Di' is sum of distances between all possible combinations (n) of the population included in a cluster.

3.4.2.2 Average inter cluster D^2

D^2 = Σ Distances between the population of cluster 1 and j./ni nj

Where,

ni = Number of populations in the cluster i

nj = Number of populations in the cluster j

3.4.2.3 Average intra and inter - cluster distance

$$D = \sqrt{D^2}$$

Cluster means were calculated for individual character on the basis of mean performance of the genotypes included in that cluster

3.4.2.3 Per cent contribution of characters towards total divergence

Contribution of each individual character towards divergence was calculated by ranking each character on the basis of $di = Y_{ji} - Y_{ki}$ values. Rank one was given to lowest mean difference.

3.5 Molecular diversity

3.5.1 Isolation of genomic DNA

The foxtail millet cultivars were planted in the 3rd week of July 2010 in green house at MPKV, Rahuri and DNA was extracted from the 15-20 days old seedlings of each accession according to protocol developed by Li *et al.* (1998) for RAPD and Reddy *et al.* (2009) for ISSR with some modifications.

Reagents required

1. Buffer 'S' (100 mM TrisCL (pH 8.5), 50mM EDTA (pH 8.0), 100 mM NaCl, 2% SDS)
2. Chloroform / Phenol
3. Isopropanol
4. T:E buffer (10 mM Tris HCl, pH 8.0 containing 1 mM EDTA)
5. 70 and 95 per cent ethyl alcohol.

Procedure

1. 3-4 gm leaf tissues of foxtail millet seedlings were ground in liquid nitrogen to a fine powder in a sterile chilled mortar and pestle.
2. The powder was quickly transferred to sterile centrifuge tube containing15 ml of buffer 'S'
3. The tubes were incubated in a water bath at 65^{0}C for 2 hours with shaking at 20 minutes intervals.
4. After incubation 15 ml phenol / chloroform added in tubes.
5. Tubes were mix thoroughly and centrifuged at 3000 rpm for 20 minutes.
6. Supernatant was transferred to a new tube.
7. Chloroform (15 ml) was added in supernatant.
8. Contents in tubes were mixed throughly and centrifuged at 3000 rpm for 20 minutes.
9. The DNA was precipitated from the aqueous phase with 0.6 vol. isopropanol and precipitate was spooled out with glass hook and rinsed in 70% ethanol.
10. After air drying, the precipitate was dissolved in 5ml 1x TE.

3.5.2 Purification of genomic DNA

Reagents

1. Sodium acetate (3 M, pH 5.2)

2. RNase

3. 70 and 95 per cent ethyl alcohol

4. Chloroform: iso amylalcohol (24 : 1)

Procedure

1. For purification, DNA sample was taken in a fresh eppendorf tube. The RNase was added to it in appropriate quantity (10 µl RNA) and incubated at 37^0C for an hour.

2. After incubation, the tubes were kept at room temperature for 1-2 minutes and 15 ml phenol/ chloroform was added.

3. Then the tubes were centrifuged at 3000 rpm for 20 minutes. The aqueous phase was removed and transferred to a fresh microfuge tube.

4. Resultant supernatant mixed with 1/10 vol. 3 M sodium acetate and 3 vol 95% ethanol.

5. The tubes were kept at -20^0C for 30 minutes and centrifuged at 3000 rpm for 20 minutes.

6. Supernatant was decanted carefully and DNA pellet were washed with 70 per cent cold ethanol and air dried.

7. Purified DNA was then dissolved in TE.

3.5.3 Quantification of genomic DNA

DNA quantification was carried out on nanodrop. Distilled water (1 µl) was measured as blank and then 1 µl of DNA was measured at

260 nm as well as 280 nm wavelength. The absorbance of 260/280 ratio was recorded. Ratio around 1.8 to 2.0 indicated good quantity of DNA.

3.5.4 DNA amplification

RAPD and ISSR analysis of the genomic DNA of forty four foxtail millet genotypes was carried out by PCR reaction in a palm cycler using 29 random decamer and 20 ISSR primers. Out of these 19 RAPD and 12 ISSR were scoreable. The details of the primers used are presented in Table 3.2 and 3.3.

Each ISSR primer was amplified at different temperatures to standardize the annealing temperature i.e. Gradient (Table 3.3) Amplification was performed in a 0.2 ml PCR tubes having 25 µl reaction volume. The composition of PCR mixture is presented in Table 3.4.

1. Requirements

1. Primers: Commercial kits obtained from Operon Technologies Inc., Alameda, USA was used.
2. Template DNA – DNA extracted from foxtail millet leaves
3. Palm cycler – Corbett Research, Australia.
4. Amplification mixture for PCR.

Table 3.2 List of RAPD primers used with their sequences

Sr. No.	Primer code	5ˡ to 3ˡ
1	OPA 3	AGTCAGCCAC
2	OPD 1	ACCGCGAAGG
3	OPD 5	TGAGCGGACA
4	OPD 11	GAGTCTCAGG
5	OPD 18	GAGAGCCAAC
6	OPE 3	CCAGATGCAC
7	OPE 4	GTGACATGCC
8	OPE 9	CTTCACCCGA
9	OPE 12	TTATCGCCCC
10	OPE 13	CCCGATTCGG
11	OPE 15	ACGCACAACC
12	OPE 17	CTACTGCCGT
13	OPE 18	GGACTGCAGA
14	OPE 19	ACGGCGTATG
15	OPK 9	CCCTACCGAC
16	OPL 2	TGGGCGTCAA
17	OPL 11	ACGATGAGCC
18	OPL 14	GTGACAGGCT
19	OPL 15	AAGAGAGGGG
20	OPL 16	AGGTTGCAGG
21	OPL 18	ACCACCCACC
22	OPM 5	GGGAACGTGT
23	OPM 9	GTCTTGCGGA
24	OPM 10	TCTGGCGCAC
25	OPM 12	GGGACGTTGG
26	OPM 14	AGGGTCGTTC
27	OPM 17	TCAGTCCGGG
28	OPM 18	CACCATCCGT
29	OPM 20	AGGTCTTGGG

Table 3.3 Sequences and fixed optimum annealing temperature for ISSR primers used in ISSR analysis

Sr. No.	ISSR Primers	Sequence of Primers (5'-3')	Optimum annealing Temp. (^{0}C)
1	ISSR 807	AGAGAGAGAGAGAGAGT	42.4
2	ISSR 808	AGAGAGAGAGAGAGAGC	46.8
3	ISSR 809	AGAGAGAGAGAGAGAGG	46.3
4	ISSR 810	GAGAGAGAGAGAGAGAT	42.8
5	ISSR 811	GAGAGAGAGAGAGAGAC	43.2
6	ISSR 816	CACACACACACACACAT	51.0
7	ISSR 817	CACACACACACACACAA	52.7
8	ISSR 819	GTGTGTGTGTGTGTGTA	47.8
9	ISSR 820	GTGTGTGTGTGTGTGTC	50.5
10	ISSR 822	TCTCTCTCTCTCTCTCA	45.7
11	ISSR 823	TCTCTCTCTCTCTCTCC	47.3
12	ISSR 826	ACACACACACACACACC	53.1
13	ISSR 834	AGAGAGAGAGAGAGAGYT	45.1
14	ISSR 835	AGAGAGAGAGAGAGAGYC	45.7
15	ISSR 840	GAGAGAGAGAGAGAGAYT	45.7
16	ISSR 841	GAGAGAGAGAGAGAGAYG	46.1
17	ISSR 880	GGAGAGGAGAGGAGA	49.0
18	ISSR 885	BHBGAGAGGAGAGAGAGA	46.4
19	ISSR 890	VHVGTGTGTGTGTGTGT	51.0
20	ISSR 891	HVHTGTGTGTGTGTGTG	51.9

Single letter abbreviations for mixed base positions: Y = (C, T); B = (C, G, T i.e. not A); H = (A, C, T i.e. not G); V = (A, C, G i.e. not T).

Table 3.4 Composition of PCR reaction mixture for RAPD and ISSR markers

Sr. No.	**Constituents**	**Stock Concentration**	**Volume of PCR reaction mixture per tube**
1	Taq Buffer B (for RAPD)	10x	2.5 µl
	Buffer F (For ISSR) with 100 mM-Tris (pH-9), 500 mM KCL and 1 % TritonX-100	10x	2.5 µl
2	MgCl2	25 mM	3 µl (1.5 mM)
3	$dNTP_s$	10 mM (2.5 mM each)	2µl (250 µM each)
4	*Taq* Polymerase	1 U/µl	0.33
5	Primer	1 µM	5 µl (0.200 µm each)
6	Genomic DNA	5 ng /µl	4 µl (20 ng)
7	Distilled water	--	8.17 µl
	Total volume	--	25 µl

Procedure

The 25 µl reaction mixture was gently vortexed and spinned down. The DNA amplification was carried out on a thermal cycler (Eppendorf, Master cycler gradient, Germany). The PCR conditions set for amplification were tabulated in Table 3.5.

Table 3.5 PCR programme set in thermal cycler for RAPD and ISSR markers

Step No.	Name of the steps followed	RAPD			ISSR		
		Temp	Time	Cycles	Temp	Time	Cycles
First	Denaturation	94^0 C	5 min	1	94^0 C	5 min	1
Second	Denaturation	94^{0}C	1 min	44	94^{0}C	30 sec	40
	Annealing	37^{0}C	1 min		45-55^{0}C	30 sec	
	Extension	72^{0}C	2 min		72^{0}C	30 sec	
Third	Final extension	72^{0}C	5 min	1	72^{0}C	10 min	1
Fourth	Final hold	4^{0}C	Till retrieval		4^{0}C	Till retrieval	

3.5.5 Agarose gel electrophoresis of amplified PCR products

Requirements

1. Electrophoresis unit (gel casting trough, gel combs, power pack)
2. UV transilluminater
3. Solutions required
   i. Ethidium bromide: 10 mg/ml.
   ii. Bromophenol blue (Loading dye)
   iii. Agarose
   iv. Stock solution of 10x TBE: 121 g Tris (1 M), 51.3 g Boric

acid (830 mM) and 7.6 g EDTA (10 mM) to make final volume 1000 ml and adjust pH 8.0.

4. Working solution of 1x TBE: Ten ml of 10x TBE was diluted to 100 ml using milli Q water

Procedure

Agarose (1.5 g) was added to 100 ml of 1x TBE buffer and agarose was melted by heating the solution in microwave oven. Solution was cooled to about 55-60°C and 5 µl of ethidium bromide (0.5 µl/ ml) was added in it. The agar solution was poured into the gel casting unit after keeping the gel comb in the proper place. The gel was allowed to solidify at room temperature. Gel was placed in the electrophoresis apparatus in such a way that the end with wells is in line with the cathode. The apparatus was filled with 1x TBE buffer in order to submerge the gel in the buffer to prevent the entry of air bubbles while removing the gel combs. The 25 µl PCR products to be analyzed were mixed with 2 µl tracking dye and loaded carefully in the wells of the gel. The unit was connected to a power pack, and electrophoresis was carried out at 70 volts. The power supply was switched off when the dye front is about 2 cm away from positive end (anode). The amplified PCR products were observed under UV transilluminater in gel documentation system (Flour Chem. TM Alpha innotech, USA) and image was captured.

3.5.6 Data analysis

The RAPD and ISSR products were scored as presence (1) or absence (0) of band for each primer genotype combination. A binary data matrix based on (0)/(1) was used for analysis with NTSYSpc Software Package Ver. 2.02 (Rohlf, 1997).

The polymorphism information content (PIC) value was calculated as $PIC=\Sigma (1 - Pi^2)/ n$ where n is the number of band positions analyzed in the set of accessions and Pi is the frequency of i^{th} pattern.

3.5.7 Construction of dendrogram

Jaccards similarity Coefficient was used for the construction of dendrogram by the Unweighted Pair Group Method using Arithmetic Mean (UPGMA).

4. RESULTS

The foxtail millet accessions were collected from the Millet Breeder, AICRIP on small millet, NARP, Shenda Park, Kolhapur. These accessions were evaluated and characterized for 12 quantitative traits in two seasons. They were also genotyped using 19 RAPD and 12 ISSR markers. Thus, the current study was formulated to understand the genetic variability and divergence in the genotypes for different traits in foxtail millet and to study the molecular diversity present in the genotypes by using RAPD and ISSR markers. The results of the investigation are presented below.

4.1 Analysis of variance

The analysis of variance (Table 4.1) revealed highly significant differences among genotypes for twelve characters studied. In pooled analysis also mean sum of squares for genotypes, environments and genotype × environment were significant.

4.2. Variability studies

The range, mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), genetic advance and genetic advance as percent mean of all the quantitative traits were calculated for each environment separately and for pooled data.

4.2.1 Mean performance

Means were calculated for each trait in individual and across environments. Mean performance of each accession for 12 quantitative traits for pooled data and E1, E2 are presented in Table 4.2 and appendix II and III, respectively. The estimates of mean and range are presented below.

4.2.1.1 Days to panicle initiation

The widest range of days to panicle initiation was observed in both environments (42.67-67.00 days in E1 and 43.67-68.67 days in E2) and in pooled data (43.33-67.83 days).

The mean of days to panicle initiation was almost similar in both environments (47.07 days in E1 and 49.25 days in E2) and pooled (48.16 days) and did not differ significantly from each other. The genotype KOFM 66 (42.66 days) had panicle earlier followed by KOFM 64, KOFM 46 and KOFM 51(43.00days) in E1. In E2 genotypes KOFM 46 and KOFM 64 (43.66 days) recorded early panicle initiations followed by KOFM 51 and KOFM 66 (44.33days). In pooled, early panicle initiation was recorded in the genotype KOFM 46 and KOFM 64 (43.33days) followed by KOFM 66 (43.50 days) and KOFM 51 (43.66 days). While, genotypes KOFM 94 (67.00days) and KOFM 95 (64.66 days) in E1, KOFM 94 (67.83 days) and KOFM 95 (65.50 days) in E2 and KOFM 94 (67.83 days), KOFM 95 (65.50 days) in pooled recorded late panicle initiations**.**

4.2.2.2 Days to 50 per cent flowering

The widest range of days to 50 per cent flowering was observed in both environments (54.00-80.00 in E1 and 57.67-82.00 days in E2) and in pooled (56.83-81.00 days). In E1 environment, early flowering was observed in genotype KOFM 25 (54.00days) followed by KOFM 70 and PS 4(55.33days). In E2, genotype KOFM 51 (57.66 days) followed by KOFM 33 (58.00 days), KOFM 79 (58.33days) recorded early flowering. In pooled analysis the genotypes KOFM 25 and PS 4(57.00 days) were earlier in flowering followed by KOFM 70 (57.16 days), KOFM 33 and KOFM 59(57.33 days). While, in E1, genotypes KOFM 94 (80.00 days) and KOFM 95 (76.66 days), in E2, genotypes KOFM 94 (82.00 days) and KOFM 95 (78.00 days) and in pooled genotypes KOFM 94 (81.00 days) and KOFM 95 (77.33 days) had late flowering.

4.2.2.3 Days to maturity

Days to maturity ranged from 90.67 to 121.67 days in E1, 89.00 to 125.67 in E2 and 90.00 to 123.67 in pooled.

The genotype KOFM 41 in E1 matured earlier (90.66 days) followed by KOFM 79 (91.00 days), SIA 326 and KOFM 88 (91.33 days). Whereas in E2 the KOFM 79 (89.00 days) was earlier followed by the genotypes KOFM 59 (90.00 days), KOFM 88 (92.33 days).When pooled over the two environments the genotype KOFM 79 (90.00days) was earliest followed by the genotypes KOFM 59 (91.16days) and KOFM 88 (91.83 days).

The genotypes KOFM 94 and KOFM 95 were very late in maturity in E1 (121.66 and 119.66 days), E2 (125.66 and 120.00 days) and in pooled (123.66 and 119.83 days).

4.2.2.4 Number of productive tillers per plant

The number of productive tillers per plant ranged between 0.10 to 3.90 in E1 and 0.70 to 3.60 in E2. In pooled data, it ranged from 0.90 to 3.73**.**

The mean number of productive tillers per plant was higher in E1 (2.55) and E2 (2.40) with an overall mean of 2.48 in pooled data. The genotypes KOFM 1 (3.90), PS 4 (3.86) and KOFM 94 (3.86) in E1 and PS 4 (3.60) followed by KOFM 94 (3.56) and KOFM 95 (3.43) in E2 had more number of productive tillers per plant. Whereas, in pooled data, PS 4 (3.73) followed by KOFM 94 (3.71) and KOFM 95 (3.50) had more number of productive tillers per plant. While, the mean number of productive tillers per plant was low in genotypes KOFM 88 (1.10) followed by KOFM 54 (1.37) and KOFM 79 (1.53) in E1. In E2, the genotype KOFM 88 (0.70) followed by KOFM 54 (0.80) and KOFM 89 (0.97). Whereas in pooled, genotypes KOFM 88 (0.90) followed by KOFM 54 (1.08) and KOFM 79 (1.28) had less productive tillers per plant.

4.2.2.5 Plant height (cm)

Wide range for plant height was observed in foxtail millet germplasm and the maximum range was observed in E1 (111.73 to 188.30 cm) followed by E2 (109.73 to 182.07cm). In pooled data it ranged from 110.73 to 185.18 cm.

The mean plant height was 139.84 cm in E1, 134.56 cm in E2, 137.20 cm in pooled). The genotype KOFM 93 was tallest followed by KOFM 36 and KOFM 89 in both environments and in pooled data. The genotypes KOFM 95 and KOFM 94 were dwarfest in E1, E2 and pooled data.

4.2.2.6 Panicle Length (cm)

Panicle length ranged from 8.90-22.97 cm in E1, 7.83-21.87cm in E2 and 8.82-22.42cm in pooled.

Mean panicle length was higher in E1 (18.05 cm) than E2 (17.621m) and in pooled data it was (17.83cm).

The genotypes KOFM 14 showed maximum length of panicle (22.97cm) followed by KOFM 18 (22.00cm) and KOFM 90 (21.27cm). While, KOFM 95 recorded minimum length (7.90 cm) in E1.Whereas, the genotypes KOFM 14, KOFM 18 and KOFM 54 exhibited maximum panicle length and the genotypes KOFM 95 and KOFM 94 showed minimum length of panicle in E2 environment and also across environments.

4.2.2.7 Number of panicles per plant

Maximum range for number of panicles per plant was observed in E1 (2.10-4.90) followed by E2 (1.70-4.60). In pooled data, it ranged from 1.90 to 4.73.

The mean for number of panicles was higher in E1 (3.55) than E2 (3.40) with an overall mean of 3.48 in pooled data. The maximum number of panicles per plant was observed in genotypes KOFM 1(4.90), PS4 and KOFM 95 (4.87). While, the minimum number of

panicles per plant was in KOFM 88 (2.10), KOFM 54 (2.37) and KOFM 79 (2.53) genotypes in E1.Whereas in E2, the genotypes PS 4 (4.60) had maximum panicles followed by KOFM 94 (4.56), KOFM 95 (4.43) and KOFM 66 (4.33).While, genotype KOFM 88 (1.70) had minimum number of panicles per plant followed by KOFM 54 (1.80), KOFM 89 (1.97) and KOFM 79 (2.03).

Over two environments the genotypes PS 4 had maximum panicles per plant (4.73) followed by KOFM 94(4.71) and KOFM 95(4.50). While, the genotype KOFM 88 (1.90), KOFM 54 (2.08) and KOFM 79 (2.28) had minimum number of panicles per plant.

4.2.2.8 1000-grain weight (g)

The range of 1000-grain weight was the maximum in E1 (1.09-3.52g) followed by E2 (1.05-3.36g) and (1.07-3.44) in pooled data.

The mean 1000-grain weight was highest in E1 (2.85g) than in E2 (2.77g). Among the genotypes KOFM 89 (3.52g), KOFM 37(3.51g) and KOFM 59(3.48g) in E1 environment and KOFM 89 (3.36g), KOFM 59 and KOFM 73(3.34g) in E2 environment recorded high 1000-grain weight.

On the basis of pooled mean highest 1000-grain weight was recorded in KOFM 89 (3.44g) followed by KOFM 73 (3.39g) and KOFM 37 (3.36g).

4.2.2.9 Grain yield per plant (g)

Grain yield per plant (g) ranged from (6.87-23.87 g) in E1, (6.53-22.07 g) in E2 and (6.70-21.68 g) in pooled.

The mean grain yield per plant (g) was maximum for E1 (16.75 g) followed by E2 (15.26g). In pooled, the mean grain yield per plant was (16.01 g).

The genotypes KOFM 59 (23.87 g), KOFM 37 (23.20 g), PS 4 (22.70 g) and KOFM 52 (21.07g) gave higher yield in E1. Whereas in E2, KOFM 14 (22.07 g), PS 4 (20.67 g), SIA 326 (19.57 g) and KOFM

24 (19.43 g) were high yielding. While in pooled over environments the genotypes PS 4 (21.68 g), KOFM 59 (21.48 g), KOFM 37 (20.90g) and KOFM 24 (20.78 g) recorded high grain yield. While, KOFM 94 and KOFM 95 were lower in grain yield per plant in both E1, E2 and also in pooled.

4.2.2.10 Straw yield per plant (g)

The straw yield per plant ranged between 14.19 to 48.16 g in E1 and 12.07 to 46.23 g in E2. In pooled data, it was 13.49 to 47.19 g.

In E1, genotypes KOFM 36 (48.16g), KOFM 1 (41.41 g), KOFM 24 (38.03) and KOFM 41(37.87 g) recorded high straw yield per plant. In E2, KOFM 36 (46.23 g), KOFM 41 (36.38 g), KOFM 29 (35.15g) and KOFM 25 (34.37g) were high for straw yield per plant. Pooled analysis showed that the genotypes KOFM 36 (47.19 g), KOFM 1 (37.32g) and KOFM 41 (37.13 g) were high in straw yield per plant.

4.2.2.11 Protein Content (%)

The range for protein content was (6.97-13.65%) in E1, (7.18-13.86%) in E2 and 7.08-13.75% in pooled analysis.

In E1, the genotype KOFM 65 recorded the highest protein (13.75%) followed by KOFM 36 (12.43%), KOFM 80 (12.09%) and KOFM 28 (12.08 %). In E2 also, KOFM 65 (13.86%), KOFM 36 (12.21%) and KOFM 28 (12.16%) were highest for protein content. Over two environments the genotypes KOFM 65 (13.75 %), KOFM 36 (12.32%) and KOFM 28 (12.12%) had maximum protein content.

4.2.2.12 Iron Content (%)

Iron content varied from (0.03-0.10%) in E1, E2 and in pooled analysis. There was no environmental variation for iron content. In E1, the genotypes KOFM 53 had maximum iron content (0.098%) followed by KOFM 51 (0.078%), KOFM 14 (0.064%), KOFM 59 (0.063%). In E2, KOFM 53 (0.097%) and KOFM 51 (0.081%) recorded the highest iron content. On the basis of pooled analysis, KOFM 53 (0.098%),

KOFM 51 (0.080%) KOFM 14 (0.064%) and KOFM 59 (0.063%) were the highest.

4.2.2 Coefficient of variation

4.2.2.1 PCV and GCV

The estimates on PCV (%) and GCV (%) are given in Table 4.3 and presented in Figure 1. The value of PCV obtained with respect to various quantitative traits ranged from 6.25 to 28.83 in E1, 6.53 to 32.18 in E2 and 6.39 to 30.46 in pooled, where as values of GCV ranged from 6.08 to 27.68 in E1, 6.41 to 30.94 in E2 and 6.18 to 27.85 in pooled. The lowest and the highest values for PCV and GCV were noted for days to maturity and number of productive tillers per plant in all the environments and for pooled data. High PCV was observed for number of productive tillers per plant, number of panicles per plant, grain yield per plant, straw yield per plant and iron content in E1, E2 and pooled. While, days to panicle initiations, plant height, panicle length, 1000 grain weight and protein content had moderate PCV in E1, E2 and pooled. The same trend was observed for GCV estimates in all traits except days to panicle initiation, days to 50% flowering and plant height having low GCV values. All the traits exhibited narrow differences between PCV and GCV in both environments and pooled data.

4.2.2.2 Heritability

The estimates of broad sense heritability in foxtail millet ranged from 49.70 to 99.70 in E1, 34.90 to 99.70 in E2, and 52.70 to 99.30 in pooled. High heritability estimates for all the traits were observed in both environments as well as across the environments except plant height which had low heritability (Table 4.3 and Figure 1).

4.2.2.3 Genetic advance

The plant height recorded highest genetic advance in E1 and pooled data, however, in E2 straw yield per plant had highest genetic advance.

The estimate of genetic advance as per cent of mean was high for all the characters in both the environments and pooled data except for days to maturity and plant height (Table 4.3 and Figure 1).

4.3 Correlation studies

Understanding of the interaction of the traits among themselves and with the environment is of great use in plant breeding. Correlation studies provide information on the nature and extent of association between any two quantitative traits and it would be useful for genetic enhancement of a trait through selection of a correlated trait (associated response). Grain yield is a complex character and jointly determined by a number of related traits. An insight into the association between grain yield and other correlated traits helps to improve the efficiency of selection. In general, the correlation between yield and other characters as well as among the component characters will vary with the material handled by the breeder. The genotypic correlation for twelve characters studied is presented in Table 4.4. The only significant correlations either in positive or negative directions are described.

Genotypic correlations in both the environments and for pooled data were calculated between twelve quantitative traits. Out of total 66 correlations, 32 correlations were significant in E1 and 33 each in E2 and pooled data (Table 4.4).

4.3.1 Correlation between grain yield and other component traits

Nine out of 12 traits, days to panicle initiation, days to 50 per cent flowering, days to maturity, number of productive tillers per plant, panicle length, number of panicles per plant, 1000-grain weight

and straw yield per plant showed significant correlation with grain yield per plant (g) in both environments and in the pooled data. Number of productive tillers per plant, panicle length, number of panicles per plant, 1000-grain weight and straw yield per plant showed significant positive correlation with grain yield per plant. While, days to panicle initiation, days to 50 per cent flowering, days to maturity showed significant negative correlation with grain yield per plant. Protein content in E2 and pooled had significant positive correlation with grain yield per plant (Table 4.4).

Grain yield per plant (g) had significant positive correlations with number of productive tillers per plant in E1 (0.346), E2 (0.432) and pooled (0. 320), panicle length in E1 (0.513), E2 (0.470) and pooled (0.512), number of panicles per plant in E1(0.346), E2(0.432) and pooled (0.320), 1000-grain weight in E1 (0.729), E2 (0.656) and pooled (0.706), straw yield per plant in E1 (0.581), E2 (0.666) and pooled (0.610) and protein content in E2 (0.316) and pooled (0.319) Table 4.4.

4.3.2 Inter correlation among the yield component traits

4.3.2.1 Days to panicle initiation

Days to panicle initiation had highly significant and positive correlations with days to 50% flowering and days to maturity in both the environments and in pooled. Whereas, it had significant negative correlation with panicle length, 1000-grain weight, straw yield per plant, protein and iron content in E1, E2 and pooled data.

Days to panicle initiation showed positive correlations with days to 50% flowering (0.985 in E1, 0.921 in E2 and 0.968 in pooled), days to maturity (0.702 in E1, 0.654 in E2 and 0.688 in pooled) in both the environments and pooled. While, Panicle length (-0.483 in E1, -0.479 in E2 and -0.475 in pooled), straw yield per plant (-0.344 in E1 and -0.333 in pooled), and iron content (-0.324 in E1, -0.335 in E2 and -

0.332 in pooled), showed negative correlation with days to panicle initiation (Table 4.4).

4.3.2.2 Days to 50 per cent flowering

Days to 50 per cent flowering had highly significant and positive correlations with days to maturity in both the environments and in pooled. Panicle length, straw yield per plant and iron content had significant negative correlation with days to 50% flowering in E1, E2 and pooled data.

Days to 50 per cent flowering showed positive correlations with days to maturity (0.742 in E1, 0.786 in E2 and 0.754 in pooled) in both the environments and pooled. While, Panicle length (-0.483 in E1, -0.618 in E2 and -0.564 in pooled), straw yield per plant (-0.344 in E1, -0.342 in E2 and -0.352 in pooled), and iron content (- 0.324 in E1, -0.335 in E2 and -0.314 in pooled), showed negative correlations with days to 50 per cent flowering (Table 4.4).

4.3.2.3 Days to maturity

Days to maturity had highly significant positive correlations with number of productive tillers and number of panicles per plant in both the environments and in pooled. Plant height and panicle length had significant negative correlation with days to maturity in E1, E2 and pooled data.

Days to maturity showed positive correlations with number of productive tillers (0.468 in E1, 0.409 in E2 and 0.458 in pooled) and number of panicles per plant (0.468 in E1, 0.409 in E2 and 0.458 in pooled) in both as well as in pooled. While, plant height (-0.381 in E1, -0.330 in E2 and -0.324 in pooled) and Panicle length (-0.805 in E1, -0.781 in E2 and -0.785 in pooled), showed negative correlation with days to maturity (Table 4.4).

4.3.2.4 Number of productive tillers per plant

Number of productive tillers per plant showed significant positive correlations with number of panicles per plant (1.000, 1.000 and 1.000), 1000 grain weight (0.359, 0.400 and 0.368) and straw yield per plant (0.469, 0.546 and 0.477) in E1, E2 and pooled, respectively. While, plant height (-0.412 in E1, - 0.588 in E2 and -0.476 in pooled) and panicle length (-0.444 in E1, -0.444 in E2 and -0.468 in pooled) had significant negative correlations with number of productive tillers per plant.

4.3.2.5 Plant height (cm)

The correlation analysis revealed highly significant positive correlation of plant height with panicle length, in both environments and pooled. Panicle length had significant positive correlation in E1 (0.612), E2 (0.610) and pooled (0.535), and number of panicles per plant showed negative correlation in E1 (-0.412), E2 (-0.588) and pooled (-0.476) with plant height.

4.3.2.6 Panicle length (cm)

Panicle length showed the significant negative correlation with number of panicles per plant -0.444, -0.588 and -0.468 in E1, E2 and pooled, respectively.

4.3.2.7 Number of panicles per plant

Highly significant and positive correlation was observed between number of panicles per plant with 1000 grain weight and straw yield per plant (Table 4.4). 1000 grain weight (0.359 in E1, 0.400 in E2 and 0.368 in pooled) and straw yield per plant (0.469 in E1, 0.546 in E2 and 0.477 in pooled) had positive correlation with number of panicles per plant in both and also across environments (Table 4.4).

4.3.2.8 1000-grain weight (g)

The trait 1000-grain weight had highly significant positive correlation with straw yield per plant (g) in both the environments

(0.440 in E1 and 0.481 in E2) and pooled (0.456) (Table 4.4). Protein content revealed positive correlation with 1000-grain weight in E2 (0.303).

4.3.2.9 Straw yield per plant (g)

Straw yield per plant had highly significant positive correlation with grain yield per plant in both the environments (0.581 in E1 and 0. 666 in E2) and in pooled (0.610) (Table 4.4).

4.3.2.10 Protein content (%)

Protein content had significant and positive correlation with grain yield per plant in E2 (0.316) and in pooled (0.319).

4.3.2.11 Iron content (%)

Iron content had significant negative correlation with days to panicle initiation (0.324 in E1, 0.335 in E2 and 0.332 in pooled) and days to 50% flowering (0.308 in E1, 0.319 in E2 and 0.314 in pooled)

4.4 Path coefficient analysis

To find out direct and indirect contributions of each of the characters, path coefficient analysis was carried out. The genotypic correlation coefficient being more important was only partitioned into direct and indirect effects which are presented in Table 4.5 and fig. 2, 3 and 4 for E1, E2 and pooled, respectively.

4.4.1 Yield vs. days to panicle initiation

Path analysis revealed that days to panicle initiation showed negative direct effect (-0.216) on grain yield and it had positive indirect effects with almost all the characters except days to 50% flowering, days to maturity, number of productive tillers per plant, plant height and number of panicles per plant and thus leading to significant negative correlation with grain yield per plant (-0.438). Whereas, days to panicle initiation showed the positive direct effect on grain yield in E2 (1.215) and in pooled (0.490) leading to significant negative

correlation with grain yield per plant (-0.405) and -(0.475), respectively. Both in E2 and pooled it showed the negative indirect effect on panicle length, 1000 grain weight, straw yield per plant, protein content and iron content.

4.4.2 Yield vs. days to 50 % flowering

Days to 50 % flowering showed positive direct effect (0.210) on grain yield and it had negative indirect effects with almost all the characters except days to panicle initiation, days to maturity, number of productive tillers per plant, plant height and number of panicles per plant and thus leading to significant negative correlation with grain yield per plant (-0.452).Whereas, days to 50% flowering showed the negative indirect effect (-0.355) in E2 and (-0.350) in pooled data leading to significant negative correlation with grain yield per plant (-0.478) and (-0.503) respectively. Both E2 and pooled showed the positive indirect effect on panicle length, 1000 grain weight, straw yield per plant, protein content and iron content

4.4.3 Yield vs. days to maturity

Days to maturity showed negative direct effect on grain yield (-0.448) in E1, (-0.352) in E2 and (-0.520) in pooled. The indirect effect through plant height, panicle length, 1000 grain weight, straw yield per plant and iron content were positive. While, rest of the characters showed positive indirect effect, thus leading to significant negative correlation with grain yield per plant (-0.472) in E1, (-0.460) in E2 and (-0.510) in pooled.

4.4.4 Yield vs. number of productive tillers per plant

Number of productive tillers per plant showed positive direct effect (0.628) on grain yield in E1 and in E2 (0.446).Its indirect effect through plant height, panicle length and iron content were negative and with rest of the characters its indirect effect were positive. The

total correlation with grain yield per plant was significant and positive (0.346) in E1 and (0.320) in pooled. Whereas, in E2, its direct effect was negative (-0.479) on grain yield and correlation was significant and positive (-0.432). While, it showed positive indirect effect through plant height, panicle length and iron content.

4.4.5 Yield vs. plant height

The plant height showed positive direct effect (0.102) on grain yield. It had negative indirect effect through days to maturity, number of productive tillers per plant, number of panicles per plant and protein content. While, with the remaining characters it showed positive indirect effects. The total genotypic correlation with grain yield per plant was positive and but not significant (0.229). Whereas it showed the negative direct effect on grain yield (-1.183) in E2 and (-0.114) in pooled and positive correlation with grain yield (-0.004) in E2 and in pooled (-0.110).

4.4.6 Yield vs. panicle length

It showed positive direct effect on grain yield (0.304) in E1, (0.691) in E2 and in pooled (0.283). Its indirect effect through days to panicle initiation was negative followed by days to 50% flowering, days to maturity, number of productive tillers per plant and number of panicles per plant. It had positive indirect effect through rest of the characters. It showed the significant positive correlation with grain yield per plant (0.513) in E1, (0.470) in E2 and (0.512) in pooled.

4.4.7 Yield vs. number of panicles per plant

Number of panicles per plant was calculated on the basis of number of productive tillers per plant. As a constant increase of one was added to productive tiller number to get values for this character it reflected in strong and perfect correlation (1.00) among the two characters. Thus it has given all the direct and indirect effects as zero.

4.4.8 Yield vs. 1000-grain weight

The 1000-grain weight showed positive direct effect on grain yield (0.378) in E1, (0.890) in E2 and in pooled (0.460). It showed negative indirect effect through days to panicle initiation, days to flowering, days to maturity and iron content and rest were positive. The correlation with grain yield (0.729) in E1, (0.656) in E2 and (0.706) in pooled were positive and significant.

4.4.9 Yield vs. straw yield per plant

It showed negative direct effect (-0.089) on grain yield. Its indirect effect through days to panicle initiation, days to flowering, days to maturity and iron content were positive. It had negative indirect effect through rest of the characters. It showed positive and significant correlation with grain yield per plant (0.581). Whereas, straw yield per plant showed the positive indirect effect (0.560) in E2 and (0.033) in pooled on grain yield. Indirect effect through number of productive tillers per plant was positive followed by plant height, panicle length, number of panicles per plant, 1000-grain weight and protein content in E2 and in pooled. It had significant and positive correlation with grain yield in (0.666) E2 and in pooled (0.610).

4.4.10 Yield vs. protein content

The protein content showed positive direct effect (0.069) on grain yield in E1 and (0.118) in pooled. It had negative indirect effect through days to panicle initiation, days to 50% flowering, plant height and iron content in both E1 and in pooled. Thus leading to non significant positive correlation with grain yield per plant (0.276) in E1 and significant positive correlation (0.319) in pooled. Whereas, in E2 protein content showed negative direct effect (-0.029) on grain yield.

It had positive indirect effect through days to panicle initiation, days to 50% flowering, plant height and iron content and had significant positive correlation (0.316) with grain yield.

4.4.11 Yield vs. iron content

The iron content showed positive direct effect (0.029) on grain yield in E1, (0.324) in E2 and (0.102) in pooled. It had negative indirect effect through all other characters except plant height and panicles length in E1, E2 and pooled. Thus leading to non significant positive correlation with grain yield per plant (0.019) in E1, (0.038) in E2 and (0.035) in pooled.

4.5 Genetic diversity

4.5.1 Mahalanobis's generalized distance (D^2)

Wilk's Λ criterion showed significant differences between the genotypes for the pooled effect of the ten characters tested under study. Hence, further analysis was done to calculate D^2 values.

The calculated D^2 values ranged between 1.90 to 610.09. The lowest value being between the pair of genotypes KOFM 55 and GPUS 28, while highest between KOFM 36 and KOFM 94 followed by between KOFM 36 and KOFM 95, KOFM 41 and KOFM 94.

4.5.2 Cluster formation

The 44 genotypes were grouped into six clusters (Table 4.6). Cluster I was the largest and comprised of maximum 37 genotypes, followed by cluster II and cluster V with 2 genotypes each. Cluster III, IV and VI were solitary (Figure 5).

4.5.3 Intra and Inter cluster distance

The intra and inter-cluster distances were recorded by D^2 solutions (Table 4.7). The mean D^2 values of cluster elements were used as measure of intra and inter-cluster distance. The maximum intra-cluster distance was found in cluster V (D^2 =26.93) followed by

cluster I (D^2 =19.05) and cluster II (D^2 =3.47). The cluster III, IV and VI were being solitary, recorded no intra-cluster distance (Figure 6).

The maximum inter-cluster distance was found between cluster II and cluster VI (D^2 =594.74) followed by cluster II and cluster IV (D^2 =338.36), cluster II and cluster III (D^2 =319.28).

The cluster I showed the maximum inter-cluster distance with cluster II (D^2 =437.54) followed by cluster V (D^2 =95.60). The cluster I was closer to cluster III (D^2 =50.27).

The maximum inter-cluster distance of cluster II was observed with cluster VI (D^2 =594.74) followed by cluster IV (D^2 =338.36), cluster III (D^2 =319.28) and cluster V (D^2 = 247.22).

The cluster III was most distant from cluster VI (D^2 =113.14) followed by cluster IV (D^2 = 71.23) and cluster V (D^2 =69.11).

The maximum inter-cluster distance of cluster IV was observed with cluster VI (D^2 =142.33) and cluster V (D^2 = 30.92).

The cluster V showed the maximum inter-cluster distance with cluster VI (D^2 =169.12).

4.5.4 Cluster means

The cluster means for ten characters studied are given in Table 4.8. The mean values for individual characters are described here after.

4.5.4.1 Days to panicle initiation

The genotypes in cluster I was earliest to panicle initiation (46.27) followed by cluster III (47.00), cluster VI (50.00). However, genotypes in cluster II (66.67) had late panicle initiation**.**

4.5.4.2 Days to 50% flowering

The genotypes in cluster III were earliest to flowering (58.50) followed by cluster I (59.00) and cluster VI (61.67). However, genotypes in cluster II (79.17) flowered late.

4.5.4.3 Days to maturity

Based on the cluster means, it was observed that the genotypes in cluster III were earliest to mature (91.83) followed by cluster VI (96.17) and cluster I (96.55). Genotypes in the cluster II were very late to mature (121.75) followed by cluster V (100.50).

4.5.4.4 Number of productive tillers per plant

Cluster II exhibited maximum number of productive tillers per plant (3.61) followed by cluster I (2.53) and cluster IV (1.88). While, cluster III exhibited minimum number of productive tillers per plant (0.90) followed by cluster V (1.83).

4.5.4.5 Plant height (cm)

Cluster IV included the tallest genotypes (185.18) followed by cluster VI (181.52). While, cluster II (112.27) included dwarf genotypes followed by cluster I (134.45).

4.5.4.6 Panicle Length (cm)

Cluster V exhibited maximum length of panicle (20.01) followed by cluster VI (18.98). While, cluster II exhibited minimum length of panicle (8.21) followed by I (18.17).

4.5.4.7 Number of panicle per plant

Cluster II exhibited maximum number of panicle (4.61) followed by cluster I (3.53). While, cluster III recorded less number of panicles per plant (1.90).

4.5.4.8 1000-grain weight (g)

The genotypes in the cluster IV (3.21) had maximum 1000 grain weight followed by cluster V (3.04). While, cluster III showed minimum 1000-grain weight (1.07).

4.5.4.9 Grain yield per plant (g)

The cluster I exhibited highest grain yield per plant (16.67) followed by cluster IV (16.38). The cluster II showed lowest grain yield per plant (6.93) followed by III (10.12).

4.5.4.10 Straw yield per plant (g)

The cluster VI exhibited highest straw yield per plant (47.19) followed by cluster I (28.12). While cluster III showed lowest straw yield per plant (13.49) followed by cluster II (16.12).

Per cent contribution of various characters towards divergence

The 44 genotypes of foxtail millet were studied for various characters and the data collected were used to determine the contribution of individual character towards divergence (Table 4.9). Out of ten characters 1000-grain weight (20.93%) contributed the highest for divergence followed by straw yield per plant (20.30%), number of productive tillers per plant (19.66%), days to panicle initiation (12.37%) and days to maturity (11.52%). However, number of panicles per plant (0.00%), plant height (0.53%) and days to 50% flowering (1.37%) contributed the lowest for divergence.

4.6 Molecular diversity

4.6.1 RAPD analysis

The genomic DNA's of 44 genotypes of foxtail millet were subjected for PCR amplification using 29 random decamer primers. It was observed that out of 29 primers, 19 primers showed polymorphisms (Table 4.10).The primers namely OPD 01, OPD 11, OPD 18, OPE 09, OPE 12, OPE13, OPE 17, OPL 11, OPL 15, OPL 16 did not amplify properly. A total of 135 bands were generated by amplification, out of which 123 were polymorphic with an average of 91.11 per cent polymorphism (Table 4.10). Maximum number of bands (11) was produced by primer OPM 10 and the least (4) were produced by primer OPA 03. The primers OPA 03, OPE 04, OPE 15, OPE 18, OPE 19, OPL 02, OPL 14, OPL 18, OPM 10, OPM 12, OPM 17, OPM 18 (Plate 1, 2 and 3) showed maximum polymorphism followed by primer OPK 09 (90%), OPD 5 (87.5%), OPM 14 (85.71%).While the

least polymorphism (62.5%) was shown by OPM 05 and OPM 20 followed by OPE 03 (77.77%).

Random operon primer OPA 03 amplified four RAPD bands whereas, OPE 15, OPE 18, OPE 19 generated five bands each. OPL 2 and OPL 18 produced six bands. OPE 4, OPL 14 and OPM 18 amplified seven fragments. OPM 12 and OPM 18 amplified eight bands each. All the bands generated by these primers were polymorphic i.e. with 100 per cent polymorphism.

OPD 05 random operon primer amplified eight RAPD markers. Out of which seven were polymorphic with 87.5 per cent polymorphism.

OPE 03 random operon primer amplified nine RAPD markers, out of which seven were polymorphic with 77.77 per cent polymorphism.

OPK 9 amplified with 10 RAPD markers; out of which one was monomorphic and nine were polymorphism with 90 per cent polymorphism.

OPM 05 amplified eight RAPD markers, out of these five were polymorphic and three were monomorphic with 62.50 per cent polymorphism. OPM 09 random operon primer amplified only six RAPD markers with 83.33 per cent polymorphism.

The primer OPM 14 amplified seven RAPD markers, out of which one were monomorphic and six were polymorphic with 85.71 per cent polymorphism.

OPM 20 random operon primer amplified eight RAPD markers, out of which three were monomorphic and five were polymorphic with 62.5 per cent polymorphism. The Jacquards' Similarity coefficient was estimated with NTSYS pc software package are presented in appendix IV. The Jacquards' similarity coefficient ranged from 0.374 to 0.964.

Maximum similarity was observed between KOFM 95 and KOFM 94 followed by KOFM 53 and KOFM 48, KOFM 66 and KOFM 65. A minimum similarity coefficient of about 0.374 was present between pairs KOFM 95 and KOFM 14, KOFM 94 and KOFM 37, KOFM 95 and KOFM 37, and KOFM 95 and KOFM 41.

The UPGMA based dendrogram generated using RAPD data is depicted in Figure 7. It was observed that three major clusters were formed, with 22 genotypes in one cluster, 20 genotypes in second cluster and two genotypes in third. Cluster I comprised of two sub clusters of which KOFM 1, KOFM 28, KOFM 29, KOFM 36, KOFM 33, KOFM 48, KOFM 53, KOFM 54, KOFM 51 and KOFM 52 were in a separate sub cluster, while KOFM 2, KOFM 6, KOFM 17, KOFM 14, KOFM 18, KOFM 24, KOFM 25, KOFM 37, KOFM 42, KOFM 41, KOFM 46 and KOFM 44 were in another sub cluster of cluster I.

The second cluster contained KOFM 55, KOFM 58, KOFM 64, KOFM 59, KOFM 61, KOFM 62, PS 4, KOFM 77, KOFM 79, KOFM 65, KOFM 66, KOFM 93, KOFM 73, GPUS 28, SIA 326, KOFM 90, KOFM 70, KOFM 88, KOFM 89 and KOFM 80 genotypes.

KOFM 94 and KOFM 95 genotypes were in third cluster.

4.6.2 ISSR analysis

The genomic DNA's of 44 genotypes of foxtail millet were subjected for PCR amplification using 20 Inter simple sequence repeats (ISSR) primers).It was observed that out of 20 primers used, 12 primers showed polymorphism (Plate 4 and 5). A total of 77 bands were generated by amplification out of which 72 were polymorphic with an average of 93.50 per cent polymorphism (Table 4.11). Maximum numbers of bands (9) were produced by primer ISSR 810 and ISSR 823 and the least (4) were produced by primer ISSR 811. Out of 12 primers, nine showed 100 percent polymorphism. Whereas,

primers ISSR 807, ISSR 808 and ISSR 809 showed 80%, 75%, and 66.66% polymorphism, respectively.

ISSR 807 primer amplified five markers, out of which four were polymorphic bands and one1 were monomorphic with 80 per cent polymorphism.

ISSR 808 primer amplified eight markers. Out of which six were polymorphic bands and two were monomorphic with 75 per cent polymorphism.

ISSR 809 primer amplified six RAPD markers out of which two were monomorphic and four were polymorphic bands with 66.66 per cent polymorphism.

ISSR 820 and ISSR 8885, ISSR 810 and ISSR 823 amplified five and nine markers, respectively. The primers ISSR 811, ISSR 826, ISSR 880 and ISSR 834 generated four, six, seven and eight markers, respectively. All the markers amplified were polymorphic with 100 per cent polymorphism.

The Jaccards similarity coefficients were estimated with NTSYS pc software package are presented in appendix V. The Jaccards similarity coefficients ranged from 0.358 to 0.987. Maximum similarity was observed between KOFM 94 and KOFM 95 followed by KOFM 59 and KOFM 61, KOFM 64 and KOFM 65. A minimum similarity coefficient about 0.358 was present between KOFM 61and KOFM 94, KOFM 94 and PS 4.

The UPGMA based dendrogram generated with 12 ISSR marker data using NTSYSpc program is depicted in Fig. 8. It was observed that three major cluster were formed, with 22 genotypes in one cluster, 20 genotypes in second cluster and 2 genotypes in third cluster. Cluster I comprised of two sub clusters. The first sub cluster of cluster I contained KOFM 1, KOFM 2, KOFM 6, KOFM 36, KOFM 37, KOFM 28, KOFM 29, KOFM 14, KOFM 48 and KOFM 51.While,

KOFM 17, KOFM 44, KOFM 52, KOFM 42, KOFM 53, KOFM 18, KOFM 25, KOFM 54, KOFM 24, KOFM 46, KOFM 33 and KOFM 41 were in another sub cluster of cluster I.

The cluster II contained KOFM 55, PS 4, KOFM 59, KOFM 61, KOFM 66, KOFM 62, KOFM 64, KOFM 65, KOFM 70, SIA 326, KOFM 90, GPUS 28, KOFM 93, KOFM 73, KOFM 88, KOFM 89, KOFM 58, KOFM 77, KOFM 79 and KOFM 80 genotypes and Cluster III comprised KOFM 94 and KOFM 95 genotypes.

Table 4.11 Per cent polymorphism shown by different ISSR primers

Sr. No.	Primer	Total number of band generated	Total number of monomorphic	Total number of polymorphic	Per cent Polymorphism (%)	PIC Values
1	ISSR 807	5	1	4	80.00	0.466
2	ISSR 808	8	2	6	75.00	0.816
3	ISSR 809	6	2	4	66.66	0.725
4	ISSR 810	9	0	9	100.00	0.847
5	ISSR 811	4	0	4	100.00	0.702
6	ISSR 817	5	0	5	100.00	0.749
7	ISSR 820	5	0	5	100.00	0.703
8	ISSR 823	9	0	9	100.00	0.839
9	ISSR 826	6	0	6	100.00	0.732
10	ISSR 834	8	0	8	100.00	0.745
11	ISSR 880	7	0	7	100.00	0.779
12	ISSR 885	5	0	5	100.00	0.703
	Total	77	5	72	93.50	--

5. DISCUSSION

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is one of the oldest cereal crops contributed to human civilization not only in the past, but also still used as a staple food in China and India (Wang *et al.*, 2009). Its importance has continually decreased in the last 80 years (Austin, 2006) mainly because of poor seedling establishment, need for hand weeding and lack of breeding efforts to improve yield (Ahanchede *et al.*, 2004) beside lack of proper utilization of existing genetic resources.

In general, foxtail millet is valued as a short duration crop which is fairly resistant to insect pests and diseases (Upadhyaya *et al.*, 2008), adaptable to varied soil and environmental conditions due to its high photosynthesis and water use efficiency. It has high nutritional and medicinal value and also it has low glycemic index (GI), which makes it as an ideal food for people suffering from diabetes (Anju and Sarita, 2010; Thathola *et al.*, 2010). Foxtail millet grains contain vitamins B1, B2, B6, C and E (Coulibaly and Chen, 2011). It can be cooked and eaten like rice, either as whole grain or broken; flour used for making porridge and puddings. Foxtail millet is also used as bird feed for feeding cage birds and the by-product of the foxtail millet is used as animal feed extensively in China (En *et al.*, 2008). At present, foxtail millet is regaining its value and emerging as important crop after realizing the nutritional and health benefits and adaptability to changing climate.

In view of the several merits of this crop and very limited research undergone, there is a need for the study of genetic and molecular diversity for its effective utilization in development of improved cultivars. Therefore, the present investigation was formulated to understand the genetic variability and divergence in the genotypes for different traits in foxtail millet and to study the

molecular diversity present in the genotypes by using RAPD and ISSR markers. The results obtained on genetic and molecular diversity aspects are discussed under following headings.

5.1 Variability studies

Progress in any crop improvement program depends mainly on the variability existing in the metric traits of the base population. Genetic variability studies provide basic information regarding the genetic properties of the population based on which breeding methods are formulated for further improvement of the crop. These studies are also helpful to know about the nature and extent of variability that can be attributed to different causes, sensitive nature of the crop to environmental influences, heritability of the characters and genetic advance that can be realized in practical breeding. Hence, to have a comprehensive idea, it is necessary to have an analytical assessment of yield components.

5.1.1 Range of variability and genetic parameters

Based on analysis of variance for various characters the mean sum of squares due to treatments was significant for all the characters.

The variability studied among 44 genotypes indicated the presence of good amount of variation for all the characters studied. Variability observed for grain yield per plant ranged between 6.00g to 20.32g. Likewise, other characters showed wide range of variation as, days to panicle initiation (45.50-71.17days), days to 50 per cent flowering (57.00-79.00 days), days to maturity (90.00-123.00days), number of productive tillers per plant(0.35-3.92), plant height (109.00-184.00cm), number of panicles per plant(1.02-5.70), panicle length (8.27-22.42 cm), 1000-grain weight (1.07-3.48 g), straw yield per plant(13.80-42.35 g), protein content(7.08-13.75%) and iron content (0.03-0.11%). Seetharam *et al.* (1983), Kumar and

Parmeswaran (1998) reported similar variation for protein content and Philip and Maloo (1996) for iron content in foxtail millet.

Among the genotypes studied, KOFM 6 for early panicle initiation, KOFM 1 for early flowering and KOFM 79 for early maturity, KOFM 48 for number of productive tillers per plant, KOFM 93 for plant height, KOFM 14 for length of panicle, KOFM 95 for number of panicles per plant, KOFM 62 for 1000 grain weight, PS 4 for grain yield per plant, KOFM 70 for straw yield per plant, KOFM 65 for protein content and KOFM 53 for iron content recorded highest per se performance for respective characters. Similar reports were found by Cill and Randhawa (1975), Chidambaram and Palanisamy (1995), Reddy *et al.* (2006) and Nirmalakumari and Vetriventhan (2010).

Genotypes evaluated exhibited superior performance than the checks (PS 4 and SIA 326) for days to maturity (14), plant height (6), panicle length (19), 1000-grain weight (2), and straw yield per plant (2), protein content (2) and Iron content (10). Whereas, for days to panicle initiation, days to 50% flowering, number of productive tillers per plant, number of panicles per plant and grain yield most of the genotypes were on a par with the superior check.

5.1.2 Genotypic and phenotypic coefficients of variation

Genetic variability is the basis for any heritable improvement in the crop plants. Additive genetic variance is heritable portion of the total variation. Looking to the GCV and PCV indices of variability it was observed that PCV estimates were enormously greater than GCV for all characters revealing role environmental factor in expression of variation. This also suggested that in variability studies one should not rely upon phenotypes alone. It is always better to consider PCV and GCV together with highest magnitude of PCV and GCV. In the present study, the traits such as number of productive tillers per

plant, number of panicles per plant, grain yield per plant, straw yield per plant and iron content in E1, E2 and on pooled basis showed high estimates of PCV and GCV, and narrow difference between them in both the environments and for pooled data indicated the low effects of environments and greater role of genetic factors on the expression of these traits. Similar findings have been reported in foxtail millet for number of basal tillers (Nirmalakumari and Vetriventhan, 2010; Nirmalakumari et al., 2008; Reddy and Jhansilakshmi, 1991a; Cill and Randhawa, 1975), grain yield (Nirmalakumari and Vetriventhan, 2010; Basheeruddin and Sahib, 2004; Selvarani and Gomathinayagam, 2000b; Rathod, 1995; Cill and Randhawa, 1975) and iron content (Philip and Maloo, 1996).

Days to panicle initiation, plant height, panicle length, 1000 grain weight and protein content showed moderate PCV in E1, E2 and on pooled basis. Similar findings have been reported in foxtail millet for plant height (Nirmalakumari and Vetriventhan, 2010; Basheeruddin and Sahib, 2004; Selvarani and Gomathinayagam, 2000b; Reddy and Jhansilakshmi, 1991a), panicle length (Reddy and Jhansilakshmi, 1991a; Nirmalakumari and Vetriventhan, 2010; Lakshmanan and Guggeri, 2001), and 1000 grain weight (Cill and Randhawa, 1975; Reddy and Jhansilakshmi, 1991a).

5.1.3 Heritability and genetic advance

Heritability is a quantitative measure which provides information about the correspondence between genotypic variance and phenotypic variance, i.e., the ratio of variance due to heritable differences (σ^2g) to the total phenotypic variance (σ^2p) and expressed as per cent. Genetic coefficient of variation alone would not indicate proportion of total heritable variation. However, the heritability estimates are better indicators of heritable portion of the variation. The broad sense heritability percentage includes the contribution of

additive gene effects, allelic interactions due to dominance and non allelic due to epistasis. While, genetic advance provides knowledge about expected genetic gain for particular trait after selection.

When heritability is moderate to high with higher magnitude of expected genetic advance for a particular trait indicates the additive gene action and if the results are reversed and either of the conditions like high heritability coupled with low genetic advance is observed for any given trait, then presence of non-additive gene action may be suspected.

In the present study, all the traits viz., days to panicle initiations, days to 50 per cent flowering, Days to maturity, number of productive tillers per plant, panicle length, number of panicles per plant, 1000-grain weight, grain yield per plant, straw yield per plant, protein content and iron content showed the high estimates of broad sense heritability (h^2_b) indicating the reliability of the estimates for variation between genotypes and effectiveness of selection in this genotypes for these traits. Populations which are genetically more uniform are expected to show lower heritability than the genetically variable population. Also, more variable environmental condition reduces the magnitude of heritability and more uniform environmental condition increases it (Dabholkar, 1999). Hence, high heritability of these traits in this study may be due to highly variable and genetically diverse accessions due to uniform conditions within environments.

Since heritability is also influenced by environment, the information on heritability alone may not help in pin pointing characters enforcing selection. Nevertheless, the heritability estimates in conjunction with the predicted genetic gain will be more reliable (Johnson *et al.*, 1955). Heritability gives the information on the magnitude of inheritance of quantitative traits, while genetic advance will be helpful in formulating suitable selection procedures.

The grain yield and its components like days to panicle initiation, days to 50 per cent flowering, number of productive tillers, panicle length, number of panicles per plant, 1000-grain weight, grain yield per plant, straw yield per plant, protein content and iron content showed high genetic advance as per cent of mean coupled with high estimates of h^2_b indicating that, the variation are attributable to high level of heritable variation and selection would be effective for improvement of these traits. The high estimates of heritability coupled with high genetic advance as per cent of mean reported in the earlier studies in foxtail millet for days to panicle initiation (Cill and Randhawa, 1975), days to 50 per cent flowering (Nirmalakumari *et al.*, 2008), number of productive tillers per plant (Islam *et al*, 1990), panicle length (Nirmalakumari and Vetriventhan, 2010; Nirmalakumari *et al.*, 2008; Lakshmanan and Guggeri, 2001), 1000 grain weight (Nirmalakumari and Vetriventhan, 2010; Selvarani and Gomathinayagam, 2000b; Cill and Randhawa, 1975) , grain yield (Nirmalakumari and Vetriventhan, 2010; Nirmalakumari *et al.*, 2008; Lakshmanan and Guggeri, 2001; Selvarani and Gomathinayagam, 2000b) and iron content (Phillip and Maloo, 1996)

Days to maturity exhibited high heritability and low genetic advance. Similar result was reported by Cill and Randhawa, 1975.

5.2. Correlation studies

Understanding the interaction of the traits among themselves and with the environment is of great use in plant breeding. Correlation studies provide information on the nature and extent of association between any two quantitative traits and it would be possible for genetic enhancement of a trait through selection of a correlated trait (associated response). Grain yield is a complex character and jointly determined by a number of related traits. An insight into the association between grain yield and other correlated traits helps to

improve the efficiency of selection. In general, the correlation between yield and other characters as well as among the component characters will vary with the material handled by the breeder.

In the present investigation, the genotypic correlations were estimated among 12 quantitative traits that are closely related to grain yield in foxtail millet.

Out of 66 correlations, a total of 32 correlations were significant in E1 and 33 in E2 and pooled data which indicated the importance of the traits investigated in this study. The grain yield per plant was significantly and positively correlated with number of productive tillers per plant, panicle length, number of panicles per plant, 1000 seed weight, straw yield per plant and protein content. It could be inferred that, selection for high yield would be effective through selection for these traits. Besides these traits showed high heritability coupled with high genetic advance as per cent of mean, hence selection is desirable. Positive correlation of number of productive tillers per plant (Nirmalakumari and Vetriventhan, 2010; Rathod *et al.*, 1996) panicle length (Nirmalakumari and Vetriventhan, 2010; Murugan and Nirmalakumari, 2006, Santhakumar, 1999; Islam *et al.*, 1990), straw yield per plant (Murugan and Nirmalakumari, 2006; Santhakumar, 1999; Chidambaram and Palanisamy, 1995) reported in foxtail millet.

Days to panicle initiation, days to 50% flowering, days to maturity, were significant and negatively correlated with grain yield per plant. Similar reports were found for days to 50% flowering (Upadhyaya *et al.*, 2008; Cill and Randhawa, 1975) and days to maturity (Reddy and Jhansilakshmi, 1991b).

5.3. Path coefficient analysis

The correlation measured the relationship existing between pairs of traits. But dependent traits are an interaction product of many

mutually associated components. The path analysis takes into account the cause and effect relationship between the variables by partitioning the association into direct and indirect effects through other independent variables.

Grain yield is the product of interaction of component traits. Apart from correlation studies, path coefficient analysis is important to obtain information about different ways in which the component characters influences the grain yield.

In this study, the direct effect of 1000 grain weight on grain yield per plant (g) was positive and high in all the environments separately and in pooled analysis (0.378 in E1, 0.890 in E2 and 0.460 in pooled) which indicated the true relationship of this trait and a direct selection through this trait will be effective. The indirect effect of number of panicles, panicle length (cm), number of productive tillers and straw yield through 1000-grain weight was positive and moderate to high. It can be inferred that, the direct selection of 1000-grain weight in foxtail millet lead to simultaneous indirect selection of number of panicles, panicle length (cm), number of productive tillers and straw yield for increased grain yield per plant. Hawlader and Hamid (1988) also reported the highest direct effect of 1000-grain weight on grain yield.

The residual effect determines how best the causal factors account for the variability of the dependent factors, yield in this case. Its estimate being 0.385 in E1, 0.296 in E2 and 0.360 pooled, explained about 61.50 per cent of variability in the yield in E1, 70.40 per cent in E2 and overall 64 per cent on pooled basis. This indicated that, the reasonable proportion of the variability was captured in foxtail millet germplasm. The residual variance was low in both the environments as well on pooled basis which indicated the importance

of the characters taken in this study and accounted more variation for grain yield.

5.4 Genetic diversity (D^2)

The most important and difficult task is initiation of hybridization programme by selecting genotypes with high per se performance for yield and yield contributing components with suitable genetic divergence among them. From the genetic variability estimates it would be possible to identify desirable genotypes but unless we have sound knowledge about average between them it's difficult to expect any extra ordinary results from their progeny.

D^2 statistics a concept developed by Mahalanobis (1936) is important tool to plant breeder. It is a useful tool to study the degree of divergence between biological population at genotypic level and to assess the relative contribution of different components to the total divergence at both intra and inter-cluster level. Rao (1952) suggested the application of this technique for the assessment of genetic diversity in plant breeding.

The basic idea behind formation of clusters is to get the intra and inter-cluster distances. This serves as index for selection of parents with diverse origin. The intra and inter-cluster values are means derived from D^2 values of cluster elements. The crossing between the genotypes placed in clusters with large inter cluster distance will be more correct approach to get desirable results.

Analysis of variance revealed highly significant differences among the genotypes for all the characters. The estimates of D^2 values ranged from 1.90 to 610.09. The minimum D^2 value was noticed between the pair of genotypes KOFM 55 and GPUS 28, while highest between KOFM 36 and KOFM 94. This indicated the relationship

between genotypes and individual distances from each other with clustering pattern.

Cluster formation

The aim of cluster formation and finding out intra and inter-cluster divergence is to provide the basis for selecting parents for hybridization programme. The theoretical concept behind such grouping is that the genotypes grouped into the same cluster presumably are less diverse from each other than those belonging to different clusters (Rao, 1952) and thus crossing between the genotypes belonging to the same clusters will not give expected desired heterotic response and desired segregants in further generations. Consequentially breeding programme should be so diverse that the parents selected for crossing should be from different clusters. Greater is the distance between the two clusters, wider is the genetic diversity in the genotypes.

The six clusters formed in the present study indicated that the available genotypes possess variability for different characters under study. The cluster I had larger number of 37 genotypes followed by cluster II and V with 2 genotypes each, cluster III, IV and VI were solitary. Wide range of diversity was also reported by Murugan and Nirmalakumari (2006) in foxtail millet they grouped seventy five genotypes into nine clusters, Selvarani and Gomathinayagam (2000a) grouped fifty genotypes into six clusters, Maloo and Bhattachargee (1999) grouped forty genotypes into four clusters, Shriff and Shivashankar (1992) grouped 225 genotypes into 33 clusters. Nagrajan and Prasad (1980) grouped fifty genotypes into fifteen clusters. In D^2 analysis scattering of genotypes due to heterogeneity, genetic architecture of the population, past history of selection for development and degree of good combining ability of the parents evolved in particular genotype (Murty and Arunachalam, 1966).

Genotypes falling between cluster II and cluster VI exhibited maximum inter cluster distance, (D^2 =594.74) followed by cluster II and cluster IV (D^2 =338.36) and cluster II and cluster III (D^2 =319.28) indicating that genetic makeup of genotypes falling in these clusters may be entirely different from one another. The clustering pattern has clearly suggested the wide diversity between the genotypes.

The maximum intra-cluster distance was observed for the genotype falling in cluster V (D^2 =26.93) followed by cluster I and cluster II. This implies that these clusters have the genotype with varied genetic architecture, while other clusters showed zero intra-cluster distance due to monogenotypic nature.

The cluster mean presented in Table 4.8 revealed that, cluster II and cluster VI varied considerably for most of the characters from those clusters. This indicates that the genotypes included in these clusters (KOFM 94, KOFM 95 and KOFM 36) might have entirely different genetic architecture from the genotypes included in the other clusters. Similarly, cluster I and II had more inter cluster distance suggesting high divergence among the genotypes included in these two clusters. The characters viz., 1000-grain weight (g), straw yield per plant (g), number of productive tillers plant, days to panicle initiation and grain yield per plant (g) appeared as the major forces of differentiation. Murugan and Nirmalakumari (2006) also observed highest contribution of straw yield per plant, days to maturity and 1000 grain weight towards divergence. Similar findings were reported by Sheriff (1992), Maloo and Bhattachargee (1999).

The results obtained in the present investigation indicated that yield indicators responsible for divergence varied substantially and may be attributed to differences in the genetic constitution of material and the environment in which they were grown.

Taking into account the cluster mean for grain yield components, the various clusters and respective genotypes which can provide the desired parents for hybridization programme for improvement in the particular characters (shown against them) are listed below :

Characters	**Source (Cluster)**	**Genotypes**
1000 grain weight (g)	I, IV, V	KOFM 59, KOFM 93, KOFM 89
Straw yield per plant (g)	I, IV, VI	KOFM 1, KOFM 90, KOFM 36
No. of productive tillers per plant	I , II	PS 4, KOFM 94
Days to panicle initiation	I, III, VI	KOFM 46, KOFM 64, KOFM 88, KOFM 36
Days to maturity	I, III, VI	KOFM 51, KOFM 88, KOFM 36
Grain yield per plant (g)	I, V	KOFM 37, KOFM 59, PS 4, KOFM 89

On the basis of inter-cluster distances, cluster means and the per se performance observed in the present study the genotypes KOFM 36, KOFM 59, KOFM 88, KOFM 89 and PS 4 were found superior to be suitable for crop improvement.

In general most of the genotypes were grouped in to a single large cluster indicating the similarity among them and the low variability. However a few accessions were remarkably different from rest of the genotypes for the characters like plant height, productive tillers per plant, seed size, panicle length, panicle shape (Plate 6 and Plate 7).

5.5 Molecular diversity by RAPD and ISSR markers

Understanding the distribution of genetic diversity among individuals, populations and gene pools is crucial for the efficient management of germplasm collections and breeding programs. Diversity analysis is routinely carried out using sequencing of selected gene(s) or by molecular markers. The DNA based markers are promising and effective tools for measuring genetic diversity in plant germplasm and for elucidating their evolutionary relationships (Pervaiz *et al.*, 2009). Germplasm characterization based on molecular markers has gained importance due to the speed and quality of data generated. It would also provide information on the population structure, allelic richness, and diversity parameters to help breeders use genetic resources for cultivar development more effectively.

Genetic diversity is still studied in many crops on the basis of morphological markers, however availability of special markers is lacking in many cultivars. The mapping of these is also tedious and time consuming (Akhare *et al.*, 2008). Further, biochemical marker like proteins and isoenzymes were used but it has less polymorphism and also influenced by environmental factors. This has shifted a focus to DNA based molecular markers. Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. Application of molecular markers developed during last few decades which overcome phenotype based markers. The technology like use of RAPD which has advantages for its simplicity, rapidity, required small quantity of DNA and ability to generate more polymorphism (Cheng *et al.*1997). Thus, it has been proved as powerful and useful tool for genetic analysis (Chapco, 1992)

In contrast to morphological markers, which are based on visible traits and biochemical markers which are based on proteins

produced by genes, molecular markers rely on DNA assay. DNA based genetic markers are being increasingly utilized in cultivar development, quality control of seed production, measurement of genetic diversity for conservation management, varietal identification and intellectual property protection.

There are different kinds of molecular markers like restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), microsatelite and single nucleotide polymorphisms (SNPs). PCR based method like RAPDs are increasingly being used in the analysis of genetic diversity because of the relative ease with which PCR assay are carried out and also the prior knowledge about the genome is not known.

Very limited molecular diversity analyses have been reported in foxtail millet. In a genetic diversity study using RFLPs, Fukunaga *et al.* (2002) found that foxtail millet landraces have differentiated genetically between different regions and that Chinese landraces were highly variable. This is in contrast to the results obtained by de Wet *et al.* (1979) and Jusuf and Pernes (1985), who reported that Chinese cultivars were uniform for storage protein and enzyme alleles.

A few of the earlier workers had used RAPDs for the analysis of genetic diversity in foxtail millet (Li *et al.* 1998; Schontz and Rether 1999). Since codominant marker systems such as SSRs were not available in foxtail millet, this analysis was undertaken using the random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers techniques.

Random amplified polymorphic DNA markers (Williams *et al.*, 1990) have been successfully used for species identification in most of the plants due to technical simplicity and speed of RAPD technology (Gepts, 1995). The degree of polymorphism detected by different

primers varied and thus there was considerable variation in the ability of individual primer to detect DNA polymorphism. Molecular markers can be used to better document genetic diversity between possible parental material of breeding programme to accelerate the individual selection that combine favorable alleles and to establish the distinctness (Charcosset and Gallais, 2002).

ISSR PCR is technique that overcomes most of limitations of RAPD, AFLP, SSR or microsatellite markers (Zietkiewicz *et al.*, 1994; Gupta *et al.,* 1994; Wu *et al.,* 1994; Meyar *et al.,* 1993). In this method SSRs are used as primers to amplify mainly the inter SSR regions (Reddy *et al.,* 2002). ISSR have been successfully used to estimate the extent of genetic diversity at inter and intra-specific level in wide range of crop species which include rice (Joshi *et al.*, 2000), wheat (Nagaoka and Ogihara., 1997), fingermillet (salimath *et al.*, 1995), vigna (Ajibade *et al.*, 2000) and sweet potato (Huang and Sun, 2000).

Out of 29 RAPD and 20 ISSR primers surveyed 19 RAPD and 12 ISSR primers were selected for the present study based on the extent of polymorphism observed in the amplicons. The amplification obtained with RAPD and ISSR primers was good and consistent. A total of 212 scoreable amplification products (135 RAPD and 77 ISSR) were generated. The number of amplicons generated by each primer varied from four (OPA 03) to eleven (OPM 10) for RAPDs with an average of 7.1 amplicons per primer and four (ISSR 807, ISSR 809 and ISSR 811) to nine (ISSR 810 and ISSR 823) for ISSRs. Average number of alleles generated by ISSR was 6.41. The average number of polymorphic bands amplified for each primer (7.1) recorded in the present study were comparable with the earlier reports in foxtail millet by Li *et al.*, (1998) (7.78); Schontz and Rether, (1998) (6.25); Ratna Kumari *et al.*, (2011) (7.18) in foxtail millet and Fakrudin *et al., (*2004) (6.86); Kumari and Pande, (2010) (6.64) in finger millet. While Gupta

et al. (2010) reported 8.5 bands per primer in finger millet which were higher than that obtained in the present study.

Percent polymorphism shown by RAPD primers varied from 62.50 to 100 %. It was found that, total 135 bands were generated by amplification out of which 123 were polymorphic with an average of 91.11 % polymorphism. Similar results were found by Li et al (1998) using 19 RAPD primers in 20 accession of foxtail millet with 72.80 % polymorphism. Fakrudin *et al.* (2004) using RAPD primers among 12 selected finger millet accessions and reported 85.82% polymorphism. Similarly, Kalyan Babu *et al.* (2006) have shown 91 per cent polymorphism among 32 finger millet accessions using 50 RAPD primers. In ISSR analysis out of 77 bands, 72 were polymorphic with an average of 93.50 percent polymorphism. Ratna Kumari *et al.* (2011) also reported similar values for percent polymorphism (37.5 to 100) using RAPD and ISSR markers in foxtail millet.

The polymorphic information content (PIC) value as a relative measure of polymorphism level ranged between 0.440 (OPA 03) to 0.882 (OPK 09) in RAPD and it was ranged between 0.466 (ISSR 807) to 0.847 (ISSR 810) for ISSR.

The average PIC value for RAPD (0.74) and ISSR (0.73) was higher than that of Jia *et al.,* (2009) (0.69 for SSR) in foxtail millet and Panwar *et al., (*2010) (0.35 and 0.505 for RAPD and SSR, respectively) and Gupta et al., (2010) (0.51 and 0.19 for RAPD and ISSR, respectively) in finger millet. It was similar to that of Liu *et al.,* (2011) (0.72 for SSR) in foxtail millet.

The higher PIC value indicated the informativeness of the primer. Among the primers used in the study three primers each from RAPD (OPK 09, OPM 10 and OPM 05) and ISSR (ISSR 810, ISSR 823 and ISSR 808) exhibited the PIC values from 0.882 to 0.816. These primers can provide the basis for foxtail millet DNA profiling system. To

examine the genetic relationship among the 44 foxtail millet genotypes under study based on the RAPD and ISSR results, the data scored from 19 RAPD and 12 ISSR primer were compiled and analyzed separately using NTSYSpc programme (Rohlf,1997). The similarity matrix was computed using RAPD and ISSR markers based on Jaccard's coefficient. The genetic similarity matrices based on the Jaccard's coefficient ranged from 0.374 to 0.964 and 0.35 to 0.98 for RAPD and ISSR, respectively. The genetic similarity matrix also revealed that the KOFM 95 and KOFM 14, KOFM 94and KOFM 37 and KOFM 41 were distantly related which was indicated from the lowest genetic similarity coefficient (0.374), while KOFM 95 and KOFM 94 were closely related with a genetic similarity coefficient of 0.964.

Interestingly, the dendrograms generated based on UPGMA method of cluster analysis using RAPD and ISSR marker data revealed exactly similar grouping of genotypes into three major clusters (Fig.7 and Fig. 8, respectively). Cluster I comprised of two sub clusters of which KOFM 1, KOFM 28, KOFM 29, KOFM 36, KOFM 33, KOFM 48, KOFM 53, KOFM 54, KOFM 51 and KOFM 52 were in a separate sub cluster, while KOFM 2, KOFM 6, KOFM 17, KOFM 14, KOFM 18, KOFM 24, KOFM 25, KOFM 37, KOFM 42, KOFM 41, KOFM 46 and KOFM 44 were in another sub cluster of cluster I. The second cluster contains KOFM 55, KOFM 58, KOFM 64, KOFM 59, KOFM 61, KOFM 62, PS 4, KOFM 77, KOFM 79, KOFM 65, KOFM 66, KOFM 93, KOFM 73, GPUS 28, SIA 326, KOFM 90, KOFM 70, KOFM 88, KOFM 89 and KOFM 80 genotypes. KOFM 94 and KOFM 95 genotypes were in third cluster.

The distribution of the genotypes in the dendrogram was mostly consistent with the known pedigree information and the morphological attributes of the genotypes. The close grouping between KOFM 94 and KOFM 95 which may be attributed to the selections of these genotypes

from the closely related parents Sie 1598 and Sie 1599. These two genotypes exhibited high similarity value (0.967).

To sum up, considerable diversity existed among the foxtail millet accessions. This study identified diverse genotypes e.g. KOFM 95, KOFM 14, KOFM 94, KOFM 37 and KOFM 41 for use in hybridization program for foxtail millet improvement.

Grouping of genotypes under study based on morphological diversity (D^2) and RAPD and ISSR analysis revealed concurrence for some clusters. For instance, the genotypes KOFM 94 and KOFM 95 were grouped together in a separate cluster, indicating similarity among them at morphological and molecular level. Moreover, most of the genotypes grouped in to single large clusters at morphological and also at molecular level.

There were some differences in grouping of a few genotypes. Such differences between morphological and molecular diversity may be due to screening or use of limited number of RAPD and ISSR markers and less number of genotypes selected for fingerprinting. However, from our results it is observed in some genotypes show similar trend in both studies of diversity which shown in Table 5.1.

Table: 5.1 Reflection of diversity at field and molecular level

Sr. No.	Grouping of genotypes at field level	Grouping of genotypes at molecular level
1	KOFM 94, KOFM 95	KOFM 94, KOFM 95
2	KOFM 89, KOFM 90	KOFM 89, KOFM 90
3	PS 4, GPUS28, SIA 326	PS 4, GPUS28, SIA 326

To summerise the genotypes from diverse clusters may be inter-crossed to generate higher variability. Hence genotypes KOFM 1, KOFM 14 and KOFM 36 (Cluster I) can be crossed with KOFM 89,

KOFM 90 (Cluster II), KOFM 94 and KOFM 95 (cluster III) to create more variability.

Future line of work

Based on the results obtained in the present study and information available, the following future line of work can be proposed:

1. Most of the foxtail millet accessions used in this study were found to be similar at both morphological and molecular level. But some of the genotypes were highly diverse and possesses potential variation for economic traits. Hence, these can be used effectively for the development of genetically diverse and improved cultivars.

2. The superior most diverse accessions identified could be utilized in breeding programs to improve and to widen the genetic base of foxtail millet cultivars. Accessions with multiple superior traits could be utilized for simultaneous transfer of multiple traits/genes in crop improvement.

3. Most diverse pair of accessions identified based on phenotypic traits and molecular markers could be utilized for the development of mapping population and for the selection of superior lines in segregating generation.

4. In general both D^2 and molecular analysis suggested KOFM 14, KOFM 36, KOFM 88, KOFM 89, KOFM 90, KOFM 93, KOFM 94 and KOFM 95 were the most diverse genotypes which can be used in crossing programme.

6. SUMMARY AND CONCLUSIONS

Genetic and molecular characterization and identification of genetically diverse sources is important for enhanced utilization of foxtail millet genetic resources in development of improved cultivars. Hence, the current study was undertaken to understand the genetic variability and divergence in the genotypes for different traits in foxtail millet and to study the molecular diversity present in the genotypes by using RAPD and ISSR markers.

The genetic materials used in this study were 44 accessions of foxtail millet that were evaluated in two seasons. In both the environments, the experiment was conducted in RBD with three replications. The data on 12 quantitative traits were recorded. For the molecular diversity study, a total of 19 RAPD and 12 ISSR markers were used. The results are summarized below.

6.1 Variability studies

- ANOVA for individual environments and on pooled basis environments indicated that variance components due to genotype (σ^2_g) and genotype X environment (σ^2_{ge}) interaction were significant for all the traits. This indicated sufficient variability for all the traits in foxtail millet accessions.
- The wider range was observed for most of the characters in both environments. Means of almost all characters did not differ significantly in two environments indicating less influence of the environment on the expression of these traits.
- The estimates of genotypic as well as phenotypic coefficients of variation were high for number of productive tillers per plant, number of panicles per plant, grain yield per plant, straw yield per plant and iron content and narrow difference between them in both the environments and for pooled data indicated the low effects of

environments and greater role of genetic factors on the expression of these traits.

- All the traits except plant height had the maximum heritability in E1, E2 and on pooled basis. This indicated the importance of these characters in contributing toward divergence. High genetic advance as per cent of mean coupled with high estimates of broad sense heritability (h^2_b) (>60%) were observed in both environments separately and pooled data indicating that, the variation for most traits were heritable variation and selection would be effective for improvement of these traits.

6.2 Correlations and Path analysis

- Grain yield per plant (g) was highly significant and positively correlated with number of productive tillers per plant, panicle length, number of panicles per plant, 1000-grain weight, straw yield per plant in both E1, E2 and also in pooled and protein content in E2 and pooled . It could be inferred that, the selection in positive direction for these traits with grain yield per plant (g) can be practiced for genetic enhancement of grain yield in foxtail millet.
- The path coefficient analysis revealed that 1000-grain weight had the highest positive direct effects on grain yield per plant. The indirect effect of number of panicles, panicle length (cm), number of productive tillers and straw yield through 1000-grain weight was positive and moderate to high. It can be inferred that, the direct selection for 1000-grain weight in foxtail millet will lead to simultaneous indirect selection for number of panicles, panicle length (cm), number of productive tillers and straw yield for increased grain yield per plant.

6.3 Diversity analysis

- In D^2 analysis 44 genotypes were grouped into six clusters. Maximum D^2 value was observed between KOFM 36 and KOFM 94. This suggested that, KOFM 36 and KOFM 94 were genetically most divergent. Cluster I comprised maximum number of genotypes (37), cluster II and V with 2 and all other were solitary. The inter-cluster distance was maximum between cluster II and cluster VI followed by between cluster II and cluster IV. The intra cluster distance was maximum for genotypes falling in cluster V followed by cluster I and II.
- The 1000 grain weight showed highest percentage of contribution towards divergence. The traits straw yield per plant and number of productive tillers per plant also had substantial contribution in diversity.
- The cluster means informed that the genotypes in cluster I and III were earliest to panicle initiation, 50% flowering and earliest to mature, genotypes in cluster IV and VI were tallest for plant height at maturity, genotypes in cluster II were superior for productive tillers and number of panicles per plant. Maximum length of panicle recorded in cluster V. Highest grain yield and straw yield per plant exhibited the genotypes in cluster I and V.
- In general most of the genotypes were grouped in to a single large cluster indicating the similarity among them and the low variability.

6.4 Molecular diversity analysis

- A total of 29 RAPD and 20 ISSR markers were used to genotype the foxtail millet germplasm. Of these, 19 RAPD and 12 ISSR markers produced clear, scorable and polymorphic marker profiles and were used for further analysis.

- The amplification obtained with RAPD and ISSR primers was good and consistent. A total of 212 scoreable amplification products (135 RAPD and 77 ISSR) were generated. The number of amplicons generated by each primer varied from four (OPA 03) to eleven (OPM 10) for RAPDs with an average of 7.1 amplicons per primer and four (ISSR 807, ISSR 809 and ISSR 811) to nine (ISSR 810 and ISSR 823) for ISSRs. Average number of alleles generated by ISSR was 6.41.
- Percent polymorphism shown by RAPD primers varied from 62.50 per cent to 100 per cent. In ISSR analysis out of 77 bands, 72 were polymorphic with an average of 93.50 percent polymorphism. The average PIC value of 0.74 and 0.73 was revealed by RAPD and ISSR markers, respectively.
- The higher PIC value indicated the informativeness of the primers. Among the primers used in the study three primers each from RAPD (OPK 09, OPM 10 and OPM 05) and ISSR (ISSR 810, ISSR 823 and ISSR 808) exhibited the PIC values from 0.882 to 0.816. These primers can provide the basis for foxtail millet DNA profile system.
- The genetic similarity matrices based on the Jaccards coefficient ranged from 0.374 to 0.964 and 0.35 to 0.98 for RAPD and ISSR, respectively. The genetic similarity matrix also revealed that the KOFM 95 and KOFM 14, KOFM 94 and KOFM 37 and KOFM 41 were distantly related which was indicated from the lowest genetic similarity coefficient (0.374), while KOFM 95 and KOFM 94 were closely related with a genetic similarity coefficient of 0.964.
- The dendrograms generated based on UPGMA method of cluster analysis using RAPD and ISSR marker data revealed exactly similar grouping of genotypes in to three major clusters.
- The distribution of the genotypes in the dendrogram was mostly consistent with the known pedigree information and the

morphological attributes.

- The genotypes e.g. KOFM 95, KOFM 14, KOFM 94, KOFM 37 and KOFM 41 were identified for use in hybridization program for foxtail millet improvement.
- Grouping of genotypes under study based on morphological diversity (D^2) and RAPD and ISSR analysis revealed concurrence for some clusters.

Conclusions

- High genotypic coefficient of variation among the genotypes was observed for number of productive tillers per plant, number of panicles per plant, grain yield per plant, straw yield per plant and iron content.
- High heritability accompanied with high genetic advance as percentage of mean indicated additive gene action in the inheritance of days to panicle initiation, days to flowering, number of productive tillers per plant, length of panicle, number of panicles per plant, 1000 grain weight, straw yield per plant, protein content and iron content.
- KOFM 65, KOFM 36, KOFM 28, KOFM 80, KOFM 18 recorded high protein content. These genotypes provide a source for improvement of this trait along with high yield in foxtail millet. Similarly, KOFM 53, KOFM 51, KOFM 14 and KOFM 59 had the high iron content.
- Evaluation of germplasm revealed the low level of diversity as indicated by grouping of most of the genotypes in single large cluster. This suggested the greater amount of similarity among most of the genotypes. However some of the genotypes were totally diverse and exhibited great scope for generating variation for the important economic traits in foxtail millet.

- RAPD and ISSR analysis revealed that maximum genetic diversity was observed between genotypes viz., KOFM 1, KOFM 14, KOFM 36, KOFM 61, KOFM 89, KOFM 90 and KOFM 94 .Thus these genotypes can be considered as parents of interest and crossed with elite material to develop new breeding population in foxtail millet.
- Grouping of genotypes based on their phenotypic performance data was mostly similar to that based on RAPD and ISSR markers. However molecular markers gave more accurate and detailed grouping of the genotypes studied.

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8. APPENDICES

Appendix-I

DETERMINATION OF PROTEIN

The protein content was determined by Micro Kjeldahl method (A.O.A.C., 1990)

Reagents

1. Concentrated sulphuric acid (sp.gr. 1.84, N-free)
2. Catalyst moisture – potassium sulphate (99 g), mercuric oxide (4.1g) and copper sulphate (0.89g) were weighed and mixed thoroughly. .
3. Sodium hydroxide (50% w/v) – Sodium hydroxide pillets (50g) and sodium thiosulphate (5g) were dissolved in distilled water separately, mixed and the volume was made to 100 ml.
4. Boric acid (4%, w/v)- Boric acid (4g) was dissolved in distilled water and the volume was made to 100 ml.
5. Hydrochloric acid (0.02N)- 0.177 ml of hydrochloric acid (sp. gr.,1.18, 35%) was dissolved in distilled water and the volume was made to 100 ml.
6. Hydrogen peroxide (30%,v/v).
7. Mixed indicator – mixed indicator was prepared by dissolving bromocresol green (0.1g) and methyl red (0.1g) in 100 ml of 95 percent (v/v) ethyl alcohol separately. Bromocrecol green solution (10 parts) and methyl red solution (2 parts) were mixed together and transferred to a bottle provided with stopper.

Procedure

Powdered sample of the grains (200mg) of each of the foxtail millet cultivars was accurately weighed and transferred carefully to digestion flasks, to which the catalyst (1g) was added and thoroughly mixed with the sample. Concentrated sulphuric acid (5 ml) and hydrogen peroxide (5ml) were carefully added and sample was digested in digestion chamber. Initially, the flasks were heated slowly for 10 to 15 min and then the temperature was raised gradually so that the contents boiled briskly. The digestion was continued until the sample became clear and colourless. The flasks were then cooled and minimum quantity of water was added to dissolve the solids in the flasks. After cooling, the contents were washed 3 to 4 times and all the washings were transferred to volumetric flask and the volume was made to 50ml.

Boric acid solution (10 ml) was pipetted into a 150ml beaker and 6 to 8 drops of mixed indicator solution were added to it. The beaker was placed under a condenser of the distillation assembly. Care was taken to ensure that the tip of the condenser dipped below the surface of the solution. Digest (5 ml) was pipetted into distillation flask and mixed with 10 ml sodium hydroxide solution (50 %, w/v). The distillation was continued to collect about 50 ml of the distillate and at the end of distillation, the tip of the condenser was washed to collect all the ammonia. So also confirmation of distillation was made with the help of litmus paper. The distillate was then titrated with standard hydrochloric acid solution. Before distillation, the colour of the boric acid plus indicator was pinkish red which changed to blue green during distillation and finally to pinkish red at the end of the titration with standard hydrochloric

acid solution. Simultaneously, blank titration was carried out each time. The percentage of nitrogen content was calculated from volume of standard hydrochloric acid required for titration. The protein content was calculated by multiplying the nitrogen content by a factor of 5.7 (A.O.A.C.1990).

Appendix-II

Mean performance of forty four genotypes of foxtail millet for various characters in E1 environment

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
1	KOFM1	45.33	56.67	98.00	3.90	147.17	19.47	4.90	2.42	18.47	41.41	9.81	0.056
2	KOFM2	46.33	59.33	96.00	2.37	128.57	18.37	3.37	2.50	15.07	28.45	9.24	0.045
3	KOFM6	43.67	56.67	93.67	1.57	125.87	18.90	2.57	2.38	13.03	21.69	10.53	0.057
4	KOFM14	47.33	60.67	93.00	2.30	145.97	22.97	3.30	2.74	19.00	27.89	10.95	0.064
5	KOFM17	45.33	57.00	93.00	1.80	141.37	17.47	2.80	2.71	12.83	23.66	6.97	0.046
6	KOFM18	44.67	57.00	93.33	2.10	150.10	22.00	3.10	2.73	19.10	26.20	12.10	0.051
7	KOFM24	45.33	57.67	97.33	3.50	127.33	17.23	4.50	3.26	22.13	38.03	11.95	0.042
8	KOFM25	44.00	54.00	96.00	2.73	142.07	19.63	3.73	2.90	16.40	31.55	11.21	0.049
9	KOFM28	44.00	56.67	95.00	1.60	142.13	19.70	2.60	2.66	14.70	21.97	12.08	0.048
10	KOFM29	48.00	60.67	94.00	2.90	141.83	19.53	3.90	2.97	19.87	36.88	10.12	0.052
11	KOFM33	46.00	56.67	97.33	2.60	130.80	17.53	3.60	2.76	16.30	34.01	8.53	0.035
12	KOFM36	48.67	60.67	96.33	1.93	185.23	19.23	2.93	2.90	16.53	48.16	12.43	0.039
13	KOFM37	46.67	57.67	96.67	3.17	133.60	18.80	4.17	3.51	23.20	34.73	10.85	0.037
14	KOFM41	44.67	57.00	90.67	2.33	145.67	19.70	3.33	2.90	15.43	37.87	10.39	0.057
15	KOFM42	44.67	57.00	96.67	2.77	133.73	18.93	3.77	2.71	16.23	34.77	9.23	0.058
16	KOFM44	45.00	58.00	92.67	2.87	125.30	17.30	3.87	3.00	18.73	32.58	10.53	0.043
17	KOFM46	43.00	57.00	94.67	2.87	123.60	15.90	3.87	3.15	17.87	31.55	10.38	0.048
18	KOFM48	46.67	58.33	93.00	1.83	130.20	19.97	2.83	2.65	17.13	20.83	10.39	0.045
19	KOFM51	43.00	56.00	95.67	1.60	143.00	17.03	2.60	2.68	14.27	19.11	10.96	0.078
20	KOFM52	45.00	57.33	96.67	2.57	144.07	18.27	3.57	3.22	21.07	26.22	11.23	0.045
21	KOFM53	44.33	56.00	99.33	2.70	132.10	15.97	3.70	2.69	18.50	25.53	10.67	0.098
22	KOFM54	45.33	57.67	97.00	1.37	130.00	21.17	2.37	2.57	13.30	16.33	9.65	0.050
23	KOFM55	44.67	57.00	97.00	2.80	138.47	17.63	3.80	3.12	16.47	26.22	11.10	0.055
24	KOFM58	50.33	61.00	92.00	2.20	128.77	17.03	3.20	2.70	14.23	22.08	10.96	0.041
25	KOFM59	44.67	56.00	92.33	3.30	137.87	19.13	4.30	3.48	23.87	29.67	8.10	0.063
26	KOFM61	44.67	57.00	98.00	3.53	144.33	18.60	4.53	3.40	20.90	31.28	10.85	0.047
27	KOFM62	45.33	57.33	99.33	2.97	142.10	18.47	3.97	3.44	16.10	27.37	10.69	0.043
28	KOFM64	43.00	56.00	96.00	3.17	123.40	17.60	4.17	3.22	18.50	28.75	11.80	0.044

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
29	KOFM65	43.67	56.33	98.67	2.13	124.17	18.13	3.13	2.77	15.20	21.62	13.65	0.043
30	KOFM66	42.67	56.33	99.33	3.60	135.07	18.30	4.60	3.13	19.20	31.74	10.39	0.040
31	KOFM70	44.67	55.33	94.00	1.90	151.30	19.20	2.90	3.17	16.93	25.14	9.81	0.039
32	KOFM73	44.00	58.33	100.67	2.50	150.53	16.37	3.50	3.43	14.23	30.35	8.68	0.060
33	KOFM77	46.33	58.67	101.33	2.47	131.93	16.20	3.47	3.20	16.53	30.06	11.52	0.053
34	KOFM79	44.33	56.67	91.00	1.53	152.77	17.70	2.53	1.91	12.87	21.96	7.54	0.060
35	KOFM80	46.33	60.00	96.00	3.00	139.20	16.60	4.00	2.80	18.00	34.68	12.09	0.052
36	PS4	44.00	55.33	98.00	3.87	131.23	16.37	4.87	3.22	22.70	34.55	11.67	0.031
37	GPUS28	45.67	56.67	96.00	2.50	143.43	19.03	3.50	2.84	16.00	24.85	10.63	0.053
38	SiA326	45.00	58.67	91.33	2.80	135.13	17.63	3.80	3.13	18.20	26.98	10.21	0.054
39	KOFM88	46.00	56.67	91.33	1.10	144.87	18.50	2.10	1.09	11.20	14.91	8.10	0.052
40	KOFM89	59.67	72.33	101.33	1.73	170.10	19.57	2.73	3.52	19.60	19.41	8.11	0.039
41	KOFM90	60.67	71.67	99.00	2.47	164.60	21.27	3.47	2.66	15.57	24.61	9.84	0.035
42	KOFM93	57.00	65.33	94.00	2.20	188.30	19.17	3.20	3.24	17.80	22.72	9.95	0.058
43	KOFM94	67.00	80.00	121.67	3.87	114.20	8.87	4.87	2.15	7.30	19.00	9.82	0.038
44	KOFM95	64.67	76.67	119.67	3.57	111.73	7.90	4.57	2.03	6.87	15.20	9.70	0.037
	Mean	**47.08**	**59.20**	**96.91**	**2.56**	**139.85**	**18.05**	**3.56**	**2.86**	**16.76**	**27.79**	**10.35**	**0.050**
	S.E.	0.72	0.64	0.81	0.12	7.78	0.76	0.12	0.06	0.77	1.00	0.23	0.000
	C.D. 5%	2.04	1.79	2.27	0.33	21.88	2.13	0.33	0.18	2.15	2.81	0.65	0.001
	C.D. 1%	2.70	2.38	3.00	0.44	28.99	2.83	0.44	0.23	2.85	3.73	0.86	0.001

Appendix-III

Mean performance of forty four genotypes of foxtail millet for various characters in E2 environment

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
1	KOFM1	49.33	60.33	99.67	2.93	139.07	18.93	3.93	2.31	15.87	33.24	9.27	0.055
2	KOFM2	53.00	62.67	98.33	2.90	118.03	17.70	3.90	2.56	15.50	32.96	9.09	0.046
3	KOFM6	46.67	59.33	94.33	2.13	120.20	18.57	3.13	2.46	14.83	26.48	10.19	0.055
4	KOFM14	52.00	62.00	93.00	2.60	139.20	21.87	3.60	2.81	22.07	30.42	10.35	0.065
5	KOFM17	48.67	60.33	95.33	2.43	145.70	17.10	3.43	2.56	13.47	29.01	7.18	0.047
6	KOFM18	48.67	60.67	96.67	1.77	146.00	21.27	2.77	2.42	16.13	23.38	11.93	0.052
7	KOFM24	49.33	61.00	97.00	2.93	121.40	16.83	3.93	3.08	19.43	33.24	11.13	0.045
8	KOFM25	46.67	60.00	99.33	3.07	135.53	19.07	4.07	2.90	17.33	34.37	11.10	0.051
9	KOFM28	46.67	59.67	95.33	1.33	137.87	20.30	2.33	2.32	12.70	19.72	12.16	0.048
10	KOFM29	52.00	64.33	96.67	2.47	135.20	19.20	3.47	2.85	16.17	35.15	10.16	0.052
11	KOFM33	49.00	58.00	99.00	2.93	125.27	16.93	3.93	2.85	18.57	32.57	8.46	0.038
12	KOFM36	51.33	62.67	96.00	1.77	177.80	18.73	2.77	2.82	13.93	46.23	12.21	0.040
13	KOFM37	50.00	61.00	97.33	2.43	126.87	18.43	3.43	3.21	18.60	32.98	11.07	0.038
14	KOFM41	46.67	61.00	94.33	2.83	139.90	20.33	3.83	3.03	17.07	36.38	10.23	0.059
15	KOFM42	45.00	60.67	98.00	3.00	131.37	18.37	4.00	2.83	18.13	34.15	9.30	0.060
16	KOFM44	46.00	61.00	97.00	2.37	119.33	16.67	3.37	2.87	14.17	31.03	10.73	0.045
17	KOFM46	43.67	61.33	99.00	2.53	119.43	15.23	3.53	3.00	15.73	25.97	10.32	0.049
18	KOFM48	49.33	62.00	97.00	1.27	124.00	19.33	2.27	2.58	12.77	16.66	10.49	0.050
19	KOFM51	44.33	57.67	97.33	1.27	139.60	16.77	2.27	2.35	11.90	16.66	10.04	0.081
20	KOFM52	46.00	60.33	98.33	2.17	132.27	17.63	3.17	3.14	16.00	23.28	11.57	0.048
21	KOFM53	47.33	60.00	103.33	2.20	126.93	15.53	3.20	2.60	13.97	22.08	10.15	0.097
22	KOFM54	47.00	60.33	97.33	0.80	123.80	20.50	1.80	2.43	9.80	12.42	9.85	0.054
23	KOFM55	46.33	64.00	99.00	2.50	130.60	17.27	3.50	3.00	16.03	24.15	10.96	0.054
24	KOFM58	52.67	64.33	94.33	2.77	120.80	16.70	3.77	2.78	15.63	25.99	11.10	0.042
25	KOFM59	45.67	58.67	90.00	2.93	132.93	18.73	3.93	3.34	19.10	27.14	8.28	0.063
26	KOFM61	46.67	60.00	98.00	3.13	137.50	17.87	4.13	3.22	16.97	28.52	11.02	0.048
27	KOFM62	47.67	61.33	103.33	2.47	138.93	17.80	3.47	3.27	12.80	23.92	10.86	0.043

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
28	KOFM64	43.67	59.33	94.67	2.83	119.83	16.93	3.83	3.13	15.13	26.45	11.98	0.046
29	KOFM65	44.67	59.00	102.00	2.90	117.53	17.90	3.90	2.85	17.67	26.91	13.86	0.043
30	KOFM66	44.33	58.67	102.67	3.33	130.47	17.87	4.33	3.07	17.47	29.90	10.45	0.042
31	KOFM70	47.00	59.00	97.00	1.77	144.60	18.53	2.77	2.99	12.17	23.99	9.84	0.041
32	KOFM73	45.00	61.33	103.00	2.83	142.30	17.83	3.83	3.34	13.83	33.24	9.03	0.061
33	KOFM77	48.33	62.67	104.33	2.77	138.53	15.67	3.77	3.29	17.67	32.66	11.86	0.055
34	KOFM79	46.33	58.33	89.00	1.03	147.20	17.20	2.03	1.86	10.97	17.63	8.03	0.063
35	KOFM80	48.67	63.33	94.33	2.73	132.90	16.00	3.73	2.76	16.80	32.37	12.00	0.055
36	PS4	46.67	58.67	99.00	3.60	123.40	15.80	4.60	3.17	20.67	32.66	11.81	0.033
37	GPUS28	47.67	60.33	96.67	3.10	139.57	18.67	4.10	2.81	18.20	29.11	10.71	0.056
38	SiA326	47.67	61.33	93.33	2.60	131.17	17.40	3.60	3.01	19.57	25.56	10.54	0.054
39	KOFM88	48.00	60.33	92.33	0.70	140.77	17.93	1.70	1.05	9.03	12.07	8.37	0.052
40	KOFM89	53.00	70.33	99.00	0.97	162.80	18.87	1.97	3.36	14.33	13.96	8.55	0.041
41	KOFM90	64.00	70.67	102.67	2.17	159.27	20.33	3.17	2.62	14.83	22.48	10.01	0.037
42	KOFM93	59.33	69.00	99.33	1.57	182.07	18.60	2.57	3.17	14.97	18.22	10.01	0.058
43	KOFM94	68.67	82.00	125.67	3.57	113.40	8.33	4.57	2.11	7.00	16.93	9.79	0.037
44	KOFM95	66.33	78.00	120.00	3.43	109.73	7.73	4.43	2.08	6.53	13.33	9.69	0.037
	Mean	**49.25**	**62.20**	**98.62**	**2.41**	**134.57**	**17.62**	**3.41**	**2.78**	**15.26**	**26.49**	**10.36**	**0.051**
	S.E.	0.65	0.55	0.72	0.12	9.36	0.78	0.12	0.10	0.75	1.23	0.22	0.000
	C.D. 5%	1.82	1.54	2.02	0.35	26.31	2.18	0.35	0.28	2.10	3.47	0.62	0.001
	C.D. 1%	2.41	2.05	2.67	0.46	34.86	2.89	0.46	0.37	2.79	4.59	0.82	0.001

Appendix-IV

Similarity coefficient values based on RAPD marker data of 44 genotypes of foxtail millet using NTSYSpc 2.02 software

	KOFM1	KOFM2	KOFM6	KOFM14	KOFM17	KOFM18	KOFM24	KOFM25	KOFM28	KOFM29	KOFM33	KOFM36	KOFM37	KOFM41	KOFM42	KOFM44	KOFM46	KOFM48	KOFM51	KOFM52	KOFM53	KOFM54
KOFM1	1.000																					
KOFM2	0.878	1.000																				
KOFM6	0.799	0.871	1.000																			
KOFM14	0.791	0.863	0.885	1.000																		
KOFM17	0.806	0.892	0.899	0.863	1.000																	
KOFM18	0.748	0.835	0.871	0.878	0.892	1.000																
KOFM24	0.763	0.835	0.842	0.849	0.878	0.892	1.000															
KOFM25	0.777	0.835	0.856	0.791	0.878	0.863	0.906	1.000														
KOFM28	0.812	0.812	0.804	0.768	0.855	0.826	0.826	0.899	1.000													
KOFM29	0.863	0.878	0.827	0.820	0.878	0.791	0.820	0.835	0.884	1.000												
KOFM33	0.878	0.863	0.799	0.835	0.849	0.806	0.820	0.791	0.841	0.892	1.000											
KOFM36	0.813	0.885	0.835	0.856	0.871	0.842	0.856	0.842	0.877	0.899	0.899	1.000										
KOFM37	0.806	0.878	0.871	0.835	0.878	0.849	0.835	0.878	0.826	0.835	0.835	0.856	1.000									
KOFM41	0.763	0.863	0.856	0.863	0.878	0.863	0.820	0.835	0.812	0.849	0.820	0.885	0.892	1.000								
KOFM42	0.748	0.835	0.885	0.835	0.892	0.863	0.806	0.849	0.812	0.849	0.791	0.842	0.906	0.906	1.000							
KOFM44	0.741	0.813	0.820	0.784	0.842	0.813	0.784	0.856	0.848	0.813	0.770	0.806	0.856	0.871	0.885	1.000						
KOFM46	0.777	0.849	0.813	0.849	0.849	0.835	0.820	0.806	0.797	0.835	0.849	0.856	0.878	0.892	0.878	0.871	1.000					
KOFM48	0.835	0.892	0.856	0.835	0.863	0.835	0.863	0.849	0.855	0.892	0.849	0.899	0.849	0.863	0.849	0.827	0.892	1.000				
KOFM51	0.849	0.863	0.813	0.820	0.849	0.791	0.820	0.791	0.812	0.878	0.863	0.871	0.791	0.820	0.806	0.770	0.835	0.921	1.000			
KOFM52	0.871	0.871	0.806	0.813	0.856	0.784	0.827	0.827	0.848	0.885	0.842	0.849	0.842	0.827	0.842	0.791	0.856	0.914	0.885	1.000		
KOFM53	0.827	0.885	0.835	0.827	0.871	0.827	0.842	0.842	0.862	0.899	0.856	0.892	0.842	0.842	0.842	0.806	0.871	0.957	0.914	0.906	1.000	
KOFM54	0.835	0.863	0.784	0.806	0.835	0.806	0.820	0.806	0.826	0.849	0.835	0.842	0.820	0.820	0.806	0.784	0.863	0.921	0.892	0.899	0.928	1.000
KOFM55	0.719	0.777	0.755	0.791	0.763	0.763	0.748	0.748	0.725	0.748	0.705	0.741	0.734	0.719	0.734	0.741	0.734	0.777	0.763	0.741	0.784	0.763
KOFM58	0.712	0.770	0.734	0.784	0.727	0.741	0.712	0.698	0.674	0.712	0.698	0.719	0.755	0.727	0.712	0.676	0.727	0.741	0.712	0.719	0.748	0.741
KOFM59	0.691	0.763	0.770	0.748	0.763	0.763	0.777	0.777	0.681	0.705	0.676	0.698	0.748	0.748	0.734	0.712	0.705	0.734	0.705	0.741	0.727	0.734
KOFM61	0.734	0.835	0.784	0.777	0.791	0.791	0.734	0.748	0.710	0.748	0.734	0.770	0.791	0.806	0.806	0.755	0.763	0.763	0.719	0.755	0.755	0.748
KOFM62	0.727	0.827	0.777	0.799	0.813	0.799	0.770	0.770	0.732	0.770	0.727	0.763	0.770	0.784	0.784	0.777	0.784	0.784	0.741	0.791	0.777	0.755
KOFM64	0.727	0.784	0.748	0.741	0.784	0.755	0.755	0.741	0.746	0.770	0.727	0.748	0.741	0.712	0.727	0.705	0.727	0.784	0.755	0.763	0.806	0.770
KOFM65	0.784	0.799	0.719	0.727	0.741	0.712	0.712	0.698	0.703	0.755	0.770	0.734	0.698	0.683	0.683	0.676	0.698	0.784	0.784	0.777	0.806	0.784
KOFM66	0.784	0.784	0.719	0.741	0.741	0.741	0.741	0.727	0.732	0.770	0.770	0.719	0.683	0.683	0.683	0.676	0.712	0.784	0.799	0.777	0.791	0.770
KOFM70	0.755	0.755	0.691	0.741	0.712	0.698	0.698	0.698	0.703	0.727	0.712	0.691	0.640	0.669	0.655	0.705	0.683	0.741	0.755	0.734	0.763	0.755
KOFM73	0.727	0.813	0.734	0.770	0.770	0.741	0.770	0.741	0.703	0.770	0.755	0.763	0.712	0.712	0.712	0.705	0.741	0.799	0.770	0.777	0.806	0.755
KOFM77	0.662	0.748	0.770	0.791	0.748	0.777	0.763	0.748	0.696	0.705	0.691	0.727	0.763	0.763	0.763	0.712	0.763	0.763	0.719	0.741	0.770	0.763
KOFM79	0.669	0.770	0.777	0.799	0.755	0.784	0.770	0.741	0.659	0.712	0.698	0.734	0.755	0.784	0.755	0.719	0.755	0.770	0.727	0.734	0.748	0.712
KOFM80	0.669	0.698	0.748	0.741	0.727	0.712	0.712	0.712	0.659	0.655	0.655	0.647	0.741	0.683	0.712	0.676	0.669	0.669	0.640	0.691	0.662	0.655
PS4	0.662	0.777	0.755	0.763	0.777	0.791	0.748	0.748	0.696	0.734	0.676	0.727	0.748	0.791	0.777	0.741	0.748	0.748	0.705	0.741	0.741	0.734
GPUS28	0.727	0.784	0.734	0.770	0.770	0.770	0.813	0.784	0.717	0.741	0.755	0.748	0.755	0.712	0.712	0.691	0.755	0.770	0.741	0.777	0.763	0.755
SIA326	0.741	0.755	0.719	0.755	0.741	0.755	0.770	0.755	0.746	0.741	0.727	0.719	0.727	0.683	0.683	0.662	0.727	0.770	0.741	0.791	0.791	0.770
KOFM88	0.712	0.712	0.676	0.669	0.683	0.698	0.727	0.712	0.688	0.669	0.727	0.676	0.669	0.640	0.655	0.633	0.655	0.712	0.727	0.719	0.719	0.712
KOFM89	0.748	0.719	0.655	0.691	0.691	0.691	0.662	0.647	0.710	0.719	0.763	0.698	0.662	0.676	0.676	0.655	0.705	0.734	0.748	0.755	0.741	0.748
KOFM90	0.698	0.755	0.676	0.683	0.669	0.655	0.712	0.669	0.674	0.727	0.683	0.734	0.669	0.669	0.655	0.647	0.683	0.784	0.784	0.734	0.748	0.712
KOFM93	0.712	0.784	0.748	0.755	0.741	0.727	0.741	0.727	0.703	0.755	0.712	0.734	0.712	0.712	0.712	0.691	0.727	0.813	0.784	0.777	0.806	0.755
KOFM94	0.446	0.417	0.410	0.388	0.403	0.417	0.403	0.403	0.420	0.417	0.446	0.381	0.374	0.388	0.388	0.410	0.417	0.432	0.460	0.439	0.439	0.460
KOFM95	0.446	0.417	0.396	0.374	0.403	0.417	0.403	0.403	0.420	0.417	0.446	0.381	0.374	0.374	0.388	0.410	0.417	0.432	0.460	0.439	0.439	0.460

	KOF M55	KOF M58	KOF M59	KOF M61	KOF M62	KOF M64	KOF M65	KOF M66	KOF M70	KOF M73	KOF M77	KOF M79	KOF M80	PS4	GPUS 28	SIA3 26	KOF M88	KOF M89	KOF M90	KOF M93	KOF M94	KOF M95
KOFM1																						
KOFM2																						
KOFM6																						
KOFM14																						
KOFM17																						
KOFM18																						
KOFM24																						
KOFM25																						
KOFM28																						
KOFM29																						
KOFM33																						
KOFM36																						
KOFM37																						
KOFM41																						
KOFM42																						
KOFM44																						
KOFM46																						
KOFM48																						
KOFM51																						
KOFM52																						
KOFM53																						
KOFM54																						
KOFM55	1.000																					
KOFM58	0.871	1.000																				
KOFM59	0.849	0.842	1.000																			
KOFM61	0.849	0.885	0.878	1.000																		
KOFM62	0.871	0.849	0.885	0.928	1.000																	
KOFM64	0.871	0.878	0.871	0.856	0.878	1.000																
KOFM65	0.856	0.878	0.842	0.842	0.849	0.892	1.000															
KOFM66	0.827	0.835	0.813	0.784	0.820	0.849	0.935	1.000														
KOFM70	0.871	0.791	0.755	0.755	0.777	0.791	0.849	0.835	1.000													
KOFM73	0.842	0.820	0.856	0.842	0.878	0.863	0.892	0.878	0.849	1.000												
KOFM77	0.820	0.871	0.878	0.863	0.871	0.856	0.827	0.813	0.741	0.856	1.000											
KOFM79	0.842	0.849	0.885	0.871	0.892	0.863	0.820	0.806	0.777	0.892	0.928	1.000										
KOFM80	0.741	0.748	0.856	0.755	0.777	0.748	0.719	0.719	0.676	0.777	0.799	0.791	1.000									
PS4	0.820	0.842	0.906	0.906	0.914	0.827	0.799	0.784	0.755	0.856	0.906	0.914	0.813	1.000								
GPUS28	0.827	0.849	0.885	0.842	0.863	0.863	0.849	0.863	0.777	0.921	0.899	0.892	0.820	0.871	1.000							
SIA326	0.813	0.849	0.856	0.813	0.863	0.892	0.863	0.863	0.763	0.863	0.899	0.863	0.791	0.856	0.921	1.000						
KOFM88	0.727	0.734	0.799	0.741	0.734	0.791	0.820	0.820	0.748	0.791	0.770	0.763	0.734	0.741	0.820	0.835	1.000					
KOFM89	0.763	0.784	0.763	0.777	0.770	0.813	0.871	0.856	0.784	0.813	0.777	0.755	0.727	0.763	0.813	0.842	0.871	1.000				
KOFM90	0.784	0.791	0.770	0.784	0.791	0.791	0.820	0.806	0.748	0.835	0.784	0.791	0.748	0.784	0.806	0.806	0.748	0.770	1.000			
KOFM93	0.842	0.849	0.842	0.813	0.849	0.878	0.892	0.878	0.791	0.892	0.856	0.863	0.763	0.827	0.863	0.892	0.835	0.856	0.863	1.000		
KOFM94	0.446	0.439	0.432	0.417	0.439	0.468	0.482	0.496	0.453	0.439	0.417	0.424	0.453	0.417	0.453	0.468	0.468	0.504	0.453	0.482	1.000	
KOFM95	0.446	0.439	0.417	0.417	0.439	0.453	0.482	0.496	0.453	0.439	0.403	0.410	0.439	0.417	0.453	0.453	0.453	0.489	0.453	0.468	0.964	1.000

Appendix-V

Similarity coefficient values based on ISSR marker data of 44 genotypes of foxtail millet using NTSYSpc 2.02 software

	KOFM1	KOFM2	KOFM6	KOFM14	KOFM17	KOFM18	KOFM24	KOFM25	KOFM28	KOFM29	KOFM33	KOFM36	KOFM37	KOFM41	KOFM42	KOFM44	KOFM46	KOFM48	KOFM51	KOFM52	KOFM53	KOFM54
KOFM1	1.000																					
KOFM2	0.936	1.000																				
KOFM6	0.897	0.910	1.000																			
KOFM14	0.872	0.859	0.821	1.000																		
KOFM17	0.821	0.859	0.872	0.795	1.000																	
KOFM18	0.808	0.846	0.782	0.731	0.859	1.000																
KOFM24	0.808	0.795	0.782	0.731	0.808	0.846	1.000															
KOFM25	0.795	0.833	0.769	0.744	0.872	0.885	0.833	1.000														
KOFM28	0.833	0.821	0.808	0.808	0.731	0.769	0.846	0.756	1.000													
KOFM29	0.897	0.885	0.872	0.872	0.795	0.782	0.782	0.769	0.910	1.000												
KOFM33	0.769	0.731	0.692	0.795	0.692	0.679	0.731	0.795	0.731	0.769	1.000											
KOFM36	0.872	0.910	0.872	0.846	0.821	0.808	0.756	0.769	0.782	0.846	0.718	1.000										
KOFM37	0.885	0.897	0.885	0.859	0.833	0.821	0.821	0.808	0.846	0.885	0.731	0.936	1.000									
KOFM41	0.808	0.821	0.782	0.833	0.808	0.769	0.744	0.859	0.744	0.782	0.833	0.833	0.846	1.000								
KOFM42	0.821	0.833	0.872	0.821	0.923	0.808	0.782	0.795	0.782	0.872	0.692	0.821	0.833	0.756	1.000							
KOFM44	0.808	0.821	0.833	0.782	0.936	0.821	0.769	0.859	0.718	0.782	0.705	0.782	0.821	0.795	0.910	1.000						
KOFM46	0.782	0.769	0.782	0.782	0.808	0.769	0.846	0.833	0.821	0.782	0.705	0.756	0.821	0.795	0.808	0.872	1.000					
KOFM48	0.846	0.833	0.795	0.897	0.846	0.808	0.782	0.821	0.808	0.846	0.795	0.821	0.833	0.808	0.872	0.833	0.833	1.000				
KOFM51	0.872	0.859	0.821	0.897	0.846	0.808	0.782	0.795	0.808	0.872	0.769	0.846	0.859	0.808	0.897	0.833	0.808	0.923	1.000			
KOFM52	0.833	0.846	0.859	0.833	0.936	0.795	0.744	0.808	0.718	0.782	0.705	0.808	0.821	0.795	0.885	0.923	0.821	0.859	0.910	1.000		
KOFM53	0.744	0.782	0.821	0.769	0.897	0.782	0.833	0.795	0.782	0.744	0.641	0.769	0.782	0.731	0.872	0.859	0.859	0.821	0.846	0.910	1.000	
KOFM54	0.833	0.872	0.808	0.782	0.885	0.897	0.846	0.910	0.795	0.808	0.705	0.833	0.872	0.795	0.833	0.872	0.821	0.859	0.833	0.846	0.833	1.000
KOFM55	0.808	0.769	0.756	0.808	0.679	0.667	0.667	0.679	0.718	0.756	0.731	0.808	0.821	0.769	0.705	0.692	0.692	0.782	0.756	0.692	0.628	0.692
KOFM58	0.756	0.718	0.705	0.782	0.654	0.641	0.718	0.628	0.846	0.782	0.679	0.731	0.744	0.692	0.679	0.641	0.744	0.782	0.731	0.667	0.705	0.667
KOFM59	0.808	0.769	0.756	0.833	0.705	0.718	0.718	0.705	0.795	0.782	0.731	0.782	0.821	0.769	0.731	0.718	0.744	0.833	0.782	0.718	0.679	0.744
KOFM61	0.808	0.769	0.756	0.808	0.705	0.718	0.718	0.705	0.795	0.782	0.705	0.808	0.846	0.769	0.731	0.718	0.744	0.808	0.782	0.718	0.679	0.744
KOFM62	0.744	0.782	0.718	0.769	0.692	0.679	0.654	0.744	0.705	0.718	0.744	0.769	0.756	0.756	0.718	0.705	0.705	0.769	0.744	0.679	0.641	0.705
KOFM64	0.821	0.833	0.795	0.795	0.769	0.731	0.705	0.718	0.731	0.795	0.667	0.846	0.833	0.731	0.795	0.731	0.705	0.821	0.846	0.782	0.744	0.756
KOFM65	0.795	0.782	0.744	0.821	0.718	0.679	0.654	0.667	0.679	0.744	0.692	0.821	0.782	0.756	0.744	0.705	0.679	0.821	0.821	0.756	0.692	0.705
KOFM66	0.795	0.756	0.744	0.846	0.692	0.679	0.679	0.744	0.731	0.769	0.795	0.769	0.808	0.808	0.718	0.731	0.756	0.795	0.795	0.731	0.667	0.705
KOFM70	0.795	0.756	0.744	0.744	0.692	0.808	0.731	0.718	0.756	0.795	0.667	0.769	0.808	0.705	0.744	0.731	0.756	0.795	0.769	0.705	0.667	0.756
KOFM73	0.769	0.731	0.718	0.769	0.692	0.679	0.731	0.692	0.756	0.769	0.744	0.718	0.782	0.731	0.692	0.705	0.731	0.795	0.744	0.705	0.667	0.731
KOFM77	0.731	0.718	0.705	0.731	0.756	0.744	0.744	0.859	0.718	0.731	0.756	0.705	0.769	0.795	0.731	0.744	0.769	0.782	0.731	0.692	0.705	0.769
KOFM79	0.692	0.654	0.641	0.718	0.641	0.603	0.603	0.718	0.654	0.718	0.769	0.692	0.756	0.756	0.641	0.679	0.705	0.718	0.667	0.628	0.564	0.654
KOFM80	0.667	0.654	0.615	0.718	0.641	0.654	0.705	0.769	0.654	0.641	0.795	0.615	0.654	0.731	0.615	0.654	0.705	0.744	0.667	0.628	0.590	0.679
PS4	0.808	0.769	0.756	0.833	0.705	0.667	0.667	0.654	0.718	0.782	0.705	0.833	0.846	0.744	0.731	0.692	0.692	0.808	0.782	0.718	0.654	0.718
GPUS28	0.718	0.731	0.692	0.692	0.667	0.705	0.756	0.692	0.782	0.744	0.590	0.718	0.782	0.628	0.718	0.679	0.756	0.744	0.718	0.654	0.692	0.756
SIA326	0.782	0.744	0.731	0.756	0.679	0.718	0.718	0.705	0.769	0.808	0.705	0.731	0.795	0.692	0.731	0.718	0.744	0.808	0.756	0.692	0.654	0.744
KOFM88	0.795	0.782	0.744	0.769	0.692	0.705	0.731	0.692	0.756	0.744	0.692	0.821	0.782	0.705	0.718	0.679	0.731	0.821	0.769	0.705	0.718	0.782
KOFM89	0.731	0.718	0.679	0.731	0.679	0.718	0.744	0.705	0.769	0.731	0.679	0.756	0.769	0.692	0.731	0.718	0.744	0.782	0.731	0.667	0.705	0.795
KOFM90	0.782	0.744	0.731	0.756	0.756	0.769	0.769	0.756	0.744	0.756	0.654	0.782	0.846	0.718	0.782	0.769	0.769	0.833	0.808	0.744	0.731	0.821
KOFM93	0.731	0.692	0.679	0.731	0.679	0.692	0.769	0.731	0.769	0.731	0.679	0.679	0.744	0.718	0.731	0.718	0.821	0.782	0.731	0.667	0.731	0.718
KOFM94	0.372	0.385	0.397	0.397	0.423	0.385	0.436	0.449	0.436	0.397	0.449	0.397	0.385	0.436	0.423	0.436	0.487	0.423	0.372	0.385	0.474	0.385
KOFM95	0.385	0.397	0.410	0.410	0.436	0.397	0.449	0.462	0.449	0.410	0.462	0.410	0.397	0.449	0.436	0.449	0.500	0.436	0.385	0.397	0.487	0.397

	KOF M55	KOF M58	KOF M59	KOF M61	KOF M62	KOF M64	KOF M65	KOF M66	KOF M70	KOF M73	KOF M77	KOF M79	KOF M80	PS4	GPUS 28	SIA3 26	KOF M88	KOF M89	KOF M90	KOF M93	KOF M94	KOF M95
KOFM1																						
KOFM2																						
KOFM6																						
KOFM14																						
KOFM17																						
KOFM18																						
KOFM24																						
KOFM25																						
KOFM28																						
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KOFM44																						
KOFM46																						
KOFM48																						
KOFM51																						
KOFM52																						
KOFM53																						
KOFM54																						
KOFM55	1.000																					
KOFM58	0.846	1.000																				
KOFM59	0.923	0.897	1.000																			
KOFM61	0.897	0.846	0.949	1.000																		
KOFM62	0.859	0.756	0.859	0.859	1.000																	
KOFM64	0.859	0.756	0.833	0.885	0.872	1.000																
KOFM65	0.885	0.782	0.859	0.885	0.872	0.949	1.000															
KOFM66	0.910	0.808	0.910	0.885	0.897	0.846	0.872	1.000														
KOFM70	0.833	0.756	0.859	0.885	0.769	0.846	0.821	0.821	1.000													
KOFM73	0.808	0.782	0.859	0.885	0.769	0.821	0.795	0.821	0.821	1.000												
KOFM77	0.821	0.744	0.821	0.821	0.833	0.808	0.756	0.859	0.808	0.808	1.000											
KOFM79	0.833	0.731	0.782	0.808	0.795	0.769	0.769	0.846	0.769	0.795	0.859	1.000										
KOFM80	0.782	0.731	0.808	0.782	0.821	0.692	0.718	0.821	0.744	0.795	0.859	0.795	1.000									
PS4	0.923	0.846	0.897	0.923	0.833	0.885	0.910	0.885	0.833	0.833	0.795	0.859	0.756	1.000								
GPUS28	0.756	0.756	0.808	0.859	0.795	0.821	0.769	0.744	0.846	0.795	0.756	0.744	0.769	0.808	1.000							
SIA326	0.872	0.821	0.872	0.897	0.808	0.859	0.859	0.833	0.910	0.859	0.821	0.859	0.782	0.897	0.859	1.000						
KOFM88	0.808	0.782	0.808	0.808	0.769	0.821	0.846	0.769	0.795	0.718	0.705	0.718	0.692	0.833	0.795	0.808	1.000					
KOFM89	0.795	0.795	0.846	0.846	0.782	0.782	0.808	0.782	0.833	0.731	0.744	0.731	0.731	0.821	0.859	0.846	0.910	1.000				
KOFM90	0.846	0.769	0.872	0.897	0.782	0.859	0.833	0.833	0.885	0.833	0.846	0.782	0.756	0.872	0.859	0.872	0.808	0.846	1.000			
KOFM93	0.821	0.821	0.846	0.821	0.808	0.782	0.782	0.859	0.833	0.782	0.846	0.782	0.756	0.795	0.808	0.846	0.782	0.846	0.872	1.000		
KOFM94	0.436	0.436	0.410	0.359	0.423	0.397	0.397	0.449	0.372	0.423	0.487	0.474	0.397	0.359	0.372	0.410	0.397	0.385	0.385	0.513	1.000	
KOFM95	0.449	0.449	0.423	0.372	0.436	0.410	0.410	0.462	0.385	0.436	0.500	0.487	0.410	0.372	0.385	0.423	0.410	0.397	0.397	0.526	0.987	1.000

9. VITA

SMITA NARENDRA SHINGANE

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

CYTOGENETICS AND PLANT BREEDING

2012

Title of thesis : "Assessment of genetic and molecular diversity for different traits in foxtail millet (*Setaria italica* (L.) P. Beauv)"

Major field : Cytogenetics and Plant breeding

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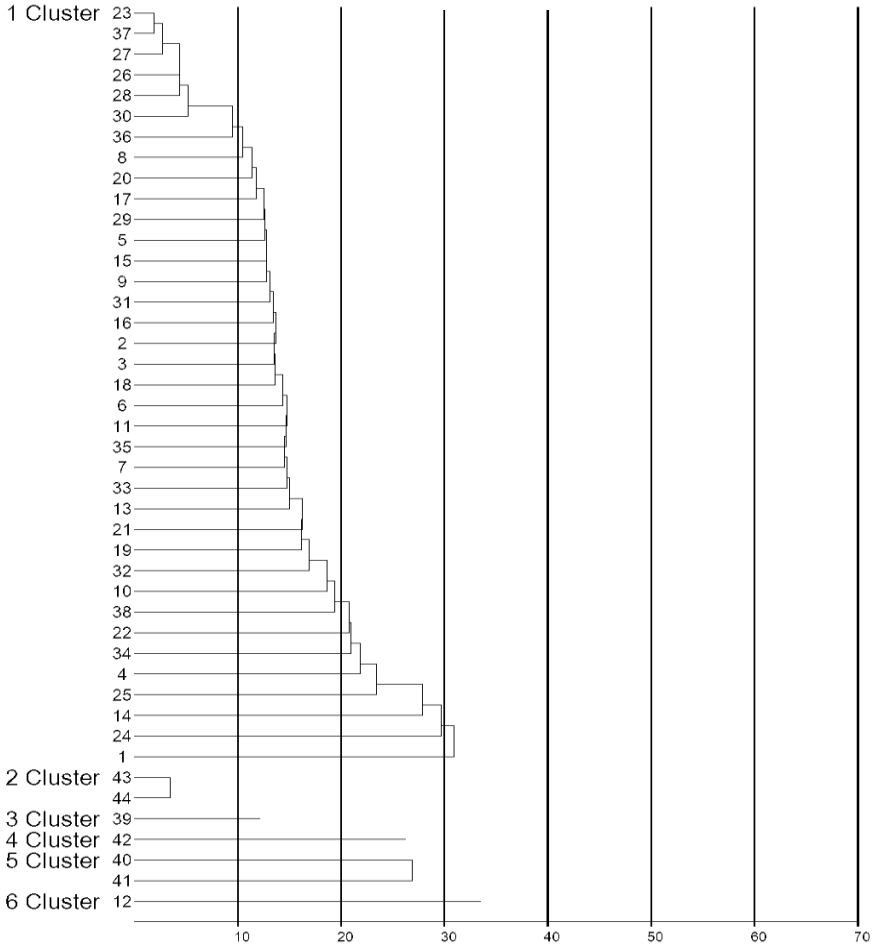
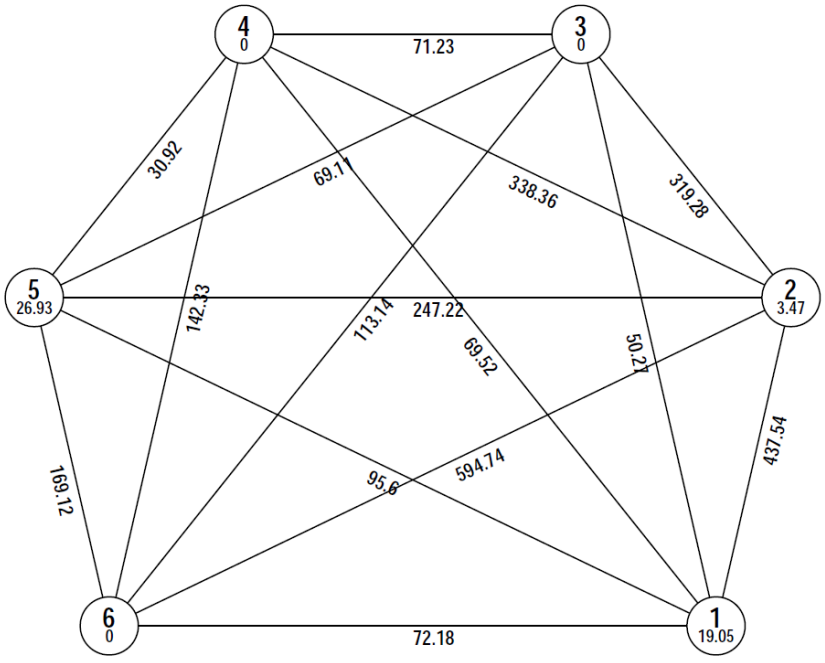


Fig.5: Tree diagram for 44 foxtail millet genotypes based on 10 traits



(Not to scale)

Fig.6: Intra and inter cluster distances among six clusters based on morphological data in foxtail millet

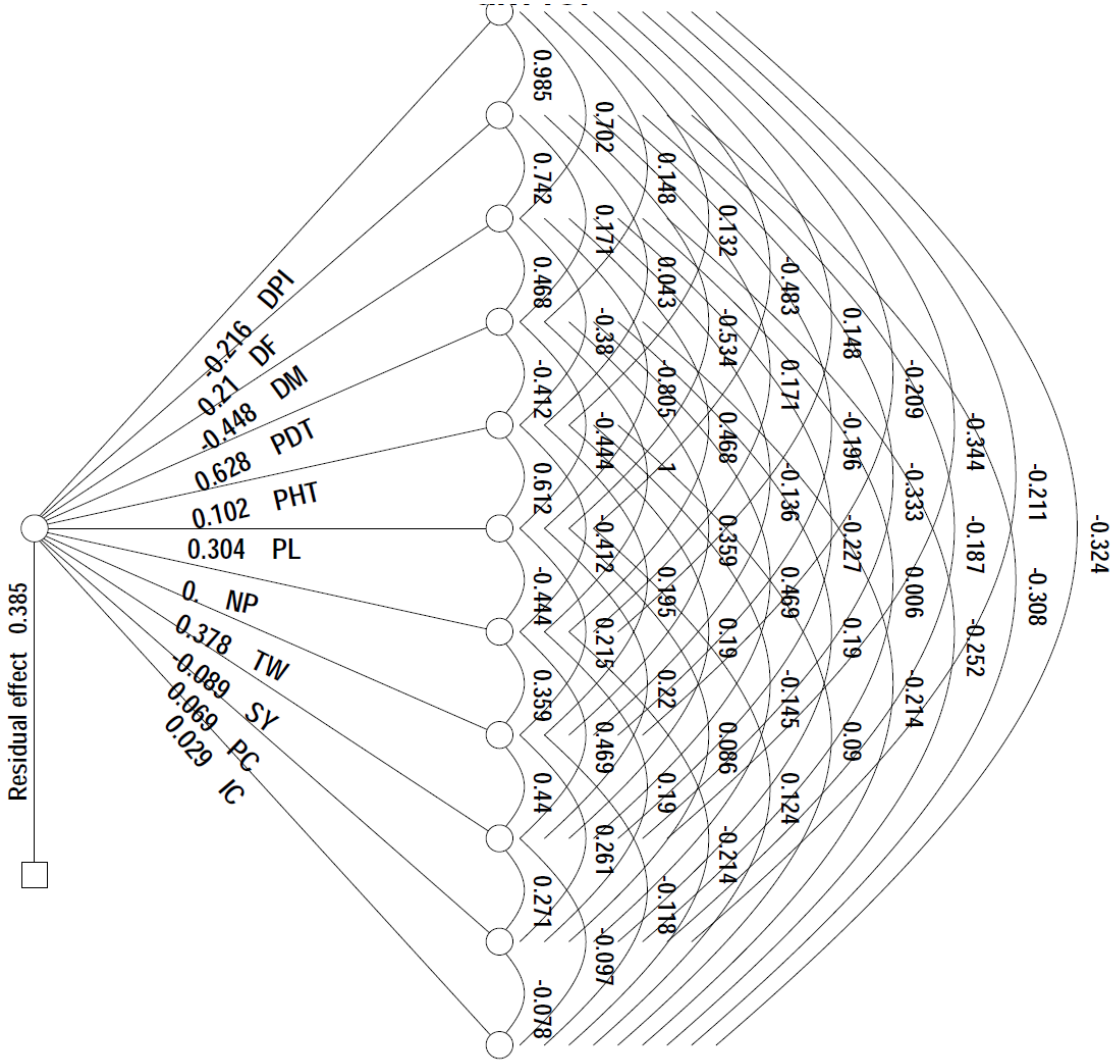


Fig. 2: A path diagram showing relationship between Grain yield per plant and some growth parameters in E1 environment. DPI (Days to panicle initiation), DF (Days to 50% flowering), DM (Days to maturity), PDT (Productive tillers per plant), PHT (Plant height), PL (Panicle length), NP (Number of panicles per plant), TW (1000 grain weight), SY (Straw yield per plant), PC (Protein content) and IC (Iron content).

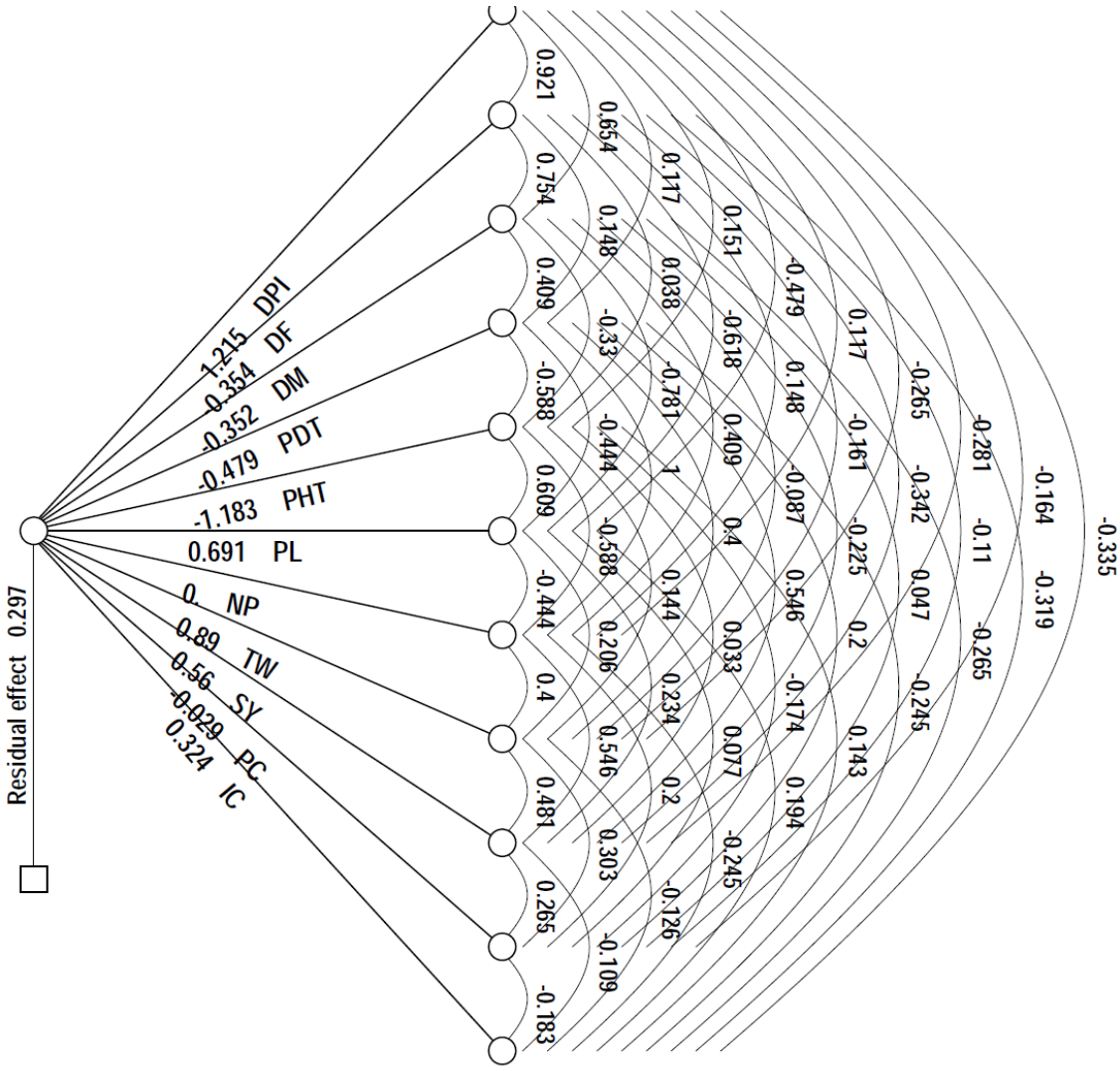


Fig. 3: A path diagram showing relationship between Grain yield per plant and some growth parameters in E2 environment. DPI (Days to panicle initiation), DF (Days to 50% flowering), DM (Days to maturity), PDT (Productive tillers per plant), PHT (Plant height), PL (Panicle length), NP (Number of panicles per plant), TW (1000 grain weight), SY (Straw yield per plant), PC (Protein content) and IC (Iron content).

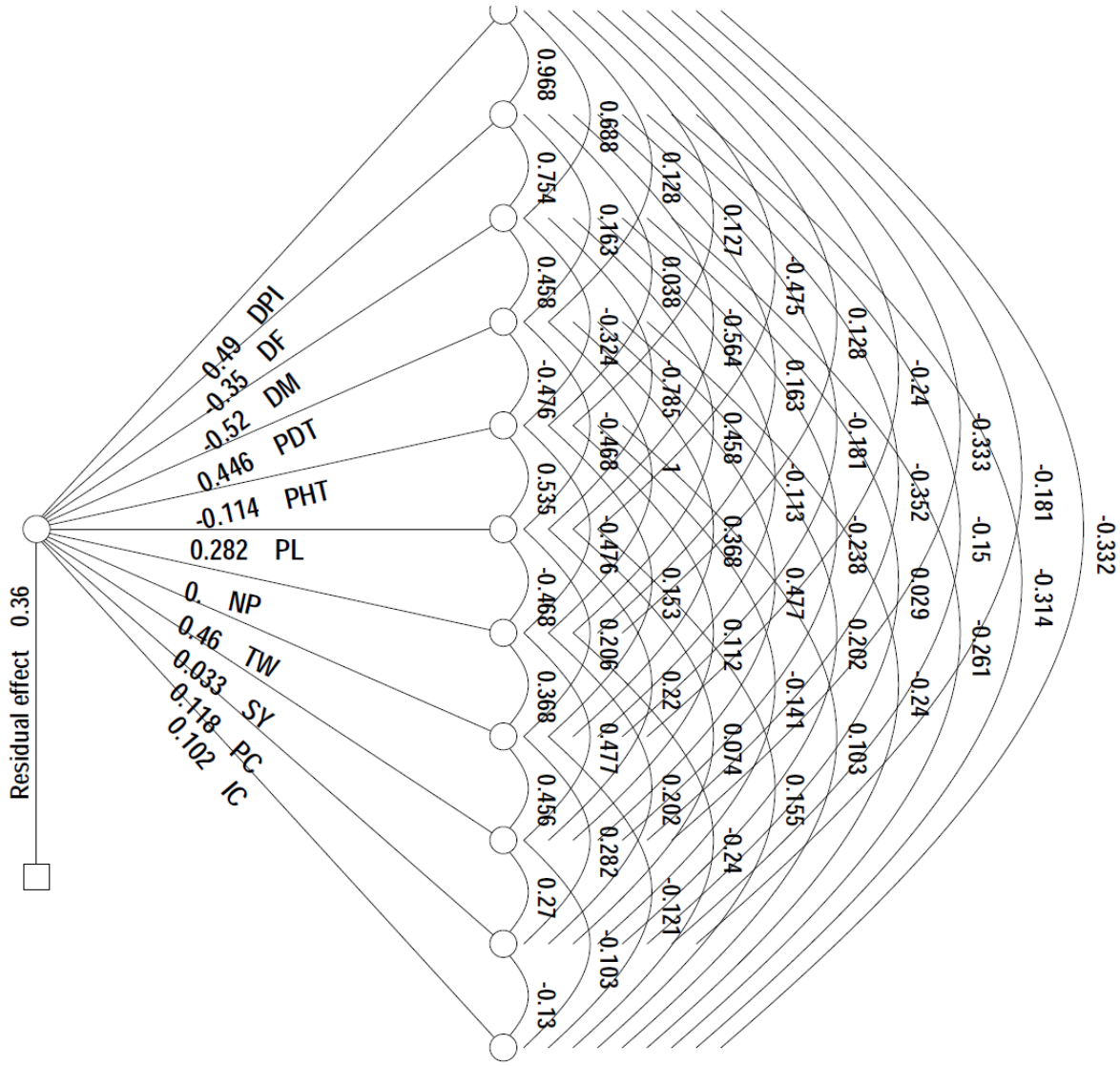


Fig. 4: A path diagram showing relationship between Grain yield per plant and some growth parameters across environments. DPI (Days to panicle initiation), DF (Days to 50% flowering), DM (Days to maturity), PDT (Productive tillers per plant), PHT (Plant height), PL (Panicle length), NP (Number of panicles per plant), TW (1000 grain weight), SY (Straw yield per plant), PC (Protein content) and IC (Iron content).

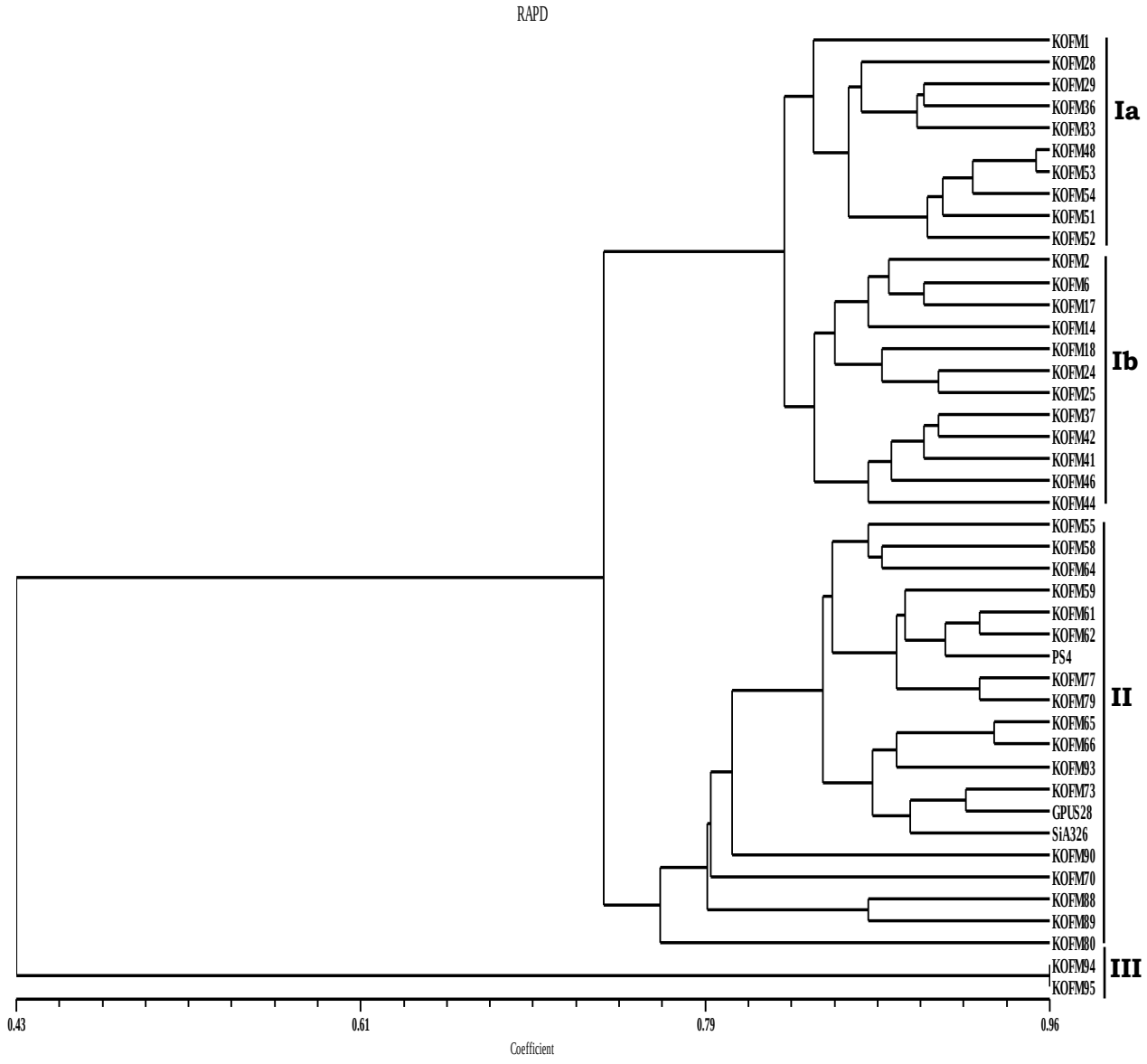


Figure 7: Dendrogram constructed with NTSYSpc ver.2.02 using UPGMA clustering algorithm from the pair-wise genetic similarity matrix to compare 44 foxtail millet genotypes based on allelic information from 19 RAPD markers

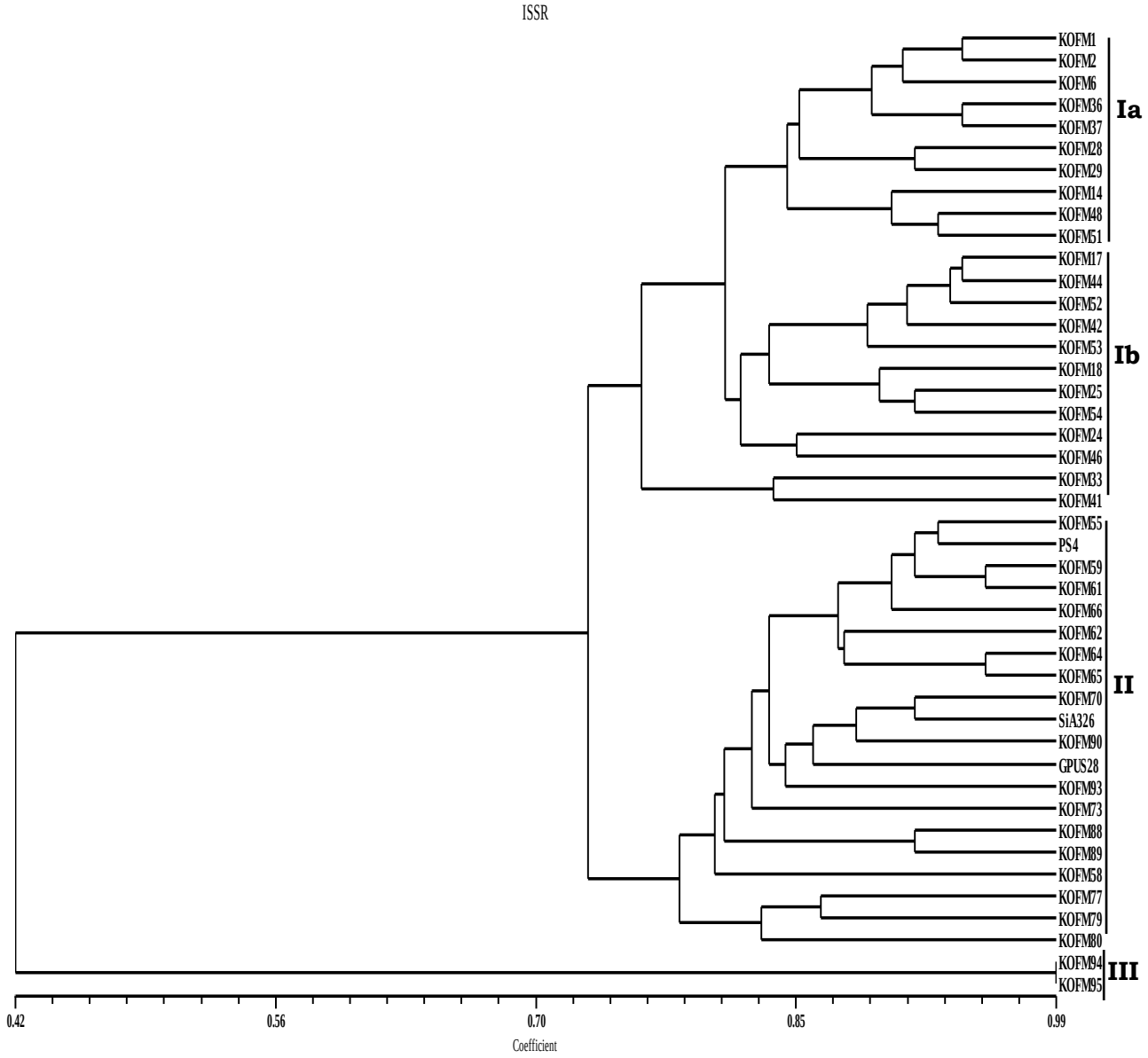


Figure 8: Dendrogram constructed with NTSYSpc ver.2.02 using UPGMA clustering algorithm from the pair-wise genetic similarity matrix to compare 44 foxtail millet genotypes based on allelic information from 12 ISSR markers

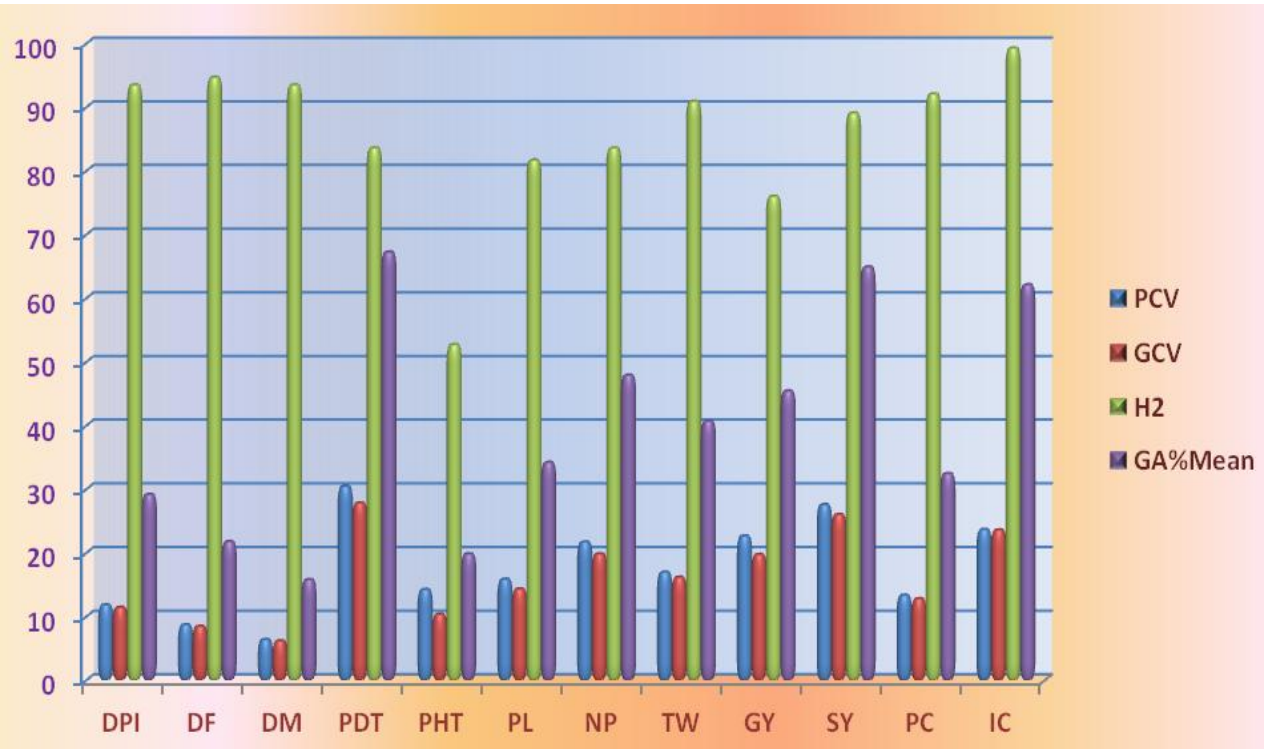


Fig. 1: Phenotypic, genotypic coefficients of variation and heritability (%) for grain yield and yield contributing traits in foxtail millet. DPI (Days to panicle initiation), DF (Days to 50% flowering), DM (Days to maturity), PDT (Productive tillers per plant), PHT (Plant height), PL (Panicle length), NP (Number of panicles per plant), TW (1000 grain weight), SY (Straw yield per plant), PC (Protein content) and IC (Iron content).

Table 4.1. Analysis of variance (MSS) over the years for 12 characters in foxtail millet

Sr. No.	Environment	E1		E2		Pooled		
	Components of variance	σ^2g	SE	σ^2g	SE	σ^2g	σ^2ge	SE
1	Days to panicle initiations	96.46**	1.57	92.90**	1.25	184.39**	157.03**	2.12
2	Days to 50% flowering	92.39**	1.22	71.36**	0.90	160.56**	300.18**	1.48
3	Days to maturity	106.16**	1.94	121.62**	1.54	222.07**	100.24**	2.53
4	No. of productive tillers/ plant	1.54**	0.04	1.70**	0.04	2.96**	0.79**	0.09
5	Plant height	720.27**	181.71	685.04**	262.67	1390.37**	1702.95**	180.7
6	No. of panicles/ plant	1.54**	0.04	1.70**	0.04	2.96**	0.79**	0.09
7	Panicle length	20.86**	1.72	20.13**	1.80	40.74**	12.60**	1.46
8	1000 grain weight	0.67**	0.01	0.61**	0.02	1.26**	0.24**	0.02
9	Grain yield/ plant (g)	38.59**	1.75	33.51**	1.67	63.15**	76.55**	3.16
10	Straw yield/ plant (g)	153.38**	3.00	167.81**	4.56	305.87**	74.95**	6.08
11	Protein content (%)	5.67**	0.15	5.17**	0.14	10.71**	0.74**	0.15
12	Iron content (%)	0.0004**	0.0000	0.0004**	0.00	0.0008**	0.00005**	0.000001

*,** = significant at 5% and 1% level, respectively

Table 4.3. Variability parameters for various characters in foxtail millet in E1, E2 and across environments

Sr No.	Characters		Range	General mean	PCV	GCV	Heritability h^2 (bs)	Genetic advance	Genetic advance as % mean
1	Days to panicle initiations	E1	42.67-67.00	47.7	12.24	11.94	95.30	14.49	30.78
		E2	43.67-68.67	49.25	11.45	11.22	96.10	14.30	29.03
		P	43.33-67.83	48.16	11.83	11.44	93.50	14.06	29.20
2	Days to 50% flowering	E1	54.00-80.00	59.20	9.49	9.31	96.10	14.26	24.10
		E2	57.67-82.00	62.20	7.94	7.79	96.30	12.55	20.18
		P	56.83-81.00	60.70	8.71	8.48	94.70	13.22	21.78
3	Days to maturity	E1	90.67-121.67	96.90	6.25	6.08	94.70	15.14	15.62
		E2	89.00-125.67	98.62	6.53	6.41	96.30	16.38	16.61
		P	90.00-123.67	97.77	6.39	6.18	93.50	15.44	15.79
4	No. of productive tillers/ plant	E1	1.10-3.90	2.55	28.83	27.68	92.20	1.79	70.20
		E2	0.70-3.60	2.40	32.18	30.94	92.40	1.88	78.52
		P	0.90-3.73	2.48	30.46	27.85	83.60	1.66	67.25
5	Plant height	E1	111.73-188.30	139.84	13.59	9.58	49.70	24.93	17.83
		E2	109.73-182.07	134.56	14.92	8.81	34.90	18.50	13.75
		P	110.73- 185.18	137.21	14.25	10.34	52.70	27.21	19.83
6	Panicle length	E1	7.90- 22.97	18.05	15.77	13.99	78.70	5.91	32.77
		E2	7.73-21.87	17.61	15.97	14.02	77.20	5.73	32.53
		P	7.82-22.42	17.84	15.87	14.34	81.70	6.10	34.22
7	No. of panicles/ plant	E1	2.10- 4.90	3.55	20.72	19.90	92.20	1.79	50.47
		E2	1.70-4.60	3.40	22.73	21.85	92.40	1.88	55.46
		P	1.90-4.73	3.48	21.71	19.85	83.60	1.66	47.93
8	1000 grain weight	E1	1.09-3.52	2.85	16.85	16.41	94.90	1.20	42.21
		E2	1.05-3.36	2.77	17.07	15.91	86.90	1.08	39.14
		P	1.07-3.44	2.82	16.96	16.17	90.90	1.14	40.71

Sr No.	Characters		Range	General mean	PCV	GCV	Heritability h^2 (bs)	Genetic advance	Genetic advance as % mean
9	Grain yield/ plant	E1	6.87-23.87	16.75	22.35	20.90	87.50	8.65	51.63
		E2	6.53-22.07	15.26	22.97	21.34	86.30	7.99	52.36
		P	6.70- 21.68	16.01	22.66	19.75	76.00	7.27	45.44
10	Straw yield/ plant	E1	14.91- 48.16	27.78	26.23	25.48	94.40	18.15	65.34
		E2	12.07-46.23	26.49	28.99	27.84	92.30	18.70	70.61
		P	13.49-47.19	27.14	27.58	26.04	89.10	17.61	64.92
11	Protein content (%)	E1	6.97-13.65	10.35	13.66	13.10	92.00	3.43	33.18
		E2	7.18-13.86	10.35	13.03	12.50	92.0	3.28	31.67
		P	7.08-13.75	10.35	13.35	12.81	92.10	3.36	32.46
12	Iron content (%)	E1	0.03-0.10	0.04	24.24	24.21	99.70	0.03	63.83
		E2	0.03-0.10	0.05	23.14	23.11	99.70	0.03	60.93
		P	0.03-0.10	0.05	23.68	23.61	99.30	0.03	62.13

Table 4.6. Distribution of 44 foxtail millet genotypes into different clusters

Cluster Number	Number of genotypes	Genotypes
I	37	KOFM 55, GPUS 28, KOFM 62, KOFM 61, KOFM 64, KOFM 66, PS 4, KOFM 25, KOFM 52, KOFM 46, KOFM 65, KOFM 17, KOFM 28, KOFM 42, KOFM 44, KOFM 2, KOFM 6, KOFM 48, KOFM 80, KOFM 24, KOFM 33, KOFM 77, KOFM 70, KOFM 37, KOFM 18, KOFM 53, KOFM 51, KOFM 73, KOFM 29, SIA 326, KOFM 54, KOFM 79, KOFM 14, KOFM 59, KOFM 41, KOFM 58, KOFM 1
II	2	KOFM 94, KOFM 95
III	1	KOFM 88
IV	1	KOFM 93
V	2	KOFM 89, KOFM 90
VI	1	KOFM 36

Table 4.9. Per cent contribution of various characters to divergence in foxtail millet

Sr. No.	Characters	Per cent contribution
1	Days to panicle initiation	12.37
2	Days to 50 per cent flowering	1.37
3	Days to maturity	11.52
4	No. of productive tillers	19.66
5	Plant height (cm)	0.53
6	Panicle length (cm)	6.03
7	No. of panicles	0.00
8	1000-seed weight (g)	20.93
9	Grain yield per plant (g)	7.29
10	Straw yield per plant(g)	20.30
	Total	100

Table 4. 7. Average intra (diagonal) and inter (above diagonal) clusters D^2 values in six clusters of 44 genotypes of foxtail millet

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**
Cluster I	**19.05**	437.54	50.27	69.52	95.60	72.18
Cluster II		**3.47**	319.28	338.36	247.22	594.74
Cluster III			**0.00**	71.23	69.11	113.14
Cluster IV				**0.00**	30.92	142.33
Cluster V					**26.93**	169.12
Cluster VI						**0.00**

Table 4.10 Per cent polymorphism shown by different RAPD primers

Sr. No.	Primer	Total number of band generated	Total number of monomorphic bands	Total number of polymorphic bands	Per cent Polymorphism (%)	PIC Values
1	OPA 03	4	0	4	100.00	0.440
2	OPD 05	8	1	7	87.50	0.789
3	OPE 03	9	2	7	77.77	0.529
4	OPE 04	7	0	7	100.00	0.701
5	OPE 15	5	0	5	100.00	0.701
6	OPE 18	5	0	5	100.00	0.754
7	OPE 19	5	0	5	100.00	0.704
8	OPK 09	10	1	9	90.00	0.882
9	OPL 02	6	0	6	100.00	0.613
10	OPL 14	7	0	7	100.00	0.797
11	OPL 18	6	0	6	100.00	0.707
12	OPM 05	8	3	5	62.50	0.849
13	OPM 09	6	1	5	83.33	0.787
14	OPM 10	11	0	11	100.00	0.878
15	OPM 12	8	0	8	100.00	0.814
16	OPM 14	7	1	6	85.71	0.651
17	OPM 17	8	0	8	100.00	0.816
18	OPM 18	7	0	7	100.00	0.779
19	OPM 20	8	3	5	62.50	0.838
	Total	135	12	123	91.11	--

Table 4.11 Per cent polymorphism shown by different ISSR primers

Sr. No.	Primer	Total number of band generated	Total number of monomorphic bands	Total number of polymorphic bands	Per cent Polymorphism (%)	PIC Values
1	ISSR 807	5	1	4	80.00	0.466
2	ISSR 808	8	2	6	75.00	0.816
3	ISSR 809	6	2	4	66.66	0.725
4	ISSR 810	9	0	9	100.00	0.847
5	ISSR 811	4	0	4	100.00	0.702
6	ISSR 817	5	0	5	100.00	0.749
7	ISSR 820	5	0	5	100.00	0.703
8	ISSR 823	9	0	9	100.00	0.839
9	ISSR 826	6	0	6	100.00	0.732
10	ISSR 834	8	0	8	100.00	0.745
11	ISSR 880	7	0	7	100.00	0.779
12	ISSR 885	5	0	5	100.00	0.703
	Total	77	5	72	93.50	--

Table 4. 2. Mean performance of forty four genotypes of foxtail millet for various characters over two seasons

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers per plant	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
1	KOFM1	47.33	58.50	98.83	3.42	143.12	19.20	4.42	2.36	17.17	37.32	9.54	0.056
2	KOFM2	49.67	61.00	97.17	2.63	123.30	18.03	3.63	2.53	15.28	30.70	9.17	0.046
3	KOFM6	45.17	58.00	94.00	1.85	123.03	18.73	2.85	2.42	13.93	24.09	10.36	0.056
4	KOFM14	49.67	61.33	93.00	2.45	142.58	22.42	3.45	2.77	20.53	29.16	10.65	0.064
5	KOFM17	47.00	58.67	94.17	2.12	143.53	17.28	3.12	2.64	13.15	26.34	7.08	0.047
6	KOFM18	46.67	58.83	95.00	1.93	148.05	21.63	2.93	2.57	17.62	24.79	12.02	0.052
7	KOFM24	47.33	59.33	97.17	3.22	124.37	17.03	4.22	3.17	20.78	35.63	11.54	0.044
8	KOFM25	45.33	57.00	97.67	2.90	138.80	19.35	3.90	2.90	16.87	32.96	11.16	0.050
9	KOFM28	45.33	58.17	95.17	1.47	140.00	20.00	2.47	2.49	13.70	20.85	12.12	0.048
10	KOFM29	50.00	62.50	95.33	2.68	138.52	19.37	3.68	2.91	18.02	36.01	10.14	0.052
11	KOFM33	47.50	57.33	98.17	2.77	128.03	17.23	3.77	2.80	17.43	33.29	8.50	0.037
12	KOFM36	50.00	61.67	96.17	1.85	181.52	18.98	2.85	2.86	15.23	47.19	12.32	0.040
13	KOFM37	48.33	59.33	97.00	2.80	130.23	18.62	3.80	3.36	20.90	33.86	10.96	0.037
14	KOFM41	45.67	59.00	92.50	2.58	142.78	20.02	3.58	2.97	16.25	37.13	10.31	0.058
15	KOFM42	44.83	58.83	97.33	2.88	132.55	18.65	3.88	2.77	17.18	34.46	9.27	0.059
16	KOFM44	45.50	59.50	94.83	2.62	122.32	16.98	3.62	2.93	16.45	31.80	10.63	0.044
17	KOFM46	43.33	59.17	96.83	2.70	121.52	15.57	3.70	3.08	16.80	28.76	10.35	0.049
18	KOFM48	48.00	60.17	95.00	1.55	127.10	19.65	2.55	2.62	14.95	18.74	10.44	0.047
19	KOFM51	43.67	56.83	96.50	1.43	141.30	16.90	2.43	2.52	13.08	17.89	10.50	0.080
20	KOFM52	45.50	58.83	97.50	2.37	138.17	17.95	3.37	3.18	18.53	24.75	11.40	0.046
21	KOFM53	45.83	58.00	101.33	2.45	129.52	15.75	3.45	2.64	16.23	23.81	10.41	0.098
22	KOFM54	46.17	59.00	97.17	1.08	126.90	20.83	2.08	2.50	11.55	14.38	9.75	0.052
23	KOFM55	45.50	60.50	98.00	2.65	134.53	17.45	3.65	3.06	16.25	25.19	11.03	0.055
24	KOFM58	51.50	62.67	93.17	2.48	124.78	16.87	3.48	2.74	14.93	24.04	11.03	0.042
25	KOFM59	45.17	57.33	91.17	3.12	135.40	18.93	4.12	3.41	21.48	28.41	8.19	0.063
26	KOFM61	45.67	58.50	98.00	3.33	140.92	18.23	4.33	3.31	18.93	29.90	10.94	0.048
27	KOFM62	46.50	59.33	101.33	2.72	140.52	18.13	3.72	3.36	14.45	25.65	10.77	0.043
28	KOFM64	43.33	57.67	95.33	3.00	121.62	17.27	4.00	3.18	16.82	27.60	11.89	0.045
29	KOFM65	44.17	57.67	100.33	2.52	120.85	18.02	3.52	2.81	16.43	24.27	13.75	0.043

Sr. No.	Character / Variety	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers per plant	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
30	KOFM66	43.50	57.50	101.00	3.47	132.77	18.08	4.47	3.10	18.33	30.82	10.42	0.041
31	KOFM70	45.83	57.17	95.50	1.83	147.95	18.87	2.83	3.08	14.55	24.57	9.83	0.040
32	KOFM73	44.50	59.83	101.83	2.67	146.42	17.10	3.67	3.39	14.03	31.79	8.86	0.060
33	KOFM77	47.33	60.67	102.83	2.62	135.23	15.93	3.62	3.24	17.10	31.36	11.69	0.054
34	KOFM79	45.33	57.50	90.00	1.28	149.98	17.45	2.28	1.89	11.92	19.80	7.78	0.062
35	KOFM80	47.50	61.67	95.17	2.87	136.05	16.30	3.87	2.78	17.40	33.53	12.04	0.054
36	PS4	45.33	57.00	98.50	3.73	127.32	16.08	4.73	3.20	21.68	33.61	11.74	0.032
37	GPUS28	46.67	58.50	96.33	2.80	141.50	18.85	3.80	2.83	17.10	26.98	10.67	0.055
38	SiA326	46.33	60.00	92.33	2.70	133.15	17.52	3.70	3.07	18.88	26.27	10.37	0.054
39	KOFM88	47.00	58.50	91.83	0.90	142.82	18.22	1.90	1.07	10.12	13.49	8.24	0.052
40	KOFM89	56.33	71.33	100.17	1.35	166.45	19.22	2.35	3.44	16.97	16.69	8.33	0.040
41	KOFM90	62.33	71.17	100.83	2.32	161.93	20.80	3.32	2.64	15.20	23.55	9.92	0.036
42	KOFM93	58.17	67.17	96.67	1.88	185.18	18.88	2.88	3.21	16.38	20.47	9.98	0.058
43	KOFM94	67.83	81.00	123.67	3.72	113.80	8.60	4.72	2.13	7.15	17.97	9.81	0.038
44	KOFM95	65.50	77.33	119.83	3.50	110.73	7.82	4.50	2.06	6.70	14.27	9.70	0.037
	Mean	**48.16**	**60.70**	**97.77**	**2.48**	**137.21**	**17.84**	**3.48**	**2.82**	**16.01**	**27.14**	**10.35**	**0.050**
	S.E.	0.60	0.50	0.65	0.12	5.49	0.49	0.12	0.06	0.73	1.01	0.16	0.000
	C.D. 5%	1.66	1.39	1.81	0.35	15.30	1.38	0.35	0.16	2.02	2.81	0.44	0.001
	C.D. 1%	2.19	1.83	2.39	0.46	20.17	1.82	0.46	0.22	2.67	3.70	0.58	0.001

Table 4.8. Cluster mean performance for 10 characters in foxtail millet

Characters / Cluster	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers per plant	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Grain yield per plant (g)	Straw yield per plant (g)
I	46.27	59.00	96.55	2.53	134.45	18.17	3.53	2.88	16.67	28.12
II	66.67	79.17	121.75	3.61	112.27	8.21	4.61	2.10	6.93	16.12
III	47.00	58.50	91.83	0.90	142.82	18.22	1.90	1.07	10.12	13.49
IV	58.17	67.17	96.67	1.88	185.18	18.88	2.88	3.21	16.38	20.47
V	59.33	71.25	100.50	1.83	164.19	20.01	2.83	3.04	16.08	20.12
VI	50.00	61.67	96.17	1.85	181.52	18.98	2.85	2.86	15.23	47.19

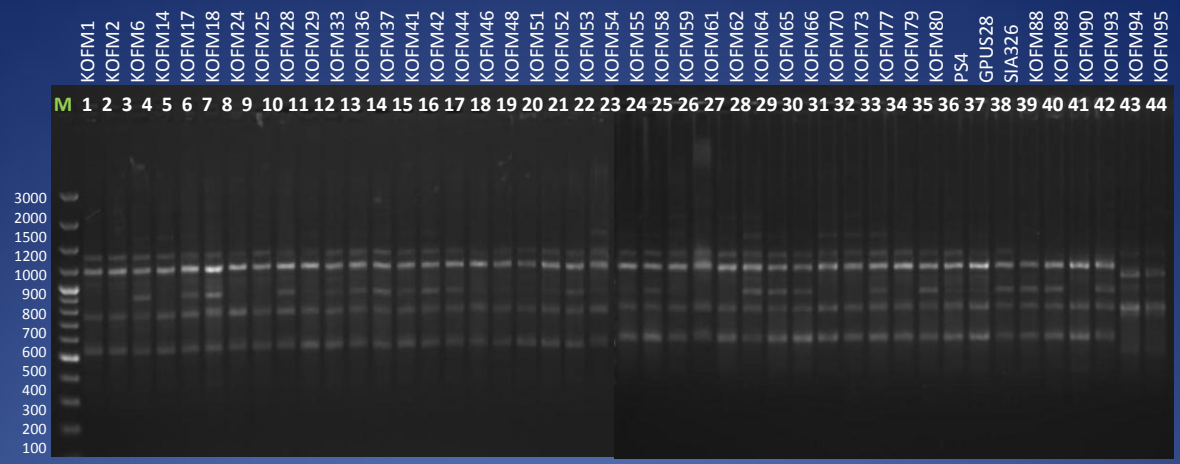
Table 4.4. Genotypic correlation coefficients between 12 characters in foxtail millet in E1, E2 environments and pooled

Sr. No.	Characters	Env	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers per plant	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
1	Days to panicle initiation	E1	**1.000**	0.985**	0.702**	0.148	0.132	-0.483**	0.148	-0.209	-0.344*	-0.211	-0.324*
		E2	**1.000**	0.921**	0.654**	0.117	0.151	-0.479**	0.117	-0.265	-0.281	-0.164	-0.335*
		Pooled	**1.000**	0.968**	0.688**	0.128	0.127	-0.475**	0.128	-0.240	-0.333*	-0.182	-0.332*
2	Days to 50% flowering	E1		**1.000**	0.742**	0.171	0.043	-0.534**	0.171	-0.196	-0.333*	-0.187	-0.308*
		E2		**1.000**	0.754**	0.148	0.038	-0.618**	0.148	-0.161	-0.342*	-0.110	-0.319*
		Pooled		**1.000**	0.754**	0.163	0.038	-0.564**	0.163	-0.181	-0.352*	-0.150	-0.314*
3	Days to maturity	E1			**1.000**	0.468**	-0.381*	-0.805**	0.468**	-0.136	-0.227	0.006	-0.252
		E2			**1.000**	0.409**	-0.330*	-0.781**	0.409**	-0.087	-0.225	0.047	-0.265
		Pooled			**1.000**	0.458**	-0.324*	-0.785**	0.458**	-0.113	-0.238	0.029	-0.261
4	No. of productive tillers per plant	E1				**1.000**	-0.412**	-0.444**	1.000**	0.359*	0.469**	0.190	-0.214
		E2				**1.000**	-0.588**	-0.444**	1.000**	0.400**	0.546**	0.201	-0.245
		Pooled				**1.000**	-0.476**	-0.468**	1.000**	0.368*	0.477**	0.202	-0.240
5	Plant height(cm)	E1					**1.000**	0.612**	-0.412**	0.195	0.190	-0.145	0.090
		E2					**1.000**	0.610**	-0.588**	0.144	0.033	-0.174	0.143
		Pooled					**1.000**	0.535**	-0.476**	0.153	0.112	-0.141	0.103
6	Panicle length (cm)	E1						**1.000**	-0.444**	0.215	0.220	0.086	0.124
		E2						**1.000**	-0.444**	0.207	0.234	0.077	0.194
		Pooled						**1.000**	-0.468**	0.206	0.221	0.074	0.155
7	No. of panicles per plant	E1							**1.000**	0.359*	0.469**	0.190	-0.214
		E2							**1.000**	0.400**	0.546**	0.201	-0.245
		Pooled							**1.000**	0.368*	0.477**	0.202	-0.240
8	1000 grain weight (g)	E1								**1.000**	0.440**	0.261	-0.118
		E2								**1.000**	0.481**	0.303*	-0.126
		Pooled								**1.000**	0.456**	0.282	-0.121
9	Straw yield per plant(g)	E1									**1.000**	0.271	-0.097
		E2									**1.000**	0.265	-0.109
		Pooled									**1.000**	0.270	-0.104
10	Protein content(%)	E1										**1.000**	-0.078
		E2										**1.000**	-0.183
		Pooled										**1.000**	-0.130
12	Grain yield per plant (g)	E1	-0.438**	-0.452**	-0.472**	0.346*	0.229	0.513**	0.346*	0.729**	0.581**	0.276	0.019
		E2	-0.405**	-0.478**	-0.460**	0.432**	0.004	0.470**	0.432**	0.656**	0.666**	0.316*	0.038
		Pooled	-0.475**	-0.503**	-0.510**	0.320*	0.110	0.512**	0.320*	0.706**	0.610**	0.319*	0.035

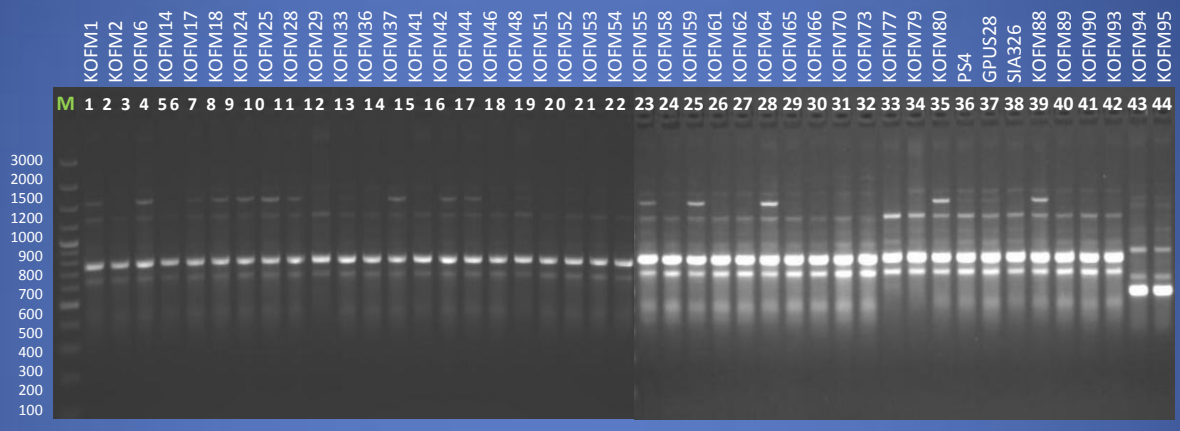
***,** -Significant at 5% and 1% level, respectively**

Table 4.5. Direct and indirect effects of 12 quantitative traits on grain yield in foxtail millet in E1, E2 environments, and pooled.

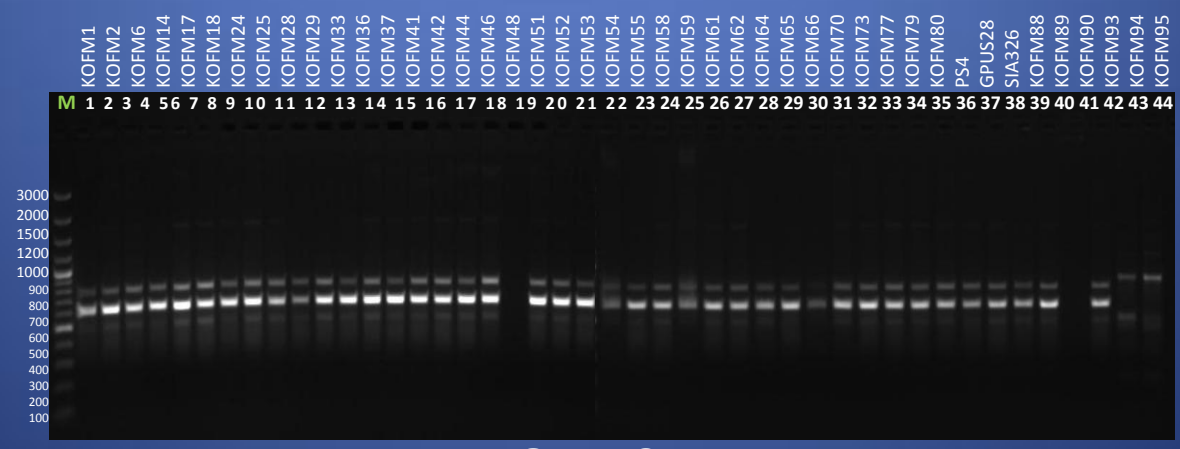
Sr. No.	Characters	Env	Days to panicle initiation	Days to 50 % flowering	Days to maturity	No. of productive tillers per plant	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	1000 grain weight (g)	Straw yield per plant (g)	Protein content (%)	Iron content (%)
1	Days to panicle initiation	E1	**-0.216**	-0.213	-0.152	-0.032	-0.029	0.104	-0.032	0.045	0.074	0.046	0.070
		E2	**1.215**	1.119	0.795	0.142	0.184	-0.582	0.142	-0.322	-0.341	-0.199	-0.407
		Pooled	**0.490**	0.474	0.337	0.063	0.062	-0.233	0.063	-0.118	-0.163	-0.089	-0.163
2	Days to 50% flowering	E1	0.207	**0.210**	0.155	0.036	0.009	-0.112	0.036	-0.041	-0.070	-0.039	-0.065
		E2	-0.327	**-0.355**	-0.267	-0.053	-0.013	0.219	-0.053	0.057	0.121	0.039	0.113
		Pooled	-0.339	**-0.350**	-0.264	-0.057	-0.013	0.198	-0.057	0.063	0.123	0.053	0.110
3	Days to maturity	E1	-0.315	-0.333	**-0.448**	-0.210	0.171	0.361	-0.210	0.061	0.102	-0.003	0.113
		E2	-0.230	-0.266	**-0.352**	-0.144	0.116	0.275	-0.144	0.031	0.079	-0.017	0.093
		Pooled	-0.358	-0.392	**-0.520**	-0.238	0.169	0.408	-0.238	0.059	0.124	-0.015	0.135
4	No. of productive tillers per plant	E1	0.093	0.108	0.294	**0.628**	-0.259	-0.279	0.628	0.226	0.295	0.120	-0.134
		E2	-0.056	-0.071	-0.196	**-0.479**	0.281	0.212	-0.479	-0.191	-0.261	-0.096	0.117
		Pooled	0.057	0.073	0.204	**0.446**	-0.213	-0.209	0.446	0.164	0.213	0.090	-0.107
5	Plant height(cm)	E1	0.013	0.004	-0.039	-0.042	**0.102**	0.062	-0.042	0.020	0.019	-0.015	0.009
		E2	-0.179	-0.045	0.391	0.696	**-1.183**	-0.721	0.696	-0.171	-0.039	0.206	-0.169
		Pooled	-0.014	-0.004	0.037	0.054	**-0.114**	-0.061	0.054	-0.017	-0.013	0.016	-0.012
6	Panicle length (cm)	E1	-0.147	-0.162	-0.245	-0.135	0.186	**0.304**	-0.135	0.066	0.067	0.026	0.038
		E2	-0.331	-0.427	-0.540	-0.307	0.421	**0.691**	-0.307	0.143	0.162	0.053	0.134
		Pooled	-0.134	-0.159	-0.222	-0.132	0.151	**0.283**	-0.132	0.058	0.062	0.021	0.044
7	No. of panicles per plant	E1	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000
		E2	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000
		Pooled	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	0.000
8	1000 grain weight (g)	E1	-0.079	-0.074	-0.051	0.136	0.074	0.081	0.136	**0.378**	0.166	0.099	-0.045
		E2	-0.236	-0.143	-0.077	0.356	0.128	0.184	0.356	**0.890**	0.428	0.269	-0.112
		Pooled	-0.110	-0.083	-0.052	0.169	0.070	0.095	0.169	**0.460**	0.210	0.130	-0.056
9	Straw yield per plant(g)	E1	0.031	0.030	0.020	-0.042	-0.017	-0.020	-0.042	-0.039	**-0.089**	-0.024	0.009
		E2	-0.157	-0.191	-0.126	0.306	0.018	0.131	0.306	0.269	**0.560**	0.148	-0.061
		Pooled	-0.011	-0.012	-0.008	0.016	0.004	0.007	0.016	0.015	**0.033**	0.009	-0.003
10	Protein content(%)	E1	-0.015	-0.013	0.000	0.013	-0.010	0.006	0.013	0.018	0.019	**0.069**	-0.005
		E2	0.005	0.003	-0.001	-0.006	0.005	-0.002	-0.006	-0.009	-0.008	**-0.029**	0.005
		Pooled	-0.022	-0.018	0.004	0.024	-0.017	0.009	0.024	0.033	0.032	**0.118**	-0.015
11	Iron content (%)	E1	-0.009	-0.009	-0.007	-0.006	0.003	0.004	-0.006	-0.003	-0.003	-0.002	**0.029**
		E2	-0.109	-0.103	-0.086	-0.080	0.046	0.063	-0.080	-0.041	-0.036	-0.060	**0.324**
		Pooled	-0.034	-0.032	-0.027	-0.025	0.011	0.016	-0.025	-0.012	-0.011	-0.013	**0.102**
12	Grain yield per plant (g)	E1	-0.438	-0.452	-0.472	0.346	0.229	0.513	0.346	0.729	0.581	0.276	0.019
		E2	-0.405	-0.478	-0.460	0.432	0.004	0.470	0.432	0.656	0.666	0.316	0.038
		Pooled	-0.475	-0.503	-0.510	0.320	0.110	0.512	0.320	0.706	0.610	0.319	0.035



OPD 5

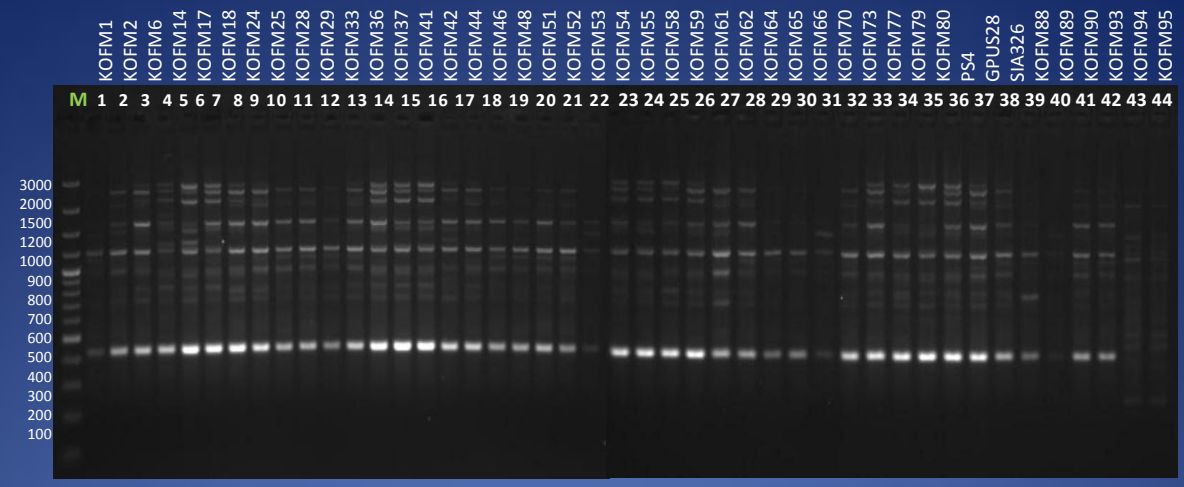


OPE 4

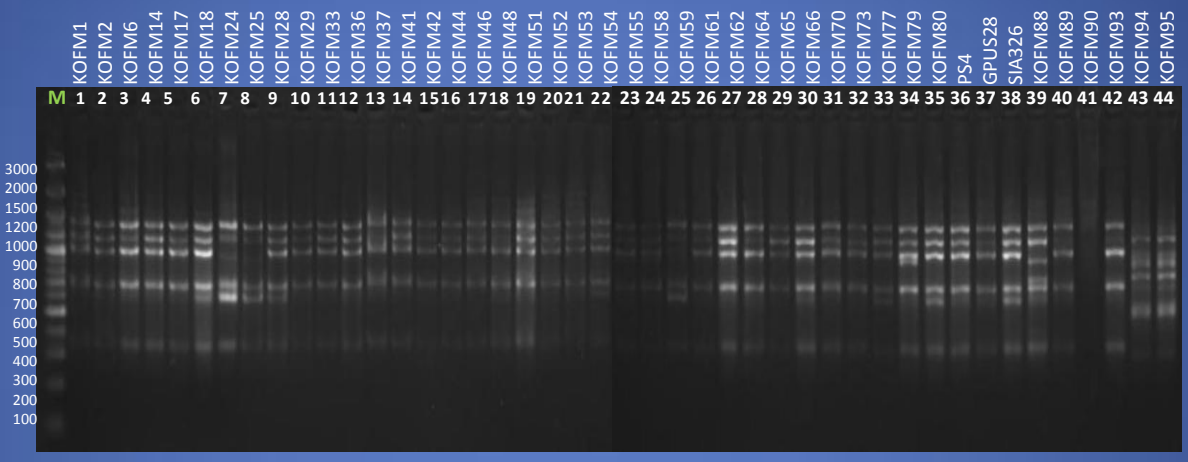


OPE 18

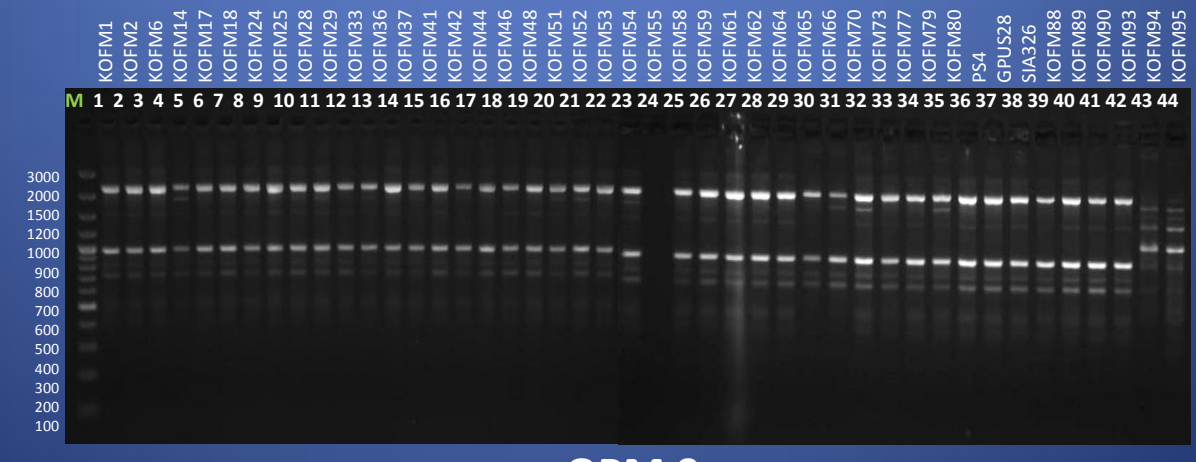
Plate 1: Random amplified DNA polymorphism of Foxtail Millet



OPK 9

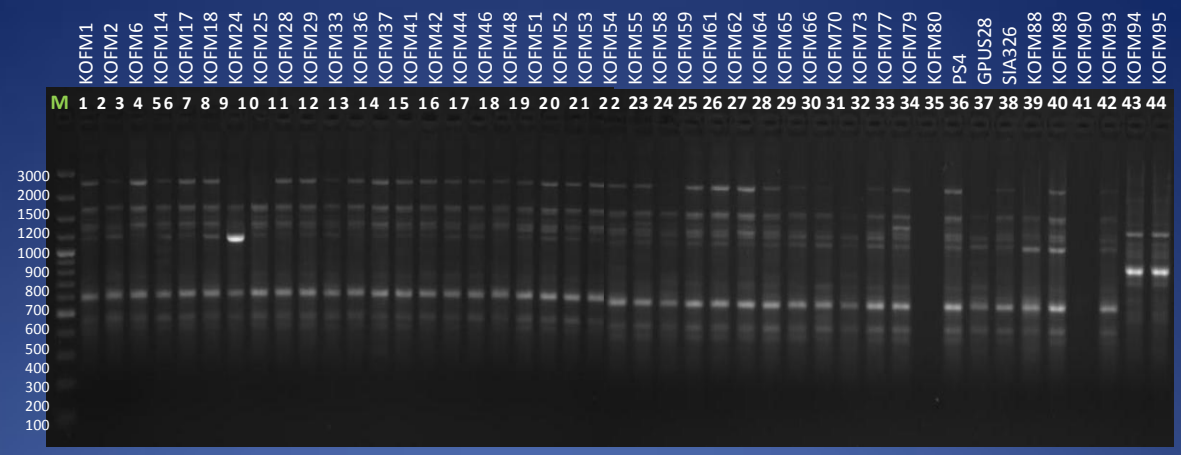


OPL 2

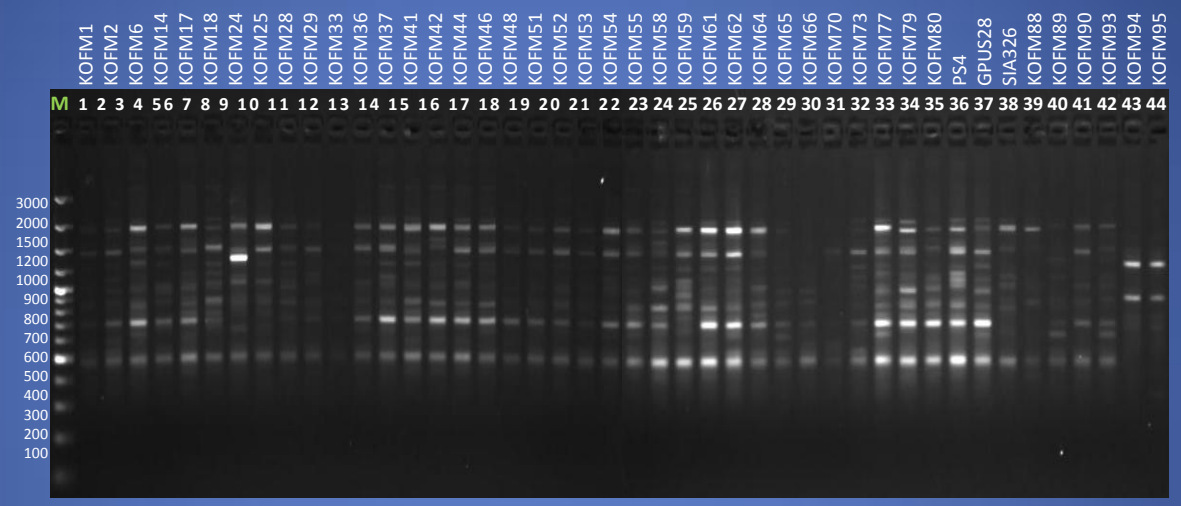


OPM 9

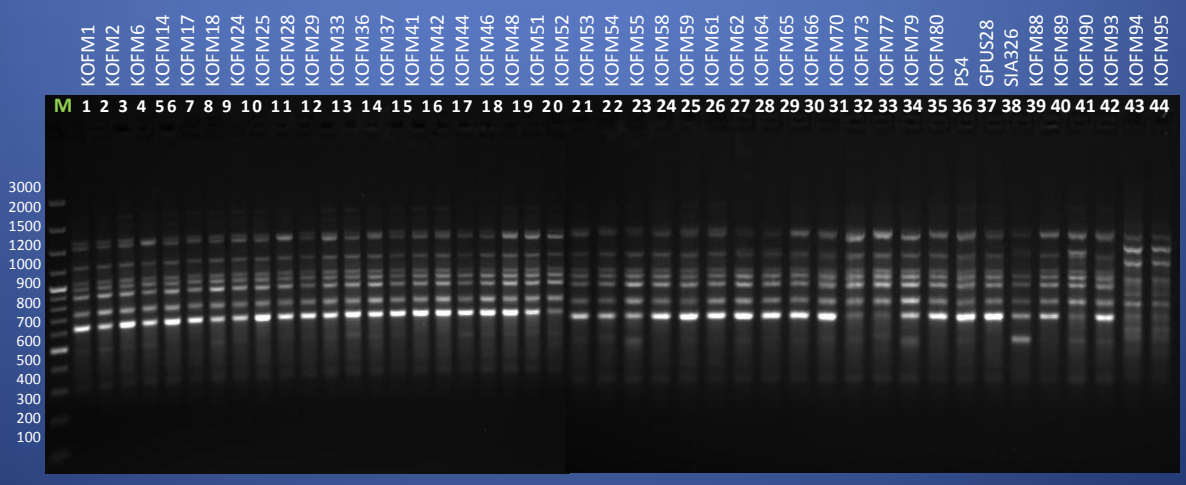
Plate 2: Random amplified DNA polymorphism of Foxtail Millet



OPM 17

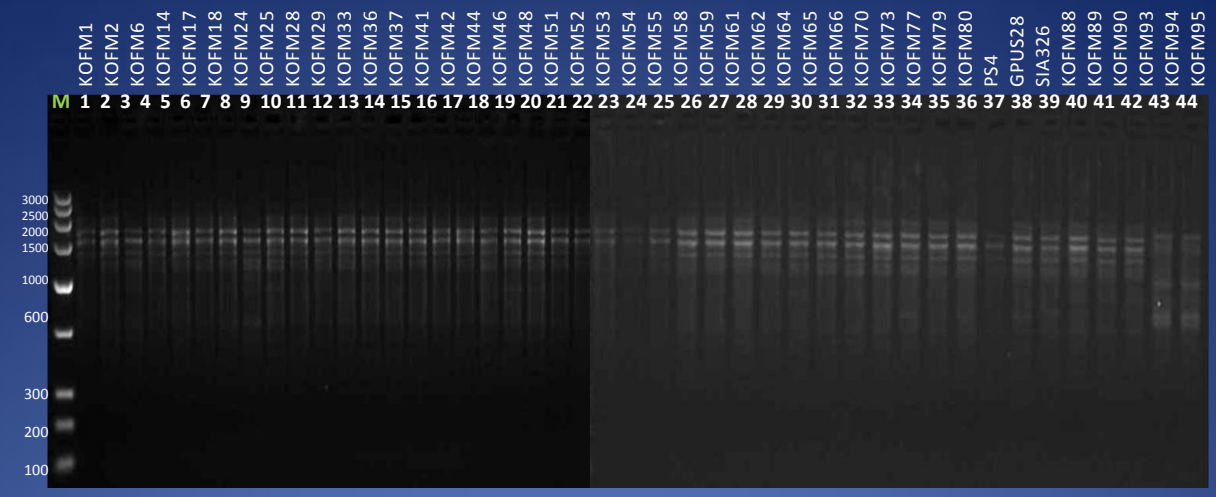


OPM 18

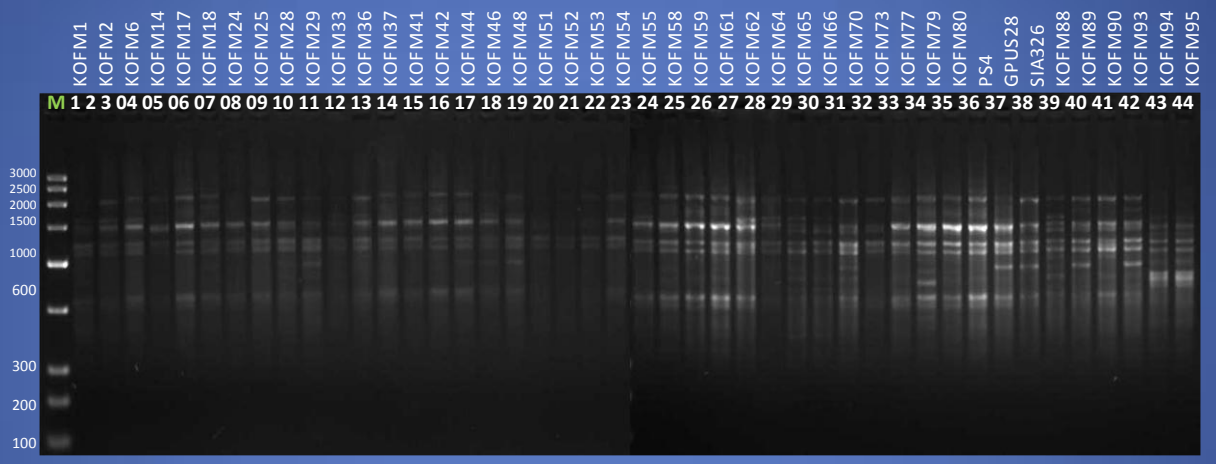


OPM 20

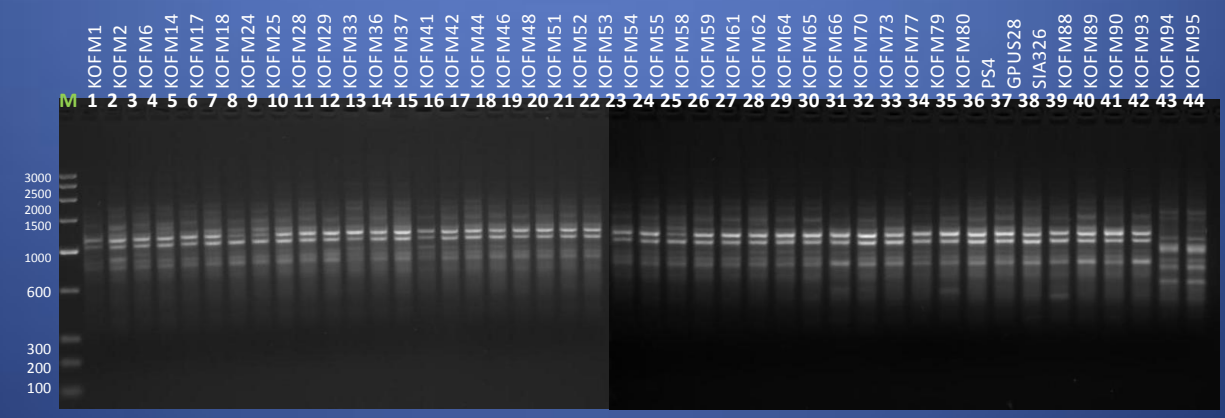
Plate 3: Random amplified DNA polymorphism of Foxtail Millet



ISSR 807

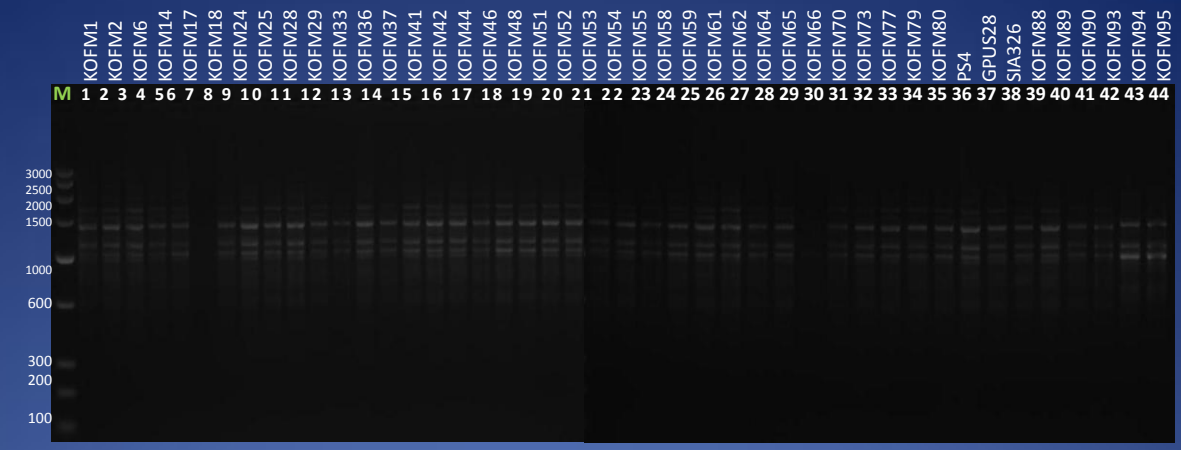


ISSR 808

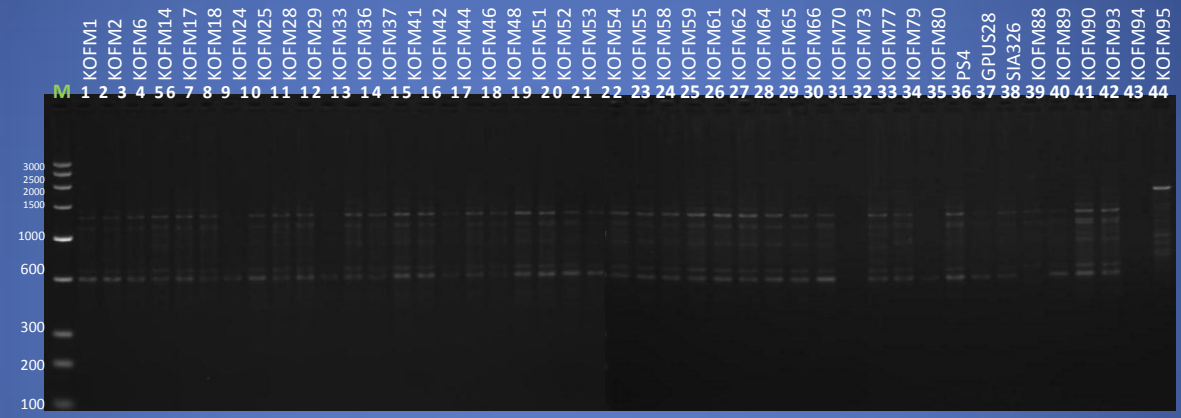


ISSR 809

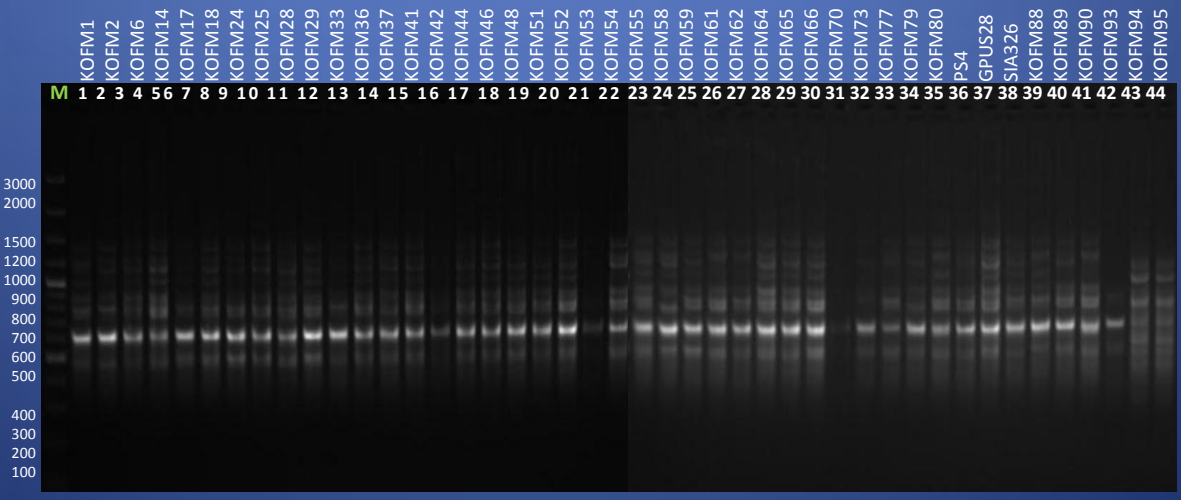
Plate 4: ISSR amplified DNA polymorphism of Foxtail Millet



ISSR 811



ISSR 880



ISSR 890

Plate 5: ISSR amplified DNA polymorphism of Foxtail millet

KOFM 94

KOFM 90

KOFM 36

KOFM 93

KOFM 36

KOFM 54

Plate 6: Morphological diversity in foxtail millet

KOFM 1 to KOFM 93

KOFM 94 and KOFM 95

Plate 7: Diversity for panicles and seed colour in foxtail millet