# GENETIC DIVERSITY IN GRAIN AMARANTHUS
# [*Amaranthus hypochondriacus* L.]

by

***Mr. RAUT VIJAYKUMAR RAKHAMAJI***
(Reg. No. 10/038)

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,**

**RAHURI – 413 722; DIST. AHMEDNAGAR,**

**MAHARASHTRA (INDIA).**

*In partial fulfilment of the requirements for the degree*
Of

**MASTER OF SCIENCE (AGRICULTURE)**

In

**AGRICULTURAL BOTANY**

**(GENETICS AND PLANT BREEDING)**

**DEPARTMENT OF BOTANY**
**POST GRADUATE INSTITUTE,**
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**2013**

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Approved by

**Prof. S.S. DODAKE**
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RAHURI – 413 722; DIST. AHMEDNAGAR (M.S.) INDIA
**2013**

# CANDIDATE'S DECLARATION

**I hereby declare that this thesis or part there of**

**has not been submitted by me or any other**

**person to any other University**

**or Institute for Degree**

**or Diploma**

**Place :** M.P.K.V., Rahuri **(V.R.Raut)**

**Date : / / 2013**

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# CERTIFICATE

This is to certify that the thesis entitled, **“GENETIC DIVERSITY IN GRAIN AMARANTHUS [*Amaranthus hypochondriacus* L.]”,** submitted to the faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra State) in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL BOTANY** (**GENETICS AND PLANT BREEDING)**, embodies the results of a piece of bonafide research carried out by **Mr.RAUT VIJAYKUMAR RAKHAMAJI,** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and sources of reference have been duly acknowledged.

Place : M.P.K.V., Rahuri **(S.S. Dodake)**

Date : / /2013

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Associate Dean,
Post Graduate Institute,
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Maharashtra State (India).

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Place : M.P.K.V., Rahuri (**S.G.Borkar**)
Date : / /2013

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Place : M.P.K.V ; Rahuri

Date : / /2013 **(V.R.Raut)**

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# LIST OF ABBREVIATIONS

–: minus
% : Percentage
$\sigma^2$ e : Environmental variance
$\sigma^2$ g : Genotypic variance
$\sigma^2$ p : Phenotypic variance
/ : Per
+ : Plus
< : Less than
= : Equal to
> : Greater than
C.D. : Critical difference
C.V. : Coefficient of variance
cm : Centimeters
D : Divergence
DC : Divergence classes
DC1 : Divergence class 1
DC2 : Divergence class 2
DC3 : Divergence class 3
DC4 : Divergence class 4
*et al.* : et all (and others)
g : Gram
GA : Genetic advance
GCV : Genotypic coefficient of variation
$H^2$ (b.s.) : Heritability in broad sense
i.e. : That is
mm : millimeters
No. : Number
PCV : Phenotypic coefficient of variation
*Per se* : Actual
R : Residual effect
S.E. : Standard error
*via* : through
*viz.,* : Videlicent (namely)
X : General mean
x : Multiplication
Y : Yield

%: Percent

# ABSTRACT

**"GENETIC Diversity IN GRAIN AMARANTHUS**
**(*Amaranthus hypochondriacus* L.)"**

by

**Mr. RAUT VIJAYKUMAR RAKHAMAJI**

A candidate for the degree
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Research Guide : Prof. S.S. DODAKE
Major discipline : Genetics and Plant Breeding

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The investigation on "Genetic Diversity in Grain Amaranthus (*Amaranthus hypochondriacus* L.)" was conducted to study genetic diversity in quantitative characters and study nature and extent of genetic diversity using molecular markers.

Twenty eight genotypes were evaluated in a randomized block design with three replications, during rabi 2011. The statistical analysis was done to determine the G.C.V., P.C.V., Heritability, Genetic Advance, and $D^2$ analysis using the data on nine quantitative characters. The quantitative characters were days

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to 50% maturity, days to maturity, plant height at maturity,

length of inflorescence, width of inflorescence, stem girth, grain yield per plant, grain yield per plot and grain weight.

Phenotypic coefficient of variation (PCV) was found to be equal or more than genotypic coefficient of variation (GCV) for all nine characters indicating the role of environment for expression of the traits. The grain yield per plant exhibited the highest GCV and PCV (29.31 and 31.90 respectively). It was followed by grain yield per plot and length of inflorescence The character days to maturity had least GCV and PCV. Heritability (b.s). For the characters *viz.,* days to 50%, flowering, days to maturity, grain weight, plant height at maturity, stem girth, grain yield per plant, grain yield per plot expressed high estimate of heritability in broad scence indicating the scope for selection of these traits. Days to 50% flowering, days to maturity and grain weight all showed highest 100% heritability and width of inflorescence showed less heritability 53%.Selections based on these characters would be most effective for achieving high yields in Amaranthus.

The $D^2$ statistic showed that there was adequate diversity amongst the strains with $D^2$ values ranging from 25.35 to 208.24 On the basis of these values, twenty eight strains under study were grouped into five clusters. Cluster I contained maximum number of genotypes (12) while cluster II had 8 genotypes.

Cluster III had 5 and cluster V had 2 genotypes while cluster IV was monogenotypic. The maximum intercluster distasnce was observed between cluster III and V (208.24) followed by cluster II and V (158.62).

A total 15 ISSR primers were used to amplify genomic DNA from 28 varieties. Out of 15 ISSR primers studied, 13 yielded PCR amplification products. A total of 61 bands were generated of which 53 bands were polymorphic with an average 86.88% polymorphic. None of the band was unique and 8 bands were monomorphism in their profile. Each primer thus produced on an average 4.69 bands were average polymorphic bands produced per marker and 4.07 bands were average polymorphic bands produced per marker.

In dendrogram all the genotypes studied were distinguished from each other. The grouping observed was moderately wide (similarity index 0.71-0.95). Five clusters were observed among them with Suvarna and SKGPA26 forming most distinct single genotype clusters.

Divergence results observed in dendrogram analysis were reflected in 2D PCO scatter plot analysis. The varieties Suvarna and SKGPA-26 were much diverse from others. Group X includes genotypes having most of the desirable characters the genotypes in cluster X are SKGPA-27, SKGPA-18, SKGPA-41, SKGPA-43, SKGPA-34, SKGPA-47, SKGPA-48, SKGPA-1 and BGA-44.

Cluster X is followed by cluster Z in terms of desirable character. The genotypes in cluster Z are BGA-51, SKGPA-21, SKGPA-28, SKGPA-52, BGA-28, SKGPA-50 and RGAS-92-10-1. No correlation was observed between molecular divergence with days to 50% flowering and plant height at maturity. Inter-cluster crossing can be used for further widening of genetic variability in grain amaranth breeding programme.

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# 1. INTRODUCTION

Grain Amaranthus (*Amaranthus hypochondriacus* L.) are important pseudocereals that are widely cultivated in India in the sub-Himalayan ranges and in the Nilgiri Hills of South India under the common name of Ramdanaa or Rajgira. Amaranthus are one of the oldest food crops in the new world. It is an ancient food crop reported to have been cultivated in Mexico.Amongst the three pseudo cereals (chenopods – *Chenopodium* spp.,buckwheat, *Fagopyrum* spp. and amaranth – *Amaranthus* spp.) grain amaranthus are the most important.

The genus *Amaranthus* is one of the annual coarse herbs distributed in temperate and tropical regions of the world (Sauer, 1967). The grain *Amaranthus* also called as 'Pigweeds", comprised of the genus *Amaranthus*, a widely distributed genus of short lived herbs, occurring mostly in temperate and tropical regions. It ranges from colours of purple and red to gold.

De Candolle (1883) and Vavilov (1950) considered Indo-Burma region to be its centre of origin, like *Amaranthus cruentus* L. (*Amaranthus paniculatus* L.).The genus is dibasic with X=16 chromosomes, almost equally distributed in section Amaranth.

*Amaranthus* cultivation is most widely spread in the mountain regions above 1500 m and extends upto 3000 m elevation in the hills of India. On the South Indian hills, the grain amaranthus is mostly mixed with ragi and rarely grown as pure crop (Anon, 1983). In North Indian hills, the grain crop is planted at the break of Monsoon and in South Indian hills, it is grown throughout the year.

The grain amaranth is a nutritious pseudocereal yielding high amounts of energy. *A. cruentus* L., *A. hypochondriacus* L. contains 14.5 to 16.0 per cent crude protein. The content of crude protein, crude fat, crude fibre and carbohydrates in *Amaranthus* ranges from 6.86-12.34, 4.06-6.38, 2.90- 4.34 and 63.13-68.78 per cent, respectively on dry weight basis (Munjal *et al.* (1999). The amino acid composition (in percentage) of *A.hypochondriacus* as Arginine 14.7, Histidine 2.9, Isoleucine 6.9, Leucine 8.0, Lysine 8.2, Methionine 2.5, Phenylalanine 4.7, Threonine 4.3, Tryptophan 0.9 and Valine 6.0. The amaranthus grains are the rich source of Ascorbic acid (70.6 – 109.9 mg/100 gm) and carotene 0.95 – 8.18 mg/100 gm). The vitamin C content in *A. cruentus* were 288 mg/100 gm fresh weight, while in *A. hypochondriacus* it was 209 mg/ 100 gm fresh weight.

In India, amaranthus assumes special significance as they are consumed during the religious days of fast. The seeds of Amaranthus are popped to be used in the preparation of Laddoos. It is also added to a mixture of flour to make pastries of pancakes, while the young leaves are used in soups. It could also be incorporated in maize tortillas or mixed with other flours to enrich their protein content. The leaves of Amaranthus are eaten in soups, broths and as a vegetable and are used in protein extracts, colouring and laxatives. The inflorescence are used for decoration and the stems as animal feed and fuel (Ravindra *et al.,* 1996). It is also used as starch in the pharmaceutical industry. An interesting application of amaranth is to use it as a food for people with allergies to other grains. The seed of grain amaranth is not a grain from a cereal plant, but is rather a pseudocereal from a dicotyledonous plant. It is unrelated to any other food crops that are commonly consumed, which makes it less likely to cause problems to people who have build up allergies.

Although grain amaranth is an ancient food crop of the old and new worlds, surprisingly very less breeding work has been done in any country to produce improved varieties of this protein rich grain.Thus analysis of genetic relationship in crop species is important component of crop improvement programme as it seems to provide information about genetic diversity.

The Mahalanobis $D^2$ statistcs is a powerful tool in quantifying the degree of divergence among genotypes. The distance between two clusters is the measure of the degree of divergence. Genotypes can grouped into different clusters by following Tochers method (Rao, 1952). The wide range of genetic diversity in grain amaranthus indicates the possibility of improving its productivity.

Assessment of patterns of *Grain amaranthus* genetic variation based on morphology or pedigree has its limitations. Complex quantitatively inherited traits are difficult to trace based solely on morphology. Therefore, DNA-based methods have been employed in studies of *Grain amaranthus* genetic diversity and in genetic improvement of the crop. Molecular markers are considered constant landmarks in the genome. Genetic distance estimates determined by molecular markers help identify suitable germplasm for incorporation into plant breeding stocks.

To overcome the problem of lack of easily understanding discoverable specific and stable morphological markers, use of molecular markers is most desirable.

With a view experimentation was performed to study genetic diversity in grain amaranthus with following objectives –

1) To measure genetic diversity in quantitative characters.
2) To study nature and extent of genetic diversity using molecular markers.

# 2. REVIEW OF LITERATURE

The literature pertaining to the present investigation has been reviewed,

## 2.1 Origin, Botany and Cytological Studies in Amaranthus

### 2.1.1 Origin of Amaranthus

By the excellent establishment and wide spread distribution of grain amaranthus in Asia, early botanists and explorers thought them to be indigenous to Asia.

De Candolle (1886) and Vavilov (1951), Darlington (1963) believed that grain amaranthus like *Amaranthus cruentus* L. arise in the Indo Burma region.

However, Sauer (1950, 1967) conducted that the domestication of grain Amaranthus began in America, based on the data from geographical, morphological, archeological, ethnobotanical and physiological studies.

The genus Amaranthus in represented in the country Senegal by 4 species, *Amaranthus hybridus* sub spp. *cruentus, A. graccizans, A.spinosus* and *A. viridis* and other two are *A. caudatas var viridis* and *A.caudatus var. purpureus.* The last species could be considered as separate species of genus Amaranthus because of its specific characters (Sarr, R.S., Noba, *et al.* 2006).

### 2.1.2 Botany

The family Amaranthaceae is dicotyledonous and consists of 60 genera and about 850 species. All plants are annual, herbaceous having almost of tropical origin, but with some adaptation to temperate climate as well.

The range of variation within the genus Amaranthus is described for the number of stem characters including morphology, epidermis, primary stem vasculature and mechanism of secondary growth.

The Amaranth plant is monoecious, cymes continuing above uppermost leaf to form large, compound terminal inflorescence, *tepals* and stamen 3-5, circumscissile utricle which allows easy threshing and winnowing. These exhibits two types of flowers staminate or pistillate.Large compound inflorescence producing enormous quantity of seeds which made this section as the member of successful grain crops.

### 2.1.3 Cytological Studies

In a recent study Mallika (1988), reported that members of section *Blitopsis* had a basic chromosome compliment of 17, while section Amaranthus had 16 and 17. The polyploidy mature of *A. dubius* (2n = 64) has also been confirmed.

## 2.2 Genetic divergence

Arunachalam and Bandyopadhyay (1984) devised method to delineate parental divergence into four divergence clases (D.C). To take into account the variable magnitude of variation in parental divergence, the mean (m) and standard deviation (s) of the values of intra and inter cluster divergence (D) were calculated.

Fatocum (1985) studied 40 accessions from twelve countries using cluster analysis of principal component analysis. Accessions formed six groups. The cluster analysis revealed that leaf length, leaf dry weight per plant and total above ground dry matter yield per plant were the most important characters in formulating the clusters.

Joshi (1986) in the study of twenty genotypes of *A.hypochondriacus* observed wide variability for height, number of leaves per plant, leaf length and width, inflorescence length, number of spikelets per plant, days to maturity and 1000 seed weight. Heritability estimates and expected genetic advance were high for 1000 seed weight, inflorescence length and height.

Tilekar (1986) studied variability and diversity in 105 strains of *A.cruentus*. The estimates of GCV, PCV, heritability and GA revealed plant height and days to 50 per cent flowering as the most important characters for improvement. These strains were grouped into 20 clusters. Clustering pattern of the strain does not follow the geographic distribution.

Joshi and Rana (1995) studied genetic divergence in 20 diverse genotypes grown during *kharif* 1991 and 1992. On the basis of D2 analysis, the genotypes were grouped into 9 clusters. These clusters were heterogeneous for geographical origin of genotypes. Popping size contributed the greatest divergence (65.48 per cent) followed by protein content (18.62 per cent). Genotypes of cluster II showed the highest grain yield, inflorescence length, spikelets or spike, leaf length and number of leaves.

Pawar (1995) observed higher estimation of GCV and PCV for number of branches per plant followed by grain yield per plant and leaf area per plant in grain amaranthus.

Lohithaswa *et al.* (1996) studied genetic variability, heritability and genetic advance for eleven characters in 144 genotypes of grain amaranth (*Amaranthus* species.). A considerable amount of phenotypic and genotypic variability was observed for fresh weight of plant, fresh weight of inflorescence, rachis per inflorescence, grain yield followed by dry weight of stem, stem girth at caller region and plant height. High heritability coupled with moderate genetic advance was observed for plant height and days to 50 per cent flowering indicating that additive genetic effects were operating for these characters and selection pressure could be applied on them for yield improvement.

Alba *et al.* (1997) studied diversity in some amaranthus entries. Twelve morphological, yield related quality and developmental traits were measured in 24 Amaranthus accessions, comprising 5 species., during 1991-1995 in South Italy. Greatest variation was observed for biological yield, yield of terminal inflorescence, grain yield and height. Following principal component analysis, the first component was associated with yield traits, qualitative traits such as oil and protein content characterized the second component, while the third component comprised traits directly connected with adaptability to agro climatic conditions. Accessions were classified into seven groups by hierarchical cluster analysis, irrespective of geographic origin or species tested.

Kamble (2000) studied genetic divergence in fifty genotypes of grain amaranthus. He observed adequate diversity among the genotypes with D2 values ranging from 7.13 to 14758.63. On the basis of D2 values, the fifty genotypes studied were grouped into eleven clusters. Clustering pattern of these genotypes did not necessarily follow the geographical distribution.

Verma *et al.* (2002) grouped sixty eight genotypes into nine clusters. The clustering pattern revealed that the genetic diversity might not be related to the geographical diversity. The average intercluster distance was maximum between clusters VIII and IX followed by VI and VIII, II and VIII and V and IX, indicating that these groups of genotypes were highly divergent from each other. The genotypes in these clusters revealed substantial differences in the means for the four traits studied.

Genotypes IC 95453, IC 35746, IC 35634 and IC 35778 were important for a breeder for exploiting their potential in breeding programme.

Shukla and Singh (2002) studied genetic divergence in 66 strains of grain amaranthus. The 66 strains could be grouped in nine clusters depending upon the genetic constitution of strains. The cluster VIII had maximum grain yield, days to maturity, plant height, inflorescence per plant and highest leaf size.

Ran Milan Pandy (2002) studied 26 acessions of grain amaranthus (*A.hypochondriascus*) including both indigenous and exotic introductions, were evaluated in the rabi season (winter crop, November-March) based on $D^2$ analysis , the accesions were grouped into Eleven clusters. Clusters I, II and II had seven, four and three accesions ,respectively cluster VII,VIII,IX and X has only one accesins in each accesions in each case. The accession in cluster V had greatest, divergence, closely followed by those clusters IV and I the maximum and minimum divergence were revealed between clusters VIII and XI and between II.

Random amplified polymorphic DNA(RAPD) analysis was performed to study the genetic diversity in the three grain amaranthus – *Amaranthus hypochondriacus, A.caudatus, A.cruentus* comparising a total of 17 accessions. As many as 13 bands were identified and the extent of polymorphism was highest in *A.cruentus* with 69.2% folllowed by *A.caudatus* 38.5%

and having 15.4%. There *A.caudatus* accessions recorded 60-80% similarity followed by 50% between two accessions of *A.cruentus,* while 12 accessions of *A.hypochondricous* displayed a similarity pattern ranging from 10-100%. The clustering pattern of gel fragments through dendrogram analysis revealved that *A.cruentus* stood apart while *A.hypochondricus* and *A.caudatus* overlapped. The study demonstrated much higher level of genetic similarity between *A.hypochondriacus* and *A.caudantus*. The RAPD profile developed from primer 1 and 2 indicated a strong possibility of a single common progeniter of these three grain amaranth species.

Pandey (2003) reviewed three different aspects in grain amaranthus research for its genetic improvement, including (i) the study on plant genetic structure and function to develop suitable plant type, (ii) the analysis of genetic structure for different plant characters and formulation of rational breeding approach and (iii) the restructuring of plants (such as *A. hypochondriacus*) by polyploidy as well as by interspecific hybridization to generate genetic variation and new gene combinations.

Kanthaswamy (2006) studied genetic divergence in 74 amaranthus genotypes from different localities using D2 statistics in an experiment conducted in Tamil Nadu, India. The genotypes were grouped in 12 clusters, with cluster I having 52 genotypes. Intracluster distance was highest between cluster XI and XII. Based on the mean performance of genotypes, genetic distance of clustering pattern, it was concluded that hybridization between genotypes from cluster XI and XII will produce highly heterotic hybrids.

# 3. MATERIAL AND METHODS

The present investigation "Studies on Genetic Diversity in Grain amaranthus [*Amaranthus hypochondriacus* ] " was carried out at All India Coordinated Research Network on Underutilized Crops, and 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

The twenty eight genotypes of *Amaranthus hypochondriacus* for the present investigation were obtained from the Plant Breeder, AICRP on Underutilized Crops , Mahatma Phule Krishi Vidyapeeth, Rahuri. The list of genotypes used is given in Table 3.1.

## 3.2 Methods

### 3.2.1 Experimental design

The experiment was conducted in Randomised block design with three replications with spacing of 45 x 15 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 genotypes in each replication and averages were worked out.

### 3.2.2.1 Days to 50 per cent flowering (No.)

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

### 3.2.2.2 Days to maturity (Nos.)

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

### 3.2.2.3 Plant height at maturity (cm)

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

### 3.2.2.4 Length of inflorescence (cm)

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

### 3.2.2.5 Width of inflorescence (cm)

Width of Inflorescence was recorded from each observational plant in centimeter.

### 3.2.2.6 Stem girth (cm)

The girth of the stem was recorded in centimeter.

### 3.2.2.7 Grain yield per plant (g)

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

### 3.2.2.8 Grain yield per plot (g)

Grain yield per plot was measured in gram by taking the total seed weight per plot.

### 3.2.2.9 Grain Weight (g/10ml)

Grain Weight was measured in g/10ml.

**Table 3.1 List of Genotypes**

| Sr.No. | Genotype | Sr.No. | Genotype |
|---|---|---|---|
| 1 | SKGPA-27 | 15 | BGA-50 |
| 2 | SKGPA-41 | 16 | SKGPA-23 |
| 3 | SKGPA-43 | 17 | SKGPA-10 |
| 4 | SKGPA-47 | 18 | SKGPA-31 |
| 5 | SKGPA-48 | 19 | SKGPA-20 |
| 6 | SKGPA-1 | 20 | BGA-43 |
| 7 | BGA-44 | 21 | BGA-42 |
| 8 | SKGPA-34 | 22 | BGA-29 |
| 9 | BGA-51 | 23 | SKGPA-16 |
| 10 | SKGPA-28 | 24 | SKGPA-26 |
| 11 | SKGPA-21 | 25 | BGA-49 |
| 12 | SKGPA-52 | 26 | SKGPA-18 |
| 13 | BGA-28 | 27 | RGAS-92-10-1(C) |
| 14 | SKGPA-50 | 28 | Suvrna(c) |

### 3.3 Statistical analysis

The mean value of randomly selected observational plants for nine 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 according to Panse and Sukhatme (1985) in the following form.

| **Source of variation** | **D.F.** | **MSS** | **Expected mean sum of squares** |
|---|---|---|---|
| Replications | r-1 | MSr | $\sigma^2 e + t\, \sigma^2 r$ |
| Treatments | t-1 | MSt | $\sigma^2 e + r\, \sigma^2 t$ |
| Error | (r-1) (t-1) | MSe | $\sigma^2 e$ |
| Total | (rt-1) | | |

Where,

r = Number of replications

t = Number of treatments

#### 3.3.2 Estimation of coefficient of variation

The genotypic and phenotypic coefficient of variation were calculated by formula suggested by Burton (1952).

i) Genotypic coefficient of variation (GCV)

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

Where,

$\sigma^2$ g = Vg = Genotypic variance

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

ii)Phenotypic coefficient of variation (PCV)

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

Where,

$\sigma^2$ p = Vp = Phenotypic variance

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

### 3.3.3 Estimation of Heritability percentage

Heritability percentage in broad sense was calculated as per the formula given by Burton (1952).

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

Where,

$h^2$ (b s) = Heritability percentage in broad sense

$\sigma^2$ g = (Vg)= genotypic variance

$\sigma^2$ p = (Vp) = phenotypic variance

### 3.3.4 Estimation of Genetic Advance

Genetic advance was calculated by the formula suggested by Johnson *et al.* (1955).

$$GA = K \times (\sigma^2 g / \sigma p) \times \sigma^2 p$$

or

$$GA = K \times h^2 \times \sigma p$$

Where,

K = Selection differential at 5 per cent selection intensity.( i.e. 2.06).

$\sigma^2$ g (Vg) = Genotypic variance.

$\sigma^2$ p (Vp) = Phenotypic variance.

$\sqrt{Vp}$ or $\sigma$ p = Phenotypic standard deviation.

### 3.3.5 Mahalanobis generalized distance ($D^2$)

The generalized distance between two populations is defined by Mahalanobis (1936) as:

$$D^2 = \sum \lambda\ i.j..\ di.\ dj.$$

Where,

$\lambda$ i.j. = Reciprocal matrix to the common dispersion matrix.

di = Difference between the mean values of two populations for $i^{th}$ character.

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

Estimation of $D^2$ values from the above formula is very complicated in the present study. Since, it requires the inversion of a thirteenth order determinant and then the evaluation of B (BH)/ 2 terms whose sum is $D^2$. It was found convenient to work with a set of uncorrelated characters constructed from the original measurements. $D^2$ with such transformed variables reduces to the evaluation of a simple sum of squares. Transformation was done by using pivotal condensation method (Singh and Chaudhary, 1977). The coefficients for the transformation were obtained by dividing the first row of the reduced matrix by the square root of the corresponding pivotal condensation elements.

### 3.3.6 Determination of gene constellations

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 a well defined term. The only criterion appeared to be that any two groups belonging to the same cluster should be at least on an average shows a smaller $D^2$ than those belonging to two different clusters. A simple device suggested by K. D. Tocher is to start with two closely associated groups and find a third group which has the smallest average $D^2$ from the first two. Similarly, the fourth is chosen to have the smallest $D^2$ from the first three and so on. If at any stage the average $D^2$ of a group from those already listed appears to be high, then this group does not fit in with the former groups and is therefore taken outside the former cluster. The groups of the 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 the inclusion of an additional group. If the changes are appreciable then the newly added group has to be considered as outside the cluster.

### 3.3.7 Average intra and inter cluster $D^2$ and D values

#### 3.3.7.1 Average intra cluster $D^2$

$$D^2 = \sum D^2 i / n$$

Where,

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

#### 3.3.7.2 Average inter cluster $D^2$

$D^2 = \sum$ distance between the population of cluster i and j./ ni, nj

Where,

ni = number of populations in the cluster i.

nj = number of populations in the cluster j.

#### 3.3.7.3 Average intra and inter cluster distance

$D = \sqrt{D^2}$

### 3.3.8 Cluster means

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

### 3.3.9 Cluster diagram

With the help of $D^2$ values between the clusters, a diagram showing the relationships between different populations was drawn.

## 3.4. Molecular marker Study

The methods followed for the molecular marker studies is described as below.

### 3.4.1 Isolation of genomic DNA

The DNA was isolated from each genotype by following CTAB extraction method (Saghai–Maroof *et al.,* 1984).

1. One to two grams of fresh young leaf (not fully expanded and less than a week old) from shoot apex was taken. The surface was cleaned with wet paper and towel. The sample was ground to a fine powder using liquid nitrogen with pre-chilled pestle and mortar.

2. The powder was transferred into a sterile 2.0 ml eppendorf tube. Then added 0.8 ml of 2% DNA extraction buffer in each tube maintained at $65^0$C in water bath, The solution was vortexed for 30-40 seconds, mixed vigorously and

incubated at 65 $^0$C for 1 hour with 2-3 times occasional shaking during incubation.

3. The solution was centrifuged at 8000 rpm for 10 min.
4. The tubes were kept for 10 min to allow them to return to the room temperature.
5. The tubes then filled with equal volume (800 µl) of chloroform and Isoamyl alcohol (CIA) solution prepared in a ratio of 24:1, and mixed gently by inverting for 5 min.
6. The solution was vortex for 40-60 seconds and centrifuged at 12000 rpm for 10 minutes.
7. The aqueous supernatant solution was pipette out and transferred to a fresh tube for DNA isolation.
8. DNA was precipitated by adding 0.6 volume (480 µl) of ice-cold isopropanol to the aqueous phase.
9. The solution was kept for an hour at freezing temperature to enhance the precipitation.
10. The Supernatant was discarded (keeping the pellet as it was).
11. The DNA pellet was washed with 70 per cent alcohol (500 µl) and centrifuged at 12000 rpm for 10 minutes, pellet was recovered.
12. The tubes were allowed to drain and then air dried at the room temperature for 30 min.

13. Then DNA was suspended in 400 µl of high salt TE (Tris EDTA) buffer in sterile eppendorf tube and kept for 45 minutes at the room temperature.
14. When the DNA was fully suspended, 1 µl of RNase (10 mg/ml) was added and the tubes were incubated at 37°C for one hour.
15. An equal volume of (400 µl )cold phenol: chloroform : iso-amylalcohol (25:24:1 v/v/v) was added and mixed well (The molecular grade phenol buffered to pH 8 was used). The solution was centrifuged for five minutes at 12,000 rpm.
16. The top layer was pipetted in to another eppendorf tube and an equal volume of chloroform:iso-amylalcohol (24:1 v/v) solution was added and centrifuged for five minutes at 12,000 rpm.
17. The top layer was pippetted in to new epperndorf tube and 0.5 volume of 7.5 mM ammonium acetate solution and two volumes (400 µl) of absolute ethanol was added, mixed thoroughly and then centrifuged at 12,000 rpm for 10 minutes.
18. The DNA pellet was removed and washed twice with 200 µl of 70% ethanol. The supernatant solution was dispensed and spin at 12000 rpm for 10 minutes.
19. The DNA was dissolved in 50 to 100 µl of TE (Tris-EDTA) buffer.

### 3.4.2 Determination of quantity and quality of isolated DNA

The genomic DNA isolated from the individual plant was quantified spectrophotometrically both at 260 ηm and 280 ηm (Nanodrop,ND-1000 USA). A pure sample of DNA shows the ratio of OD 260/280 as 1.8. The ratios less or more than this indicated contamination in the preparation either with phenol or with proteins. The values higher than this indicate presence of the RNA in the preparation. The ratios of OD at 260 ηm over OD at 280 ηm were calculated to separate the contaminants from the sample DNA. Computed OD values were used to dilute the DNA samples to working concentrations.

### 3.4.3 Agarose Gel Electrophoresis

The isolated grain amaranthus genomic DNA from twenty eight samples was run on Agarose gel to accomplish the quality determination.

**Reagents**

1. Agarose
2. TBE buffer (10 X) : 1 M Tris base, 830 mM boric acid,10mM EDTA, pH 8.0
3. TBE buffer (1 X) : The 10 X buffer was diluted to 100ml distill water.
4. Ethidium Bromide : 10 mg/ml
5. Tracking dye : 1 percent (w/v) bromophenol blue + 20 percent (w/v) Ficoll + 10 mM EDTA

**Procedure**

1. The DNA degradation and contamination with other substances were checked by electrophoresis of an aliquot of the sample in agarose gel of 0.8%. It is assumed that the large molecular weight DNA appear as a band with stripes, whereas partially degraded material forms a smear of long to small fragments.
2. The ends of gel tray were sealed with spaces and comb was inserted.
3. Agarose gel (0.8%) was prepared by adding 0.80 gm agarose to 100 ml of TAE (1X) buffer (EDTA 0.5 M at pH8).
4. The solution was boiled by keeping solution containing borosil flask in microwave oven and then cooled to the room temperature.
5. Ethidium bromide (1 µl /10ml) was added to the gel and was mixed gently.
6. The gel was poured into tray carefully without forming air bubbles. After the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank(Bio Red Sub Cell model 96 USA).
7. Approximately 500 ml of TAE (1X) buffer was poured into the electrophoresis tank, enough to cover the gel to a depth of 5mm.

8. About 1/10th volume of loading buffer (6X) bromophenol blue dye was added to the DNA samples and mixed by gentle tapping and spinning 2-3 sec in a micro centrifuge.
9. The DNA samples were loaded into the wells and power supply of about 5 V/cm (80 V) was provided to run the gel.
10. The power supply was switched off when the dye front was about 2 cm from the other end and the gel was removed from the gel apparatus.
11. After electrophoresis the band intensity of genomic DNA was visualized and photographed by using gel documentation system (Flor Chem ™ Alpha Innotech, USA) and compared with standard lambda DNA.

### 3.4.4 Polymerase Chain Reaction

Various components required for the PCR reaction are as follows.

### Template DNA

The purified genomic DNA extracts of the all the genotypes were used as template DNA.

#### 3.4.4.1 Primers

PCR amplification was performed with Inter simple Sequence Repeats (ISSR) primers. The commercial custom synthesized primers were procured from M/S Bangalore Genei Pvt. Ltd. A total of 15 ISSR primers were used for amplification.

The commercial design random ISSR primers were procured from M/S Bangalore Genei Pvt. Ltd (Table- ). The working concentrations of random primers (5 pM per µl) and specific (2 pM per µl) primers were prepared using the autoclaved sterile distilled water.

**Taq DNA polymerase**

Taq DNA polymerase (3 units per µl) and 10X Taq buffer were obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

**dNTPs**

Individual dNTPs such as dATP, dTTP, dGTP and dCTP were obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

### 3.4.4.2 PCR reaction mixture for ISSR markers

Amplification reaction mixture was prepared in 0.2 ml thin walled flat cap PCR tubes, containing the following components. The total volume of each reaction mixture was 20 µl.

### 3.4.4.3 PCR amplification

The PCR amplification for ISSR analysis was performed according to Williams *et al.* (1990) with certain modifications. The amplification conditions used were as follows given in table3.4.4.3.1 .

**Table 3.2. : ISSR markers used for the study.**

| Sr. No. | Primers | Used T anneal (°C) |
|---|---|---|
| 1. | ISSR-801 | 45 |
| 2. | ISSR-847 | 45 |
| 3. | ISSR-841 | 45 |
| 4. | ISSR-857 | 53 |
| 5. | ISSR-827 | 55 |
| 6. | ISSR-834 | 46 |
| 7. | IS-8 | 52 |
| 8. | IS-13 | 55 |
| 9 | IS-12 | 48 |
| 10. | 8081 | 48 |
| 11. | 8082 | 48 |
| 12. | 8386 | 48 |
| 13. | 8932803 | 46 |
| 14. | 8932799 | 46 |
| 15. | 8932801 | 51 |

**Table 3.3. Various chemical components required for the PCR reaction**

| Sr No. | Components | Amount in «l | |
|---|---|---|---|
| | | ISSR | Specific |
| 1. | Taq Buffer A with $MgCl_2$ | 2.0 | 2.0 |
| 2. | dNTP | 2.0 | 2.0 |
| 3. | Water | 13.7 | 13.7 |
| 4. | Primer | 1.0 | 1.0 |
| 5. | Taq DNA Polymerase(3U/µl) | 0.3 | 0.3 |
| 6. | DNA | 1.0 | 1.0 |

**Table 3.4. Step wise cycles of temperature and duration required for the PCR amplification**

| Sr. No | Steps | Temperature ($^{o}$C) | Duration | Cycles |
|---|---|---|---|---|
| 1. | Denaturation | 94 | 5 min | 1 |
| 2. | Denaturation | 94 | 30 Sec | 40 |
| 3. | Annealing | 45-55 | 30 Sec | |
| 4. | Extension | 72 | 1 min | |
| 5. | Final Extension | 72 | 10 min | 1 |
| 6. | Final hold | 4 | Until removed | |

### 3.4.5. Agarose gel electrophoresis

1. Electrophoresis unit (gel casting trough, gel combs, power pack)
2. UV transilluminator
3. Solutions required
4. Agarose

5. Ethidium bromide: 10 mg/ml.
6. Bromophenol blue (Loading dye)

i. Stock solution of 10X TBE: 107.8 g Tris (1 M), 55.03 g Boric acid (830 mM) and 43.84 g EDTA (10 mM) to make final volume 1000 ml and adjust pH 8.0.
ii. Working solution of 1X TBE: Ten ml of 10X TBE was diluted to 100 ml using milli Q water.

#### 3.4.5.1 Separation of amplified products by Agarose gel electrophoresis

Agarose gel of 1.2 per cent was prepared using the electrophoresis grade agarose in a volume of electrophoresis buffer (1x TAE) sufficient for setting a gel (100 ml). Then ethidium bromide was added at concentration of 1 μl per 10 ml (10 mg/ml) of gel. The gel was allowed to set fully before removing the comb and loading the sample. Five μl of loading dye was added to 20 μl of PCR products and mixed well before loading into the wells. Care was taken to prevent mixing of samples between the wells. A voltage of 1.5 V/cm was given for a period of three hours for separation of PCR fragments. After the run, the gel was viewed under UV light and the DNA banding pattern was observed directly in gel documentation unit (gel doc). The banding pattern itself was noted from the digital image of the gels and analyzed further for identifying polymorphic bands.

**Molecular analysis**

The amplified ISSR markers were scored as present (+) and absent (-) for each sample. Ambiguous bands that could not clearly distinguished were not scored (Williams *et al.* 1990). The similarity index of sample was calculated by using formula given by Chapco *et al.* (1992).

$$\text{Similarity index} = \frac{2\,N_{AB}}{N_A + N_B}$$

Where,

$N_{AB}$ = No. of bands shared by A and B

$N_A$ = No. of bands in A

$N_B$ = No. of bands in B

### 3.4.6. Data analysis

The clearly resolved PCR amplified bands of Grain Amaranthus species with 15 different ISSR primers were scored manually for their presence (1) and absence (0) in the data sheet. The analyses were carried out using the computer package NTSYSpc 2.02i (Rohlf, 1998). Binary Data matrix was analyzed using SIMQUAL module with Dice similarity coefficients calculated as per model suggested by Nei and Li (1979). SAHN module based on Unweighted Pair Group Method Using Arathmatic Averages (UPGMA) method was employed for clustering analysis to generate a tree (dendrogram).

Similarly to get 2D scatter plots, Principal Co-Ordinate (PCO) analysis was performed to estimate the genetic distance between each group of the varieties using NTSYS software. It involved first transforming similarity values to scalar products by DCENTER, then analysing the product matrix by EIGEN to get Eigenvectors (which is PCO) and Eigenvalues, and finally getting its 2-Dimensional scatter-plot graph.

# 4. EXPERIMENTAL RESULTS

The results of the investigation entitled "Genetic Diversity in grain amaranuthus (*Amaranthus hyporchondricus*)"are presented below.

## 4.1 Mean performance of Genotypes

The mean performance of nine characters in twenty eight genotypes of grain amaranthus is presented in Table-4.1.

### 4.1.1 Days to 50% flowering (No.)

The The genotype SKGPA-27 flowered in 81.00 days and was the earliest among all genotypes, followed by SKGPA-47 (82.00 days) and RGAS-92-10-1 (83.00 days). Maximum days for flowering were required for the genotypes SKGPA-21 (97.00 days), followed by BGA-50 (96.00 days) and SKGPA-26, SKGPA-31 (95.00 days). The general mean for this character was 89.93 days.

### 4.1.2 Days to maturity (No.)

Days required for maturity ranged between 120.00 and 141.00 days. The general mean for this character was 133.14 days. The early maturing genotypes were BGA-29 (120 days) and SKGPA-47 (122 days) and the late maturing genotypes were SKGPA-1 (141 days) and SKGPA-16 (140 days).

### 4.1.3 Plant height at maturity (cm)

SKGPA-41 (148.77 cm) was the tallest among all genotypes followed by SKGPA-1 (146.77 cm). The dwarfest genotype was SKGPA-52 (83.70 cm) followed by SKGPA-20 (86.17). The genotype mean of plant height was 106.66 cm within the range of 83.17 to 148.77 cm.

### 4.1.4 Length of inflorescence (cm)

The mean for inflorescence length was 41.84 cm having the range of 34.27 to 52.20 cm. The minimum inflorescence length was observed for SKGPA-20 (34.27 cm) followed by BGA-42 (34.67 cm) and the maximum inflorescence length was of genotypes RGAS-92-10-1 (62.20 cm) followed by SKGPA-47 (61.60 cm).

### 4.1.5 Width of inflorescence (cm)

The mean for width of inflorescence was 10.49 cm having the range of 8.67 to 13.30 cm. The minimum inflorescence width was in SKGPA-20 (8.67 cm) followed by BGA-42 (8.70 cm) and the maximum width of inflorescence was in genotype RGAS-92-10-1 (13.30 cm) followed by SKGPA-47 (13.00 cm).

### 4.1.6 Stem Girth (cm)

The mean for stem girth was 1.73 cm having the range of 0.96 to 2.67 cm. The minimum stem girth was observed in SKGPA-52 (0.96 cm) followed by SKGPA- 10(0.97 cm) and the maximum stem girth was of genotypes SKGPA-41 (2.67 cm) followed by BGA-44 (2.59 cm).

### 4.1.7 Grain yield per plant (g)

The mean for grain yield per plant was 15.25 g having the range of 7.90 to 24.83 g. the genotype with minimum grain yield per plant was BGA-49 (7.90 g) and SKGPA-23 (9.56 g) and the genotype having maximum grain yield per plant were SKGPA-18 (24.33 g) and SKGPA-28 (24.01 g).

### 4.1.8 Grain yield per plot (g)

The mean for grain yield per plot was 594.15 g having the range of 284.33 to 876.33 g The genotype with minimum grain yield per plot was BGA-49 (284.33 g) followed by SKGPA-23 (344.33 g) and the genotype with maximum grain yield per plot was SKGPA-18 (76.33 g) followed by SKGPA-28 (864.33).

### 4.1.9 Grain weight (g/10ml)

The mean for Grain weight was 8.57 g/10 ml having the range of 7.75 to 9.17 g/ 10 ml. The genotype with minimum grain weight was SKGPA-1 (7.75 g/10 ml) followed by Suvrna (7.87 g/10 ml) and the genotype having maximum grain weight was SKGPA-23 (9.17 g/10 ml) followed by BGA-29 (9.10 g/10 ml).

# Table 4.1 mean performance of twenty eight genotypes of grain amaranthus for various characters.

| Sr.No. | Character | Days to 50% flowering | Days to Maturity | Plant height at Maturity (cm) | Length of inflorescence (cm) | Width of Inflorescence (cm) | Stem girth (cm) | Grain yield per plant (g) | Grain yield per plot (g) | Grain weight (g/10ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Variety | | | | | | | | | |
| **1** | SKGPA-27 | 81.00 | 127.00 | 99.97 | 41.00 | 10.27 | 1.60 | 13.71 | 493.33 | 8.67 |
| **2** | SKGPA-41 | 87.00 | 132.00 | 148.77 | 41.87 | 10.50 | 2.67 | 17.57 | 632.67 | 8.70 |
| **3** | SKGPA-43 | 85.00 | 127.00 | 117.47 | 46.27 | 11.57 | 1.60 | 19.92 | 717.00 | 8.47 |
| **4** | SKGPA-47 | 82.00 | 122.00 | 112.87 | 51.60 | 13.00 | 1.70 | 22.73 | 818.00 | 8.64 |
| **5** | SKGPA-48 | 95.00 | 139.00 | 117.57 | 46.20 | 11.53 | 2.13 | 12.33 | 444.00 | 8.37 |
| **6** | SKGPA-1 | 94.00 | 141.00 | 146.77 | 37.73 | 9.53 | 2.43 | 18.75 | 675.33 | 7.75 |
| **7** | BGA-44 | 84.00 | 135.00 | 100.57 | 38.73 | 9.63 | 2.53 | 10.23 | 368.33 | 8.85 |
| **8** | SKGPA-34 | 94.00 | 139.00 | 118.57 | 44.67 | 11.17 | 2.13 | 16.45 | 592.33 | 8.39 |
| **9** | BGA-51 | 94.00 | 138.00 | 96.67 | 35.47 | 8.97 | 1.07 | 12.04 | 433.67 | 8.47 |
| **10** | SKGPA-28 | 95.00 | 138.00 | 104.47 | 42.13 | 10.60 | 1.60 | 24.01 | 864.33 | 8.94 |
| **11** | SKGPA-21 | 97.00 | 135.00 | 106.37 | 42.33 | 10.63 | 2.13 | 15.15 | 545.33 | 8.44 |
| **12** | SKGPA-52 | 87.00 | 124.00 | 83.70 | 46.33 | 11.66 | 0.96 | 10.73 | 386.33 | 8.42 |
| **13** | BGA-28 | 94.00 | 136.00 | 98.97 | 45.40 | 11.33 | 2.13 | 11.55 | 415.67 | 8.77 |
| **14** | SKGPA-50 | 86.00 | 125.00 | 101.87 | 45.53 | 11.43 | 2.13 | 17.38 | 625.33 | 8.52 |
| **15** | BGA-50 | 96.00 | 137.00 | 123.17 | 45.93 | 11.47 | 1.47 | 19.48 | 701.33 | 8.83 |
| **16** | SKGPA-23 | 87.00 | 131.00 | 113.87 | 42.87 | 10.66 | 1.27 | 9.56 | 344.33 | 9.17 |
| **17** | SKGPA-10 | 86.00 | 133.00 | 98.87 | 39.80 | 9.93 | 0.97 | 11.12 | 400.00 | 8.57 |
| **18** | SKGPA-31 | 95.00 | 139.00 | 87.57 | 37.67 | 9.46 | 2.13 | 15.70 | 566.33 | 8.93 |
| **19** | SKGPA-20 | 97.00 | 139.00 | 85.17 | 34.27 | 8.67 | 1.60 | 11.74 | 422.67 | 8.67 |
| **20** | BGA-43 | 84.00 | 135.00 | 90.67 | 36.13 | 9.07 | 1.47 | 12.81 | 461.33 | 8.94 |
| **21** | BGA-42 | 85.00 | 131.00 | 97.97 | 34.67 | 8.70 | 2.43 | 14.50 | 522.33 | 8.64 |
| **22** | BGA-29 | 85.00 | 120.00 | 91.87 | 38.93 | 9.73 | 1.60 | 12.61 | 454.00 | 9.10 |
| **23** | SKGPA-16 | 94.00 | 140.00 | 98.87 | 41.87 | 10.57 | 1.60 | 19.86 | 715.00 | 8.25 |
| **24** | SKGPA-26 | 95.00 | 134.00 | 91.70 | 38.73 | 9.80 | 1.47 | 10.54 | 379.67 | 8.33 |
| **25** | BGA-49 | 95.00 | 138.00 | 90.67 | 36.67 | 9.13 | 1.07 | 7.90 | 284.33 | 8.44 |
| **26** | SKGPA-18 | 96.00 | 137.00 | 111.37 | 49.93 | 12.50 | 1.47 | 24.33 | 876.33 | 8.54 |
| **27** | RGAS-92-10-1(C) | 83.00 | 125.00 | 124.70 | 52.20 | 13.30 | 1.60 | 21.26 | 765.33 | 8.32 |
| **28** | Suvrna(c) | 85.00 | 131.00 | 127.47 | 36.67 | 9.27 | 1.36 | 13.06 | 470.33 | 7.87 |
| | Mean | 89.93 | 133.14 | 106.66 | 41.84 | 10.49 | 1.73 | 15.25 | 549.15 | 8.57 |

## 4.2 Analysis of Variance

The analysis of variance for nine characters is presented in Table-4.2. It revealed that there were highly significant differences among the genotypes for all the characters under study. It indicated the presence of appreciable amount of diversity among genotypes.

## 4.3 Components of genetic variation

The range of variability estimates of genotypic and phenotypic coefficient of variation, heritability percentage in broad sense, genetic advance and genetic advance at percentage mean is presented in Table-4.3.

### 4.3.1 Coefficient of variation

It was observed that the estimates for genotypic coefficients of variation (G.C.V.) were equal to or lowest than the phenotypic coefficient of variation for all the characters.

Grain weight (g/10 ml) exhibited the lowest G.C.V. (3.75) while grain yield per plant (g) and grain yield per plot had the highest G.C.V. (29.31) as well as P.C.V. (31.90). It was followed by stem girth (29.9), length of inflorescence and width of inflorescence. The highest difference in G.C.V. and P.C.V. was observed in width of inflorescence (3.82) followed by length of inflorescence (3.59). The lowest difference in G.C.V. and P.C.V. was exhibited in days to 50% flowering (0), Days to maturity (0) followed by grain weight (0.01).

# Table 4.2 .Analysis of variance for nine characters in grain amaranthus

| Sr.No. | Character | Source of variation | | |
|---|---|---|---|---|
| | | Replication(2) | Genotypes d.f.(27) | Error (54) |
| **1.** | Days to 50% flowering | 112.00 | 88.87** | 0.00 |
| **2.** | Days to maturity | 112.00 | 107.71** | 0.00 |
| **3.** | Plant height at maturity (cm) | 1040.12 | 885.85** | 52.53 |
| **4.** | Length of inflorescence (cm) | 91.79 | 75.75** | 15.64 |
| **5.** | Width of inflorescence(cm) | 65.79 | 4.63* | 1.03 |
| **6.** | Stem girth(cm) | 0.22 | 0.70 | 0.0012 |
| **7.** | Grain yield per plant (g) | 5.80 | 63.66** | 3.68 |
| **8.** | Grain yield per plot (g) | 7515.68 | 82488.12** | 4774.43 |
| **9.** | Grain weight (g/10ml) | 0.21 | 0.31 | 0.000014 |

** significance at 1% level of significance.

### 4.3.2 Heritability (b.s)

High estimates of variability (b.s.) were observed for all the character studied.

The lowest heritability (b.s) was observed for width of inflorescence (53%). The highest heritability (b.s) was exhibited by days to 50% flowering (100%), days to maturity (100%), grain weight (100%) followed by stem girth (99%) heritability for other character ranged between grain yield per plant, grain yield per plot (84%) to length of inflorescence (56%).

### 4.3.3 Genetic advance

The characters grain yield per plot (304.66) showed highest genetic advance, followed by plant height at maturity (30.82) and days to maturity (12.34). The lowest genetic advance (0.66) was observed for grain weight, followed by stem girth (0.99).

### 4.4 Genetic divergence

Genetic divergence in 28 genotypes of amaranthus was measured following Mahalanobis (1936) $D^2$ statistics. These 28 genotypes were grouped in 5 clusters following Tochers method as described by Rao (1952). These clusters along with genotypes included under them are presented in Table-4.4.

The 28 genotypes were grouped in 5 clusters. The cluster I contained maximum i.e. 12 genotypes while in cluster II, there were 3 genotypes. Cluster III having 5 genotypes, Cluster V having 2 genotypes, Cluster IV was monogenotypic.

# Table.4.3 . Components of genetic variation in twenty eight genotypes of grain amaranthus for various characters.

| Sr. No. | Character | Range | General Mean | PCV | GCV | Heritability $h^2$ (b.s.) | Genetic advance | Genetic advance at % mean |
|---|---|---|---|---|---|---|---|---|
| 1 | Days to 50% flowering | 81-97 | 89.92 | 6.05 | 6.05 | 100 | 11.21 | 12.47 |
| 2 | Days to maturity | 120-141 | 133.14 | 4.5 | 4.5 | 100 | 12.34 | 9.27 |
| 3 | Plant height at maturity (cm) | 83.07-148.77 | 106.66 | 16.78 | 15.34 | 83 | 30.82 | 28.89 |
| 4 | Length of inflorescence (cm) | 34.67-52.20 | 41.84 | 14.28 | 10.69 | 56 | 6.91 | 16.51 |
| 5 | Width of inflorescence (cm) | 8.67-13.03 | 10.49 | 14.25 | 10.43 | 53 | 1.65 | 15.74 |
| 6 | Stem girth(cm) | 0.97-2.67 | 1.72 | 27.98 | 27.90 | 99 | 0.99 | 57.33 |
| 7 | Grain yield per plant (g) | 7.90-24.33 | 15.25 | 31.90 | 29.31 | 84 | 8.46 | 55.50 |
| 8 | Grain yield per plot (g) | 284.33-864.33 | 549.10 | 31.89 | 29.31 | 84 | 304.66 | 55.49 |
| 9 | Grain weight (g/10ml) | 7.74-9.10 | 8.57 | 3.76 | 3.75 | 100 | 0.66 | 7.73 |

## Table 4.4.Distribution of twenty eight genotypes of grain amaranthus into different clusters

| Cluster Number | Number of genotypes | Genotypes |
|---|---|---|
| I | 12 | BGA-51, BGA-49, SKGPA-52, SKGPA-43, SKGPA-21, SKGPA-34, SKGPA-48, SKGPA-26,RGAS-92-10-1, SKGPA-50, SKGPA-18, SKGPA10 |
| II | 8 | SKGPA-27, SKGPA-47,BGA-42, SKGPA-41, SKGPA-20,BGA-28,BGA-32,BGA-44 |
| III | 5 | SKGPA-28, SKGPA-31,BGA-43,BGA-29, SKGPA-23 |
| IV | 1 | SKGPA-16 |
| V | 2 | SKGPA-1,Suvarna |

### 4.4.1 Intra and Inter cluster distances

Intra and inter cluster $D^2$ and D values were worked out using $D^2$ values from divergence analysis These are presented in Table-4.5.

The minimum intra cluster distance (D) was found in cluster IV being monogenotypic equal to 0.00.

The minimum inter cluster Distance (D) was observed between cluster I and IV (37.64) followed by cluster I and cluster II (52.83), Cluster II and Cluster III (56.71), Cluster IV and Cluster V (79.41).

### 4.4.2 Cluster Means

Cluster means for nine characters presented in Table-4.6 revealed wide range of variability among 28 genotypes studied.

Cluster means for days to 50% flowering ranged from 88.25 in cluster II to 94 in Cluster IV, Cluster III, V, I have mean values 89.20, 89.50, 891.08 respectively.

Cluster mean for days to maturity ranged from 132.38 in cluster II to 140.00 in cluster IV. Cluster mean values of Cluster I, III lies close to each other i.e. 132.83, 132.60 respectively. The cluster mean value of cluster V is 136.00.

Cluster means for plant height at maturity ranged from 94.69 to 137.12. The means in cluster I, II, IV were 104.80, 108.4 and 98.87 respectively.

**Table4.5 Average intra (diagonal) and inter (above diagonal) cluster $D^2$ values in 5 cluster of 28 genotypes of grain amaranthus**

| | Cluster-I | Cluster-II | Cluster-III | Cluster-IV | Cluster-V |
|---|---|---|---|---|---|
| **Cluster-I** | **25.35** | 52.83 | 100.36 | 37.64 | 112.46 |
| **Cluster-II** | | **26.34** | 56.71 | 82.51 | 158.62 |
| **Cluster-III** | | | **30.79** | 131.21 | 208.24 |
| **Cluster-IV** | | | | **0.00** | 79.41 |
| **Cluster-V** | | | | | **31.56** |

## Table 4.6 Cluster means performance for nine characters in grain amaranthus

| Character<br>Cluster | Days to 50% flowering | Days to maturity | Plant height at maturity (cm) | Length of inflorescence (cm) | Width of inflorescence (cm) | Stem girth(cm) | Grain yield per plant (g) | Grain yield per plot (g) | Grain weight (g/10ml) |
|---|---|---|---|---|---|---|---|---|---|
| I | 91.08 | 132.83 | 104.80 | 43.68 | 10.94 | 1.56 | 14.93 | 537.47 | 8.45 |
| II | 88.25 | 132.38 | 108.43 | 41.68 | 10.45 | 2.02 | 15.19 | 546.79 | 8.72 |
| III | 89.20 | 132.60 | 97.69 | 39.55 | 9.91 | 1.61 | 14.94 | 538.07 | 9.02 |
| IV | 94.00 | 140.00 | 98.87 | 41.87 | 10.57 | 1.60 | 19.86 | 715.00 | 8.25 |
| V | 89.50 | 136.00 | 137.12 | 37.20 | 9.40 | 1.90 | 15.91 | 572.83 | 7.81 |

Cluster mean performance for length of inflorescence ranged from 37.20 in cluster V to 43.68 in Cluster I. The cluster mean values of cluster II, III, IV were 41.68, 39.55, 41.87 respectively.

Cluster mean for width of inflorescence ranged from 9.40 in cluster V to 10.94 in cluster I cluster mean for cluster II, III, IV were 10.45, 9.91, 10.57 respectively.

Cluster mean for stem girth ranged from 1.56 in cluster I to 2.02 in cluster II the cluster mean of cluster III, IV, V were 1.61, 1.60, 1.90 respectively.

Cluster mean for Grain yield per plant is ranged from 14.93 of Cluster I to 19.86 of cluster IV. Cluster mean of the cluster II, III, V were 15.19, 14.94, 15.91 respectively.

Cluster mean for Grain yield per plot is ranged from 587.47 in Cluster I to 715.00 in cluster IV. Cluster mean for cluster II, III and V are 546.79, 538.07, 572.83 respectively.

### 4.4.3 Percent contribution of various characters for divergence

The per cent contribution of 9 characters studied, to warded total divergence is presented in Table-4.7.

It was observed that grain weight contributed highest (87.83%) for divergence and it was followed by stem girth (8.20%), days to maturity (2.65%), days to 50% flowering (1.32%).However, the traits plant height at maturity , length of inflorescence, width of inflorescence, grain yield per plant, grain yield per plot had no contribution in divergence, indicating no variability.

**Table:4.7: Percent contribution of various characters to divergence in grain amaranthus**

| Sr.No. | Characters | Per cent contribution |
|---|---|---|
| 1. | Days to 50% flowering | 1.32 |
| 2. | Days to maturity | 2.65 |
| 3. | Plant height at maturity (cm) | 0.00 |
| 4. | Length of inflorescence (cm) | 0.00 |
| 5. | Width of inflorescence(cm) | 0.00 |
| 6. | Stem girth(cm) | 8.20 |
| 7. | Grain yield per plant (g) | 0.00 |
| 8. | Grain yield per plot (g) | 0.00 |
| 9. | Grain weight (g/10ml) | 87.83 |

## 4.5 Molecular Results

### 4.5.1 ISSR analysis of Grain amaranthus

A total 15 ISSR primers were used to amplify genomic DNA from 28 varieties. The results obtained with the primers are summarized below in Table-4.8.

Out of 15 ISSR primers studied, 13 yielded PCR amplification products. A total of 61 bands were generated of which 53 bands were polymorphic with an average 86.88% polymorphic. None of the band was unique and 8 bands were monomorphic in their profile. Each primer thus produced on an average 4.69 bands per marker and 4.07 bands were average polymorphic bands produced per marker.

**Table- 4.8 ISSR analysis of Grain amaranthus**

| Sr. No. | ISSR analysis | ISSR observation |
|---|---|---|
| 1. | Total No. of markers used | 15 |
| 2. | No. of marker yielding amplification | 13 |
| 3. | No. of polymorphic marker | 13 |
| 4. | Total No. of bands | 61 |
| 5. | Total No. of monomorphic bands | 8 |
| 6. | Total No. of polymorphic bands | 53 |
| 7. | Total No. of unique bands | 0 |
| 8. | Percent polymorphism | 88.86 |
| 9. | Average bands produced per marker | 4.69 |
| 10. | Average polymorphic bands produced per marker | 4.07 |

Twelve ISSR primers, i.e. ISSR-841, ISSR-867, ISSR-834, IS-8. IS-13, IS-12, 8081, 8082, 8386, 89328003, 8932799, 8932801, showed 100% polymorphism. ISSR-827 primer produced 28.57% polymorphism, while ISSR-827 showed 5 monomorphic bands and ISSR primers 8081, 8082, 89328003 amplified only one monomorphic band each.

### 4.5.2 Divergence analysis in Grain amaranthus studied using ISSR marker.

Divergence analysis was carried out with NTSYSpc. 2.021 on the basis of banding pattern on ISSR analysis of grain amaranthus 28 varieties. It was observed on dendrogram analysis that the all genotypes studied formed separate clusters, confirming their uniqueness.

**Table 4.9 Divergence analysis in Grain amaranthus studied using ISSR marker.**

| Sr. No. | ISSR primers | No.of bands generate | Polymorp-hic bands | Mon-omor-phic bands | Unique bands | percent polymorphic bands (%) |
|---|---|---|---|---|---|---|
| 1. | ISSR 841 | 7 | 7 | - | - | 100 |
| 2. | ISSR 857 | 5 | 5 | - | - | 100 |
| 3. | ISSR 827 | 7 | 2 | 5 | - | 28.57 |
| 4. | ISSR-834 | 4 | 4 | - | - | 100 |
| 5. | IS-8 | 5 | 5 | - | - | 100 |
| 6. | IS-13 | 1 | 1 | - | - | 100 |
| 7. | IS-12 | 5 | 5 | - | - | 100 |
| 8. | 8081 | 5 | 4 | 1 | - | 80 |
| 9. | 8082 | 2 | 1 | 1 | - | 50 |
| 10. | 8386 | 3 | 3 | - | - | 100 |
| 11. | 89328003 | 3 | 2 | 1 | - | 66.6 |
| 12. | 8932799 | 10 | 10 | - | - | 100 |
| 13. | 8932801 | 4 | 4 | - | - | 100 |
|  | Total | 61 | 53 | 8 | 0 |  |

In dendrogram all varieties distinguished from each other with similarity index range of 0.71-0.97. They formed different five broad group of clusters, named 1-5. Suvrna and SKGPA-26 were found to be most distinct genotypes from the rest of cultivars, while genotypes SKGPA 23 and SHGPA10 within Cluster 3b showed least variability.

As appeared in dendrogram similarity analysis lowest similarity values range (highest diversity) was observed in Suvrna (0.583-0.793) and SKGPA-26 (0.545-0.909). Lowest pair-wise similarity indices were observed between SKGPA26-RGAS-92-10-1 (0.545), SKGPA-26-Suvrna (0.583), SKGPA-50-Suvrna (0.644), SKGPA-26-SKGPA-18 (0.692)

Similarly highest pair-wise similarity indices were observed between SKGPA23-SKGPA10 (0.971), SKGPA20-BGA29 (0.966) SKGPA31-SKGPA10 (0.948) BGA29-SKGPA-23 (0.940) and BGA43-BGA29 (0.933). Similarly high similarity was also observed between SKGPA43-SKGPA47 (0.909), SKGPA47-SKGPA-43 (0.900), SKGPA48-BGA44(0.888) and SKGPA41-SKGPA-27 (0.880).

The broad cluster1 included three subcluster 1a, 1b and 1c. Subcluster 1a included genotypes SKGPA-27 and SKGPA-18. Subcluster 1b included genotypes SKGPA-41, SKGPA-43, SKGPA-34 and SKGPA-47. Subcluster 1c included genotypes SKGPA-48, SKGPA-1and BGA-44.

The broad cluster 2 included three subclusters 2a, 2b and 2c. Subcluster 2a included genotypes BGA-51 and SKGPA-21. Subcluster 2b included genotypes SKGPA-28, SKGPA-52 and BGA-28 .Subcluster 2c included genotypes SKGPA-50, RGAS-92-10-1

The broad cluster 3 included three subclusters 3a, 3b and 3c and3d. Subcluster 3a included genotype BGA-32.Subcluster 3b includes genotypes SKGPA-23, SKGPA-10, SKGPA-20, BGA-29, SKGPA-31 and BGA-43 Subcluster 3c included genotypes SKGPA-16, BGA-49 subcluster 3d included genotype BGA-42. Most distinct genotypes formed single entity clusters. The group 4 included a single genotype Suvrna while Group 5 included genotype SKGPA-26

In 2D PCO scatter plot analysis five different groups were formed namely U,V,W, X, Y and Z. The position of groups X (with 9 genotypes in cluster 1) is in between ordinates (+,+) of both X and Y axis, the group V (with single genotype Suvarna in group 5) is in between ordinates (+,+), the group Y (with six genotypes of 3bp except BGA43) is in between ordinates (-,+), the group U (with single genotype SKGPA 26) is in between ordinates (-,+), the group W ( with 4 genotypes of cluster 3c-3d and BGA43 of 3b cluster) is in between ordinates (-,-), The group Z (with 7 genotypes of cluster 2) is in between ordinates (+,-).

# 5.DISCUSSION

The genetic diversity present in any crop plays an important role in the improvement of that crop through plant breeding programme. Hence the assessment of genetic diversity in the available germplasm is of immense value to design a suitable selection procedure and its exploration of genetic upgradation of biological population.

In the present investigation, twenty eight genotypes of grain amaranthus were evaluated for their genetic diversity at field and molecular level.

## 5.1 Variability, heritability and Genetic advance.

Based on the analysis of variance for various characters the sum of squares due to treatment was significant for all characters. (Table-4.2).

In the present investigation, considerable amount of variation was judged for all the characters on the basis of knowledge of extent of variability. The wide range of variations among the genotypes paves the way to bring a desirable improvement in the crops.

### 5.1.1 Range of variability

A wide range of variability was observed in respect of days to 50% flowering (81 to 97 days), days to maturity (120 to 141 days), plant height at maturity (83.07 to 148.77 cm), length of inflorescence (34.67 to 52.20 cm), width of inflorescence (8.67 -13.03 cm) stem girth (0.97 -2.67 cm), grain yield per plant (7.90

to 24.83 g), grain yield per plot (284.33 to 864.33 g), grain weight (7.74 to 9.10 g/10 ml). This indicated an ample scope for exploration of above traits as high amount of variation was observed. Kamble (2000) also reported wide range of variability in respect of days to maturity, plant height, inflorescence length, inflorescence width and grain yield per plant.

### 5.1.2 Genotypic and Phenotypic coefficient of variation

The high estimates of genotypic and phenotypic coefficient of variation were observed for the traits *viz.,* grain yield per plant (29.31 GCV and 31.90 PCV) grain yield per plot (29.31 GCV and 31.89 PCV), grain weight (3.75 GCV and 16.78 PCV) plant height at maturity (15.34 GCV and 16.78 PCV), length of inflorescence (10.69 GCV and 14.28 PCV), width of inflorescence (10.43 GCV and 14.25 PCV) while low estimates were observed for the traits days to 50% flowering (6.05 GCV and 3.76 PCV), days to maturity (4.5 GCV and PCV), grain weight (3.75 GCV and 3.76 PCV).

These results are in conformation with Joshi (1986) showed a wide range of variability for plant height, number of leaves per plant, leaf length, leaf width inflorescence length, number of spikelets per plant days to maturity, seed yield per plant in grain amaranthus. Pawar (1995) observed higher estimation of GCV and PCV for number of branches per plant followed by grain yield per plant and leaf areq per plant in grain amaranthus. Lohitswa *et al.* (1996) reported considerable amount of phenotypic and genotypic variability for fresh weight of plant, fresh weight of inflorescence, number of rachis per inflorescence, grain yield followed by dry weight of stem, stem girth at collar region and plant height in grain amaranthus.

In some characters like width of inflorescence, length of inflorescence, shows the large difference in G.C.V and P.C.V which indicate that these characters are highly influenced by environmental factors in their expression while in case of grain weight, stem girth, days to 50% flowering, Days to maturity the estimate of G.C.V. and P.C.V. were nearly equal indicating that the variability existing in these character was due to genetic factors and had less influence of environmental factors in their expression.

### 5.1.3 Heritability (b.s.) and genetic advance.

The coefficient of variation reveals the extent of variability present for different characters and it does not indicate the heritable portion. The heritability estimates are better indicator in this respect.

The character with high heritability estimates associated with high genetic gain may be governed by additive gene effects and can effectively be improved by simple selection (Panse 1957). On the other hand the trait exhibiting high heritability and low genetic gain may be governed by the non additive gene effects (dominance and epistatis). The character showing such inheritance can be improved through hybridization. The low heritability estimates suggests that selection for such characters will not be much effective.

In the present investigation, the characters *viz.,* days to 50% flowering, days to maturity, grain weight, plant height at maturity, stem girth grain yield per plant, grain yield per plot expressed high estimates of heritability in broad sense indicating the scope for selection of these traits.

The traits *viz.,* length of inflorescence and width of inflorescence showed comparatively less heritability which suggests that the selection for such characters will not be much effective. However, Joshi (1986) reported that the high heritability estimates as a tool in selection programme for still better efficiency in selection. In the present study days to 50% flowering, days to maturity and grain weight showed highest heritability 100% coupled with genetic advance 11.21, 12.34 and 0.66 respectively.

Genetic advance for grain weight was low but when expressed as percentage means it was fairly high. In case of days to 50% flowering, days to maturity, grain weight, stem girth high heritability is coupled with low genetic advance. The trait grain yield per plot having highest genetic advance where as grain weight having lowest genetic advance.

Thus considering the estimates of genetic parameters like G.C.V., heritability and genetic advance together, it is evident that grain weight, days to 50% flowering, days to maturity, stem girth, grain yield per plant, grain yield per plot are the important characters selection for these traits could be more effective for improvement in amaranthus.

## 5.2 Genetic Divergence

The several groups of genotypes configuration may admit a description in terms of a few group constellations and their interrelationship. Mahalanobis (1936) distance statistic ($D^2$) is useful tool for this and is now well established and widely used in plant breeding for classifying the genetic stocks, on the basis of genetic divergence between population.

The cluster I comprised most of the genotypes i.e. 12 while cluster II had 8 genotypes, cluster III had 5 genotype, cluster IV is Monogenotypic and cluster V had 2 genotypes.

Perusal of Table-4.4 and fig. 5.1 revealed that the maximum intercluster distance was observed between cluster III and V (D = 208.24) suggesting that genotypes included in cluster III might have entirely different genetical architecture from the genotypes included in the cluster VIII. The cluster distance between II and V was 158.62 followed by cluster III and IV was 131.21, Cluster I and V was 112.46, Cluster I and III had 100.36.

Greater the reduction in differences between cluster distances results into higher close proximity of genotypes present in those clusters.

The $D^2$ values indicated that the cluster III and IV had maximum divergence ($D^2$ = 433.63.897) from the rest of clusters as well as from each other.

### 5.2.1 Relative contribution of characters towards divergence.

The variation of cluster means provides information on relative importance of characters towards the divergence. The Table-4.7 indicated that the variance of cluster mean was much higher for grain weight followed by stem girth, days to maturity and days to 50% flowering.

### 5.2.2 Genetic diversity and Selection of potent parents.

The selection of best parents having high potential for the economically important characters, is the success of any crop improvement. Among the different approaches of selecting parents, selection based on diversity had its own significance as diversity is the basic need of crop improvement.

Arunachalam and Bandyopadhyay (1984) devised method to delineate parental divergence into four divergence clases (D.C). To take into account the variable magnitude of variation in parental divergence, the mean (m) and standard deviation (s) of the values of intra and inter cluster divergence (D) were calculated. They defined divergence classes as below:

DC1 : D (m+s)

DC2 : D < (m+s) and m

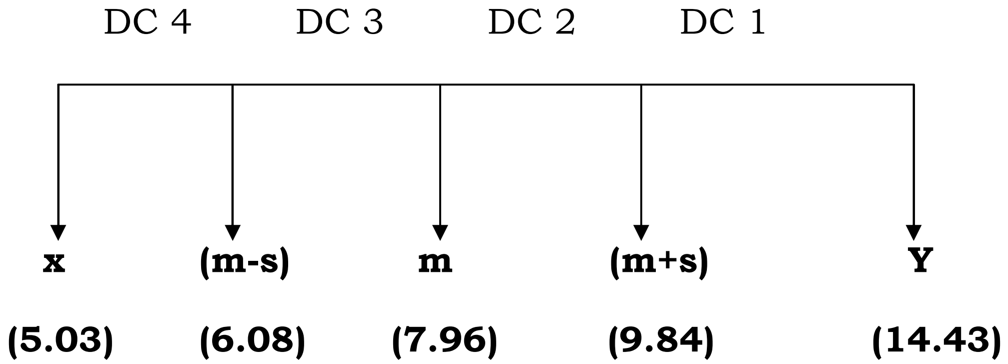
DC3 : D (m-s) and < m

DC4 : D < (m-s)

In the present study, an attempt was made to classify the cluster combinations into four divergence classes. The statistical distance (D) given in Table- 4.5 represents the index of genetic diversity among cluster. The mean of 5 inter cluster and 4 intra cluster ( as monogenotypic cluster IV had no intra cluster distance) was 7.96 and the standard deviation was 1.88. The minimum (X) and maximum (Y) values among these distances were 5.03 and 14.43 respectively. Thus the divergence classes were as presented in Table-5.1.

## Table-5.1. Distribution of different cluster combination into four divergence classes based on D values between them.

| | | |
|---|---|---|
| DC 1 | Y (14.43) | (I, III), (III, IV), (I, V), (II, V), (III, V) |
| DC 2 | m +s (9.84) | (IV, V), (II, IV |
| DC 3 | m (7.96) | (I, IV), (I, II) (II, III) |
| DC 4 | m – s (6.08) | (I, I), (II, II) (III, III), (V, V) |
| | X (5.03) | |



Grouping of cluster pairs into these divergence classes have presented in Table-5.1. On the light of the above discussion, initial choice of the parents could be made from the cluster combinations.

## 5.3 Molecular study

### 5.3.1 ISSR molecular marker analysis

The 61 bands were generated by amplification of 15 ISSR primers on an average 86.88% polymorphism was obtained by generating 53 bands. Each primer thus produced 4.07 polymorphic bands. 8932799 primer was the most informative primer with 10 bands.

**Table-5.2 ISSR analysis with different primers**

| Sr. No. | Particulars | Number of primers |
|---|---|---|
| 1. | No. of primer screened | 15 |
| 2. | Primer which does not amplify | 2 |
| 3. | Primer which did not produce uniform amplification | - |
| 4. | Primer which produce monomorphic Band | 3 (ISSR-827, 8082,8086) |
| 5. | Primers which produce polymorphic band | 12 |
| 6. | Primers that can be used as a molecular market for confirmation of a genotype | 13 |

In dendrogram all the genotypes studied were distinguished from each other the grouping observed is moderately wide (inter-cluster similarity index 0.71-0.97). Five broad groups of clusters (1-5) were observed among them with Suvarna and SKGPA26 forming most distinct single genotype clusters and lowest similarity values with rest of genotypes.

Grouping results observed in dendrogram analysis were reflected in 2D PCO scatter plot analysis. In 2D PCO scatter plot analysis five different groups were formed namely U to Z which shared genotypes as in dendrogram analysis i.e. U (with SKGPA 26); V (with Suvarna); X (9 of cluster 1), Y (six genotypes of 3bp except BGA43); W (cluster 3c-3d and BGA43 of 3b); Z (cluster 2).

When morphological data was correlated with molecular divergence data following trend was observed:

- No correlation of the genotypes was observed with respect to Days to 50% flowering and plant height at maturity.
- <u>Yield per plant</u>: The genotypes in group X (cluster 1) showed higher values in terms of yield per plant. They are followed by those of group Z (cluster 2), W (cluster 3c-3d and BGA43 of 3b) and Suvarna variety of group V (cluster 4). Genotypes of group U and Y showed fewer yield.
- <u>Stem girth</u>: The genotypes in group X (cluster 1) showed higher values of stem girth followed by group Z and group W. Genotypes of group Y shows less values of stem girth.

- Inflorescence width: The genotypes in group X, Z and W showed higher values. Lower values were observed amongst genotypes of group Y U (SKGPA-26) and group V (Suvrna).

  However, sthe same correlation was not reflected in few genotypes.

# 6. SUMMARY AND CONCLUSION

The present investigation “Genetic diversity in Grain Amaranthus (*Amaranthus hypochondriacus*)” was undertaken with twenty eight genotypes to measure genetic diversity in quantitative characters and to study the nature and extent of genetic diversity by using molecular markers.

The results obtained in present investigation are summarized as follows;

Twenty eight genotypes were planted in 45 X 15 cm. spacing in RBD design with three replication in rabi 2011-12. The genotypes were evaluated for nine quantitative characters to have clear view of yield contributing components. The observations recorded were days to 50% maturity, Days to flowering, plant height at maturity, length of inflorescence, width of inflorescence, width of inflorescence, stem girth, grain yield per plant, grain yield per plot, grain weight.

Wide range of variation was observed for all the 9 characters considered for the study. The analysis of variance exhibited significant differences among the genotypes for all the nine characters indicating the presence of substantial degree of variability.

Phenotypic coefficient of variation (PCV) was found to be more than (genotypic coefficient of variation (GCV) for all nine characters indicating the role of environment for expression of the traits. The grain yield per plant exhibited the highest GCV and PCV (29.31 GCV and 31.90 PCV respectively). followed by grain yield per plot and length of inflorescence. The character days to maturity had least GCV and PCV.

Heritability (b.s). For the characters *viz.,* days to 50% flowering, days to maturity, grain weight, plant height at maturity, stem girth, grain yield per plant, grain yield per plot expressed high estimate of heritability in broad scence indicating the scope for selection of these traits days to 50% flowering, days to maturity and Grain weight all shown highest 100% heritability and width of inflorescence shown less heritability 53%.

In the present investigation, $D^2$ values between 25.35 and 208.24. The genotypes were grouped into 5 clusters. Cluster I contain maximum number of genotypes (12) while cluster II had 8 genotypes. Cluster III had 5 and cluster V had 2 genotypes while cluster IV was monogenotypic. Intra-cluster $D^2$ values ranged between 25.35 to 208.24. The maximum inter-cluster distasnce was observed between cluster III and V (208.24) followed by cluster II and V (158.62).

A total 15 ISSR primers were used to amplify genomic DNA from 28 varieties. Out of 15 ISSR primers studied, 13 yielded PCR amplification products. A total of 61 bands were generated of which 53 bands were polymorphic with an average 86.88% polymorphic. None of the band was unique and 8 bands were monomorphic in their profile. Each primer thus produced on an average 4.69 per marker and 4.07 bands were average polymorphic bands produced per marker.

In dendrogram all the genotypes studied were distinguished from each other the grouping observed is moderately wide (similarity index 0.71-0.95). Five clusters were observed among them with Suvarna and SKGPA26 forming most distinct single genotype clusters.

Divergence results observed in dendrogram analysis were reflected in 2D PCO scatter plot analysis. The varieties Suvarna and SKGPA-26 are diverse much from others. Group X includes genotypes having most of the desirable characters the genotypes in cluster X are SKGPA-27, SKGPA-18, SKGPA-41, SKGPA-43, SKGPA-34, SKGPA-47, SKGPA-48, SKGPA-1 and BGA-44. Cluster X is followed by cluster Z in terms of desirable character. The genotypes in cluster Z are BGA-51, SKGPA-21, SKGPA-28, SKGPA-52, BGA-28, SKGPA-50 and RGAS-92-10-1. No correlation was observed between molecular divergence with Days to 50% flowering and plant height at maturity. Intercluster crossing can be used for further widening of genetic variability in grain amaranth breeding programme.

# 7. LITERATURE CITED

*Alba, E., G.B., Piligano, and Notarnicola, L. 1997. Diversity analysis in some Amaranthus entries. Agri. Mediterranea. 127 (3) : 198-204.

Allard, R.W. 1960. Principles of plant breeding. John Wiley and Sons. Inc. (New York), pp. 20-24 and 88-89.

Allard, R.W. 1961. Genetic diversity and consistency of performance in different environments. Crop Sci, 1 : 127-133.

Anand, I.J., J.N. Singh, and P.P. Khanna. 1975. Interrelationship and diversity in Indian Colza. Indian J. Agric. Sci. 45 (6) : 253-258.

Anonymous, 1970. Association of Official Agricultural Chemists. Official methods of analysis of the Association of Agricultural Chemists, 11th Edn. Washington, D.C.

Arunachalam, V. and A. Bandyopadhyay. 1984. Limits to genetic divergence of heterosis – experimental evidence from crop plants. Indian J. Genet. 49 (3): 548-554.

Batta, R.K., B.S. Sidhu, and P.D. Mehndiratta. 1996. Forage production of *Amaranthus* spp. on the Indian subcontinent. In Rangelands in a sustainable biosphere, Proceedings of the Fifth International Rangeland Congress, U.S.A. Vol. I. 41-42.

Bersani, R. and J.M Gozzales. 1987. Yield selected composition and nutritive values of 14 sections of grain amaranth representing four species. I. Sci. Food and Agric. 38 (4) : 347-356.

Burton, G.W. 1952. Quantitative inheritance in pearl millet (*P. glanacum* L.) Agron. J. 50 : 503.

*Candolle, A. De. 1883. Origine de plants cultivars, Paris. 58

*Candolle, A. De. 1886. Origin of cultivated plants.

Chocharan, W.G. and , G.M .Cox. 1957. Experimental design. John Wiley and Sons. Inc. N.Y., London, pp. 82-90 and 403-412.

Coves, G. 1950. Un hibrido inter specifico natural en. Amaranthus. Arten. Bot. Mag. (Tokyo). 47 : 556-557.

Costea, M. and D.A Demason. 2001. Stem morphology and anatomy in *Amaranthus* L. (*Amaranthaceae*) taxonomic significance. J. of Tarrey Botanical Soc. 128 (3) : 254-281.

Darlington, C.D. and A.P .Wylie. 1955. Chromosome Atlas of flowering plants. George Allen and Uncoin, London.

Darlington, C.D. 1963. Chromosome, botany and origins of cultivated plants. George Allen and Uncoin, London.

Devdas, V.A., P.K. Gopal Krishnan, and K.V. Peter.1993. A comparison of red and green amaranthus for growth and yield parameters. South Indian Horti. 41 (1) : 43-46.

Dhan Prakash, B. D. Joshi. and M.Pal. 1995. Vitamin C in leaves and seed oil composition of Amaranthus spp. International J. of Food Sci. and Nutri. 46 (1) : 47-51.

Falconer, D.S. 1964. Introduction to Quantitaive Genetics. 1st Edn. Edinburgh and London, Oliver and Boyd. pp. 365.

*Fatocum, C.A. 1985. Multivariate studies of the variability in cultivated amaranth. Beitrage. Zur. Tropischen, Landutrischaf and Vterinarnedzin, 23 (3) : 267-275.

*Giranko, M.M. and A.A. Borodkin, 1987. Variation in main economical and useful characters in Amaranthus. Genetikci seleksii. 118: 59-67.

Grant, W.F. 1959. Cytogenic studies in Amaranthus. Chromosome number and phenotypic aspects. Can. J. Genet. Cyto. 1 (4) : 113-128.

Gupta, V.K. and S. Guda. 1991. Inter-specific hybrids and possible phylogenetic relations in grain amaranthus. Euphytica. 52 : 33-38.

Hutchinson, J. 1974.. Origin and evaluation of grain amaranthus. In evolutionary studies in world crops. Ed. Joseph Hutchinson. Combridge Univ. Press.

Johnson H.W., H.E. Robinson and R.E. Comstock. 1955. Estimation of genetic and environmental variability in soybean. Agron.J. 47 (7):314-318.

Joshi, B.D. 1985. Collection, evaluation and conservation of grain amaranth, buck wheat and chenopod, IIIrd workshop of AICRP on under utilized and under exploited crop and plants, held at NBPGR, New Delhi on 30th June and 1st July, J. 985. pp. 1-20.

Joshi, B.D. 1986. Genetic variability in grain amaranth, *Amaranthus hypochondriacus* Linn. Indian J. Agric. Sci. 56 : 574 – 576.

Joshi, B.D. and J.C. Rana 1995. Genetic divergence in grain amaranth (*Amaranthus hypochondriacus*) Indian J. Agric. Sci. 65 (8) : 605 –607.60

Kamble, A.K. 2000. Genetic divergence and path analysis in grain amaranthus. M.Sc. (Agri.) thesis submitted to M.P.K.V., Rahuri.

Kanthaswamy, V. 2006. Studies on multivariate analysis in amaranthus. International J. Agric. Sci. 2 (2) : 317-319.

Katiyar, R.S., S. Shukla, and Sanjay Rai. 2000. Varietal performance of grain amaranthus (*A. hypochondriacus*) on sodic soil. Biological Science, N.B.R.I., Lucknow. 70 (2) : 185-187.

Kowal, J. 1954. Morphological and Anatomical features of the seeds of the genus Amaranthus and keys for their identification. Monogr. Bat. 2 : 162-193.

Kulakow, P. and S.Jain. 1987. Genetics of grain amaranthus, variation and early generation response to selection in *A. cruentus* L. Theoretical and Applied Genetics. 74 (1) : 113-120.

Lohithaswa, H.C., T.E. Nagraj, D.L. Savithramma and H.B. Hemareddy.1996. Genetic variability studies in grain amaranth. Mysore J. Agric. Sci. 30 (2) : 117-120.

Lush, J. L. 1949. Heritability of quantitative characters in farm animals. Proc. Cong. Genet. Heriditas. 28: 356-357.

Mahalanobis, P.C. 1936. On generalized distance in statistics. Proc. Nat. Ins. Sci. India. 2 : 49-55.

*Mallika, V.K. 1988. Genome analysis in the Genus Amaranthus (Abstr.) Amaranth News. No.3 Sept. 1988.

*Merril, E.D. 1950. Observations on cultivated plants with reference to certain American problems. Ceiba 1 : 3-36.61

Munjal, S.C., P.N. Mahajan, Y.M. Patil and S.R. Patil. 1999. Evaluation of grain amaranth cultivars for biochemical and mineral constituents. J. Maharashtra Agric. Univ. 24 (1) : 58-60.

Murray, M.J. 1940. Colchicine induced tetraploids in dioecious andmonoecious species of Amaranthaceae J. of Heridity. 31 : 477-485.

Pal, M. and T.N. Khoshoo. 1973. Evolutionary studies in world crops. Diversity and change in the Indian Sub continent. pp. 132.

Pal, M., D.Ohri and G.V.Subramanyam. 2000. A new basic chromosome number for Amaranthus (*Amaranthaceae*). Cytologia. N.B.R.I., Lucknow, India. 65 (1) : 13-16.

Pandey, R.M. 2003. Genetics of agronomic traits in Amaranthus. Sabraa J. 14 (2) : 121-129.

Panse, V.G. 1957. Chromosome number in some Indian Angiosperms – I : Proc. Indian Acad, Sci. 9 : 5 (B) : 347-350.

Panse, V.G. and P.V. Sukhatme. 1985. Statistical methods for agricultural workers. ICAR, New Delhi. Ed. 4 pp. 359.

Pawar, A.N. 1995. Genetic diversity and path analysis in amaranthus. M.Sc. (Agri.). thesis submitted to M.P.K.V., Rahuri.

Pearson, K. 1926. On the coefficient of racial likeness, Biometrika, 28 : 106-117.

Rao, C. R. 1952. Advanced statistical methods in biometrical research. John Wiley and Sons. Inc., New York. 62

Rao, C.R. 1960. Multivariate analysis : an indispensable aid in applied reseach. Sankhya. 22 : 317-318.

Ravindran, V., R.L. Hood, R.J. Gill, C.R. Kheale, and W.L.Bryden. 1996. Nutritional evaluation of grain amaranth (*A. hypochondriacus* L.) in broiler diets. Animal Feed Sci. and Technology. 63 : 1-4.

Sauer, J.D. 1950. The grain amaranths a survey of their history and classification Ann. Missouri Bot. Garden. 37 : 561-619.

*Sauer, J.D. 1955. Revision of the dioecious amaranthus. Madrone, 13 : 5-46.

Sauer, J.D. 1957. Recent migration and evaluation of the dioecious amaranthus. Evolution. 11 : 11-31.

Sauer, J.D. 1967. The grain amaranthus and their relatives : A revised taxonomic and geographic survey. Ann. Missouri Bot. Garden. 54 : 103-137.

*Schinz, H. 1934. Amaranthaceae. In Engler, A. and Paranti, K. Die naturilichen. Pflanzen Familien. 166 : 7-85.

Shukla, S. and S.P. Singh. 2002. Genetic divergence in amaranth (*A. hypochondriacus* L.). Indian J. Genet. 62 (4) : 336-337.

Singh, R.K. and B.D.Chaudhari. 1977. Biometrical techniques in Genetics and Breeding. pp. 163-191.

Singh,H. and T.A.Thomas.1978. Grain amaranthus, buck wheat, chenopods, I.C.A.R., New Delhi. pp.4.63

Subramaniam, N. and M. Shrinivisan. 1952. Vegetable protein from a new source (*A. paniculatus* L.). Proc. Soc. Biol. Chem. Indian. 10 :25-26.

*Takagi, F. 1933. A udder die chromosomes – Zahein – bei – eingien. Amaranthus Arten. Bot. Mag. (Tokyo). 47 : 556-557.

*Thellung, A. 1914. Amaranth. Ascherson and Graebneris synopsis dermittelu ropaischen. Flora. 5 (1) : 225-356.

Tilekar, S.D. 1986. Correlation, variability and genetic diversity in yield and its component in Amaranthus (*Amaranthus cruentus* L.). M.Sc. (Agri.), thesis submitted to M.P.K.V., Rahuri.

Varlaxmi,B. and V.V.P.Reddy. 1994. Variability, heritability and correlation studies in vegetable amarantus. South Indian Horti. 42 (6) : 361-364.

Vavilov, N.I. 1951. The origin, variation, immunity and breeding of plants. New York. Ronald Press. Co. pp. 364.

Verma, P.K., S.N. Gupta, M.K. Deen and B.P.S.Malik. 2002. Genetic divergence in grain amaranth. Annals of Biology, Department of Plant Breeding, Hissar. 18 (1) : 35-38.

Wills, J.C. 1973. A dictionary of the flowering plants and ferns. Cambridge Univ. Press, Cambridge, U.K. Wright, S. 1921. Correlation and causation. J. Agric. Res. 20 : 557-587.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JAand Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18:6531-6535

* Originals not seen

# VITA

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**MASTER OF SCIENCE (AGRICULTURE)**

**IN**

**GENETICS AND PLANT BREEDING**

**2013**

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**Department** : Genetics and Plant Breeding

**Major field** : Agricultural Botany

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|  | SKGPA27 | SKGPA41 | SKGPA43 | SKGPA47 | SKGPA48 | SKGPA1 | BGA44 | SKGPA34 |
|---|---|---|---|---|---|---|---|---|
| SKGPA27 | 1.0000000 |  |  |  |  |  |  |  |
| SKGPA41 | 0.8800000 | 1.0000000 |  |  |  |  |  |  |
| SKGPA43 | 0.8831169 | 0.9000000 | 1.000000 |  |  |  |  |  |
| SKGPA47 | 0.8611111 | 0.8536585 | 0.9090909 | 1.0000000 |  |  |  |  |
| SKGPA48 | 0.8611111 | 0.7733333 | 0.8311688 | 0.861111 | 1 |  |  |  |
| SKGPA1 | 0.8266667 | 0.8205128 | 0.8500000 | 0.826667 | 0.8800000 | 1 |  |  |
| BGA44 | 0.8888889 | 0.8292683 | 0.8831169 | 0.820513 | 0.888889 | 0.906667 | 1 |  |
| SKGPA34 | 0.8378378 | 0.8571429 | 0.9367089 | 0.888889 | 0.876712 | 0.868421 | 0.8500000 | 1 |
| BGA51 | 0.7012987 | 0.775 | 0.804878 | 0.779221 | 0.779221 | 0.7750000 | 0.7792208 | 0.868421 |
| SKGPA28 | 0.7105263 | 0.7088608 | 0.7654321 | 0.736842 | 0.763158 | 0.734177 | 0.710526 | 0.826667 |
| SKGPA21 | 0.7945205 | 0.7619048 | 0.7948718 | 0.775 | 0.849315 | 0.81579 | 0.85 | 0.864198 |
| SKGPA52 | 0.7948718 | 0.7654321 | 0.8192771 | 0.769231 | 0.820513 | 0.839506 | 0.846154 | 0.883117 |
| BGA28 | 0.7631579 | 0.7341772 | 0.7654321 | 0.736842 | 0.763158 | 0.78481 | 0.789474 | 0.8 |
| SKGPA50 | 0.6933333 | 0.744186 | 0.775 | 0.756098 | 0.666667 | 0.692308 | 0.731707 | 0.795181 |
| BGA32 | 0.7096774 | 0.7692308 | 0.7761194 | 0.806452 | 0.741936 | 0.769231 | 0.774194 | 0.786885 |
| SKGPA23 | 0.7428571 | 0.739726 | 0.7466667 | 0.742857 | 0.771429 | 0.767123 | 0.8 | 0.760563 |
| SKGPA10 | 0.7777778 | 0.7804878 | 0.7792208 | 0.794872 | 0.805556 | 0.8 | 0.820513 | 0.8 |
| SKGPA31 | 0.7777778 | 0.7804878 | 0.7792208 | 0.820513 | 0.805556 | 0.773333 | 0.769231 | 0.8 |
| SKGPA20 | 0.7323944 | 0.7297297 | 0.7368421 | 0.732394 | 0.760563 | 0.756757 | 0.788732 | 0.75 |
| BGA43 | 0.6969697 | 0.6764706 | 0.7323944 | 0.727273 | 0.8 | 0.794118 | 0.8 | 0.75 |
| BGA42 | 0.6875 | 0.6666667 | 0.7246377 | 0.6875 | 0.730159 | 0.69697 | 0.698413 | 0.714286 |
| BGA29 | 0.7419355 | 0.71875 | 0.7164179 | 0.709677 | 0.754098 | 0.78125 | 0.786885 | 0.735294 |
| SKGPA16 | 0.8358209 | 0.7792208 | 0.7777778 | 0.756757 | 0.818182 | 0.811594 | 0.849315 | 0.839506 |
| SKGPA26 | 0.6896552 | 0.6666667 | 0.6666667 | 0.655172 | 0.736842 | 0.7 | 0.736842 | 0.65625 |
| BGA49 | 0.7647059 | 0.7435897 | 0.739726 | 0.746667 | 0.776119 | 0.8 | 0.810811 | 0.804878 |
| SKGPA18 | 0.8888889 | 0.7887324 | 0.8235294 | 0.794118 | 0.806452 | 0.8 | 0.835821 | 0.8 |
| RGAS92101 | 0.6666667 | 0.7142857 | 0.8135593 | 0.727273 | 0.592593 | 0.701754 | 0.679245 | 0.786885 |
| Suvarna | 0.7457627 | 0.7540984 | 0.71875 | 0.711864 | 0.758621 | 0.786885 | 0.724138 | 0.738462 |

Table4.10 Similarity index

| BGA51 | SKGPA28 | SKGPA21 | SKGPA52 | BGA28 | SKGPA50 | BGA32 | SKGPA23 | SKGPA10 |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 0.839506 | 1 | | | | | | | |
| 0.871795 | 0.805195 | 1 | | | | | | |
| 0.86747 | 0.878049 | 0.86076 | 1 | | | | | |
| 0.864198 | 0.875 | 0.857143 | 0.902439 | 1 | | | | |
| 0.85 | 0.78481 | 0.809524 | 0.790124 | 0.835443 | 1 | | | |
| 0.857143 | 0.782609 | 0.75 | 0.782609 | 0.782609 | 0.852941 | 1 | | |
| 0.8 | 0.702703 | 0.760563 | 0.763158 | 0.783784 | 0.712329 | 0.870968 | 1 | |
| 0.831169 | 0.710526 | 0.8 | 0.794872 | 0.789474 | 0.731707 | 0.888889 | 0.971429 | 1 |
| 0.779221 | 0.736842 | 0.775 | 0.769231 | 0.81579 | 0.731707 | 0.875 | 0.942857 | 0.948718 |
| 0.789474 | 0.746667 | 0.75 | 0.779221 | 0.8 | 0.72973 | 0.888889 | 0.956522 | 0.957747 |
| 0.764706 | 0.716418 | 0.738462 | 0.782609 | 0.80597 | 0.716418 | 0.836364 | 0.920635 | 0.923077 |
| 0.757576 | 0.8 | 0.793651 | 0.776119 | 0.830769 | 0.769231 | 0.821429 | 0.852459 | 0.857143 |
| 0.793651 | 0.709677 | 0.766667 | 0.769231 | 0.806452 | 0.741936 | 0.88 | 0.949153 | 0.95082 |
| 0.811594 | 0.764706 | 0.837838 | 0.857143 | 0.852941 | 0.81579 | 0.785714 | 0.875 | 0.876712 |
| 0.766667 | 0.677966 | 0.77193 | 0.688525 | 0.711864 | 0.61017 | 0.754717 | 0.727273 | 0.77193 |
| 0.828571 | 0.753623 | 0.853333 | 0.816901 | 0.869565 | 0.805195 | 0.77193 | 0.861539 | 0.864865 |
| 0.75 | 0.761905 | 0.80597 | 0.787879 | 0.825397 | 0.724638 | 0.693878 | 0.766667 | 0.80597 |
| 0.821429 | 0.8 | 0.679245 | 0.807018 | 0.836364 | 0.909091 | 0.782609 | 0.679245 | 0.690909 |
| 0.7 | 0.711864 | 0.701754 | 0.709677 | 0.779661 | 0.644068 | 0.708333 | 0.75 | 0.758621 |

| SKGPA31 | SKGPA20 | BGA43 | BGA42 | BGA29 | SKGPA16 | SKGPA26 | BGA49 | SKGPA18 |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 0.929578 | 1 | | | | | | | |
| 0.923077 | 0.9375 | 1 | | | | | | |
| 0.888889 | 0.903226 | 0.870968 | 1 | | | | | |
| 0.918033 | 0.966667 | 0.933333 | 0.862069 | 1 | | | | |
| 0.849315 | 0.892308 | 0.861539 | 0.793651 | 0.918033 | 1 | | | |
| 0.736842 | 0.75 | 0.75 | 0.740741 | 0.745098 | 0.736842 | 1 | | |
| 0.837838 | 0.878788 | 0.848485 | 0.78125 | 0.918033 | 0.933333 | 0.758621 | 1 | |
| 0.776119 | 0.786885 | 0.754098 | 0.745763 | 0.819672 | 0.852941 | 0.692308 | 0.823529 | 1 |
| 0.690909 | 0.703704 | 0.703704 | 0.730769 | 0.740741 | 0.777778 | 0.545455 | 0.777778 | 0.740741 |
| 0.758621 | 0.736842 | 0.736842 | 0.690909 | 0.77193 | 0.758621 | 0.583333 | 0.758621 | 0.793103 |

RGAS92101 Suvarna

| | |
|---|---|
| 1 | |
| 0.705882 | 1 |