

VARIABILITY, CORRELATION AND PATH ANALYSIS IN GRAIN AMARANTH (*Amaranthus hypochondriacus* L.)

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ABSTRACT

VARIABILITY, CORRELATION AND PATH ANALYSIS IN GRAIN AMARANTH (*Amaranthus hypochondriacus* L.)

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ABSTRACT

Genetic variability, heritability, genetic advance, correlation, path analysis and genetic diversity through ISSR markers in grain amaranth (*Amaranthus hypochondriacus* L.) was studied at Centre for Crop Improvement, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during *rabi* season of 2018-19 in a set of forty-six genotypes grown in a Randomized Block Design (RBD) with three replications.

Molecular analysis work was carried out at Biotechnology Laboratory of the Department of Genetics and Plant Breeding, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Analysis of variance revealed that significant genotypic differences was observed for all the eleven characters under study. A wide range of variation was apparent for all the characters. The genotypic coefficient of variation, heritability and genetic advance were observed highest for the characters viz., seed yield per plant and leaf area per plant. High heritability (98.06 %) coupled with high genetic advance as per cent of mean (82.60 %) was observed for seed yield per plant indicated that phenotypic selection would be effective for genetic improvement in this trait.

Association analysis between seed yield per plant and other eleven characters revealed that seed yield per plant was significant and positively correlated with days to flowering ($r_g = 0.4094$), leaf area per plant ($r_g = 0.7676$), length of inflorescence ($r_g = 0.6043$), plant height ($r_g = 0.5034$) and harvest index ($r_g = 0.7822$) at genotypic level, which indicates the dependency of these characters with each other at genotypic level.

Path coefficient analysis indicated that harvest index ($r_g = 0.6077$) recorded highest positive direct effect on seed yield per plant followed by leaf area per plant ($r_g = 0.3544$). The trait showed low positive direct effect on seed yield per plant by width of inflorescence ($r_g = 0.1490$) and stem girth ($r_g = 0.1338$). While, days to flowering ($r_g = 0.0579$), days to maturity ($r_g = 0.0565$), length of inflorescence ($r_g = 0.0240$) and plant height ($r_g = 0.0608$) had negligible positive direct effect on seed yield per plant. On other hand test weight ($r_g = -0.0123$) and protein content showed ($r_g = -0.0459$) negligible negative effect on seed yield per plant.

Based on path analysis, it can be concluded that improving seed yield per plant in grain amaranth, due consideration should be given to early maturity, a largest size leaf area per plant, harvest index, width of inflorescence while making selection.

The genomic DNA from forty-six genotypes of grain amaranth was extracted by modified CTAB method for the molecular characterization. Total seven ISSR primers were screened across all the genotypes. All seven primers produce polymorphic band with an average of 5.71 bands per primer. The average Polymorphic Information Content (PIC) value based on ISSR primer study in the present investigation was observed 0.572.

Cluster analysis based on presence and absence of band was performed by Jaccard's similarity co-efficient based on Unweight Pair Group Method with Arithmetic Averages (UPGMA). The Jaccard's co-efficient and the dendrogram were derived using PAST software NTSYSpc (Version 2.20 N). The Jaccard's similarity co-efficient were able to classify all the genotypes of grain amaranth into sub-populations. Two genotypes named RGA-16 and RGA-17 evident maximum similarity; while the genotypes BGA-10 and BGA-4-9 shown minimum similarity by using ISSR markers. PCA plot based on ISSR primer study was constructed that distributed all genotypes in five major groups. PCoA plot analysis indicating scattered distribution of all the genotypes in all the direction around axis indicating wide genetic variation of all the genotypes.

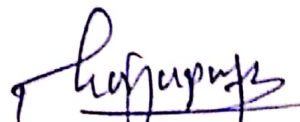
Overall, it was concluded that the genetic diversity obtained in this study will be useful in future for grain amaranth improvement programmes. Hence, studies on morphological and molecular markers are very much useful in analysing the genetic diversity in grain amaranth.

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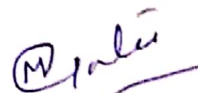
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This is to certify that the thesis entitled, **“VARIABILITY, CORRELATION AND PATH ANALYSIS IN GRAIN AMARANTH (*Amaranthus hypochondriacus* L.)”** submitted for the degree of **MASTER OF SCIENCE** in the subject of **GENETICS AND PLANT BREEDING** is a record of bonafide research work carried out by **SOLANKI RITESHKUMAR SATYANARAYAN** under my guidance and supervision and that no part of this thesis has been submitted for any other degree, diploma, associateship, fellowship or other similar titles. The assistance and help received during the course of investigation have been fully acknowledged.



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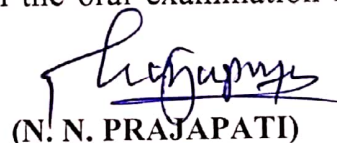
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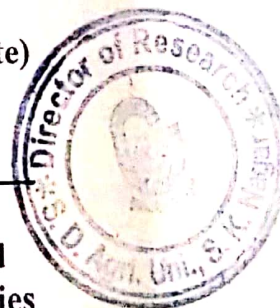
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LIST OF ABBREVIATIONS AND SYMBOLS

°C	:	Degree Celsius
ANOVA	:	Analysis of Variance
bp	:	Base Pairs
BPB	:	Bromo phenol blue
C.D	:	Critical difference
C.V.	:	Coefficient of Variation
C: I	:	Chloroform: Isoamyl alcohol
cm	:	Centimeter
Conti.	:	Continuous
CPCA	:	C. P. College of Agriculture
CTAB	:	Cetyl Trimethyl Ammonium Bromide
d.f.	:	Degree of freedom
dNTPs	:	Deoxyribo Nucleotide Triphosphate
DNA	:	Deoxyribo Nucleic Acid
etc.	:	Etcetera
EDTA	:	Ethylene Diamine Tetra Acetic Acid
et al.	:	<i>Et alii</i> (and others)
Fig.	:	Figure
g	:	Gram
GAM	:	Genetic advance as per mean
GCV	:	Genotypic coefficient of variation
h²bs	:	Heritability broad sense
i.e.	:	That is
ISSR	:	Inter-Simple Sequence Repeats
AFLP	:	Amplified fragment length polymorphism
Kb	:	Kilo Bases
M	:	Molar
mg	:	Mili Gram
ml	:	Milliliter
mM	:	Mili Molar
MS	:	Mean Square
Ng	:	Nanogram
No.	:	Number
PCA	:	Principle Component analysis
PCoA	:	Principle Co-ordinate analysis
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic coefficient of variation
PIC	:	Polymorphic Information Content
PVP	:	Polyvinyl Pyrolidone
r	:	Replication
RBD	:	Randomized Block Design
RAPD	:	Random amplified polymorphic DNA

RFLP	:	Restriction fragment length polymorphism
r_g	:	Genotypic Correlation Coefficient
r_p	:	Phenotypic Correlation Coefficient
rpm	:	Revolution Per Minute
SSRs	:	Simple sequence repeats
S.E.	:	Standard Error
S.Ed.	:	Standard error of difference
S.Em.	:	Standard Error of mean
SDAU	:	Sardarkrushinagar Dantiwada Agricultural University
TBE	:	Tris-Borate-EDTA-buffer
TE	:	Tris-EDTa
UPGMA	:	Unweighted Pair Group Method with Arithmetic average
viz.	:	Which is (Namely)
e.g.	:	Exempli gratia
vs.	:	versus
σ²_e	:	Variances due to environment
σ²_g	:	Variances due to genotype
σ²_p	:	Variances due to phenotype

SYMBOLS

=	:	is equal to
.	:	Full stop
×	:	Multiply
&	:	And
-	:	Minus
()	:	Bracket
√	:	Square root
[]	:	Square bracket
,	:	Comma
%	:	Per cent
>	:	Greater than
<	:	Less than
@	:	At the rate of
/	:	Per
Σ	:	N-ray summation
σ	:	Sigma
;	:	Semi colon
:	:	Colon
μ	:	General Mean

INTRODUCTION

I. INTRODUCTION

Grain amaranth is fast growing, dicotyledonous, belonging to genus *Amaranthus* and family *Amaranthaceae*. The word “Amaranth” is basically derived from Greek word “Anthos” which means “everlasting” according to Sankaran (1943). Grain amaranth is a nutritionally potential photo-synthetically efficient C4 plant which is highly adaptable to various climatic conditions. Leaf colour is green or red or with different shades observed. The height of mature plants varies between 0.3 m and 2.5 m, depending on the species, growth habit and environment. Some species have distinct markings on their leaves. Terminal and auxiliary inflorescences also occur. Most of the cultivated species are monoecious, wind pollinated, but the grain species with colourful inflorescence are occasionally visited by bees (Khoshoo and Pal, 1970).

Within the genus are the species *A. cruentus* L., *A. hypochondriacus* L. and *A. caudatus* L. which are the most important for the production of amaranth grain. The first two are widely distributed in Mexico, forming the most important centre of diversity (Espitia *et al.*, 2010 and Ruiz *et al.*, 2013). It is one of the suitable crops for kitchen garden and it can be cultivated in various crop rotations. Amaranths produce high edible matter per unit area and time. It can be use as food, fodder and as medicine in various pharmaceutical and cosmetic products (Dhan and Pal, 1991 and, Shukla and Singh, 2003).

The genus amaranth is dibasic with $X = 16$ and $X = 17$ chromosomes, almost equally distributed in section Amaranth. Grant (1959) has reported the information regarding the chromosome number of 30 species of *Amaranthus*. Among these, four have $2n = 32$, while all others have $2n = 34$. Among the 30 species, *A. caudatus* L. ($2n = 32$), *A. cruentus* L. ($2n = 34$) and *A. hypochondriacus* L. ($2n = 32$) are domesticated species and among these three species, *A. caudatus* L. is drooping type and rest two are erect type species.

Sauer (1993) believes the progenitor of *A. cruentus* L. and *A. hybridus* L. which is currently found over a wide range of plains and mountains in North, Central and South America. *A. hypochondriacus* L. has characteristics of both *A. cruentus* L. and the wild species *A. powelli* L. and it may be a hybrid of the two.

In addition, these crops are well adapted to harsh environmental conditions and are therefore suitable for cultivation on marginal soils. The amaranth grain is one of the valuable foods as it has high protein content and well balanced amino acid profile (Gamel *et al.*, 2004). In the last twenty years amaranth has been rediscovered as a promising food crop mainly due to its resistance to heat, drought, diseases and pests, and the high nutritional value of both seeds and leaves (National Research Council, 1984).

The exact information on grain amaranth production and productivity at national level is lacking. In Gujarat, it is grown in districts of North Gujarat and middle Gujarat as a sole crop, or taken as a mixed crop and on the borders of the field of other crops during *rabi* season. In Gujarat, Palanpur APMC market of Banaskantha district is one of the biggest market for amaranthus grain.

There are three groups of food grains. The first group of food grains is cereals which include wheat, rice, maize, barley *etc.* The second group of food grains is millets which include sorghum, pearl millet, finger millet, kodomillet *etc.* and third group of food grains comprising the grain amaranth (*Amaranth* spp.), buckwheat (*Fagopyrum* spp.) and Chenopods (*Chenopodium* spp.) belonging to three different families viz., *Amaranthaceae*, *Polygonaceae* and *Chenopodiaceae*, respectively.

Chemical composition and nutritional value of grain amaranth (Dodok *et al.*, 1994) confirms its high potential for use as human and animal nutrition as well as medicine (Teutenico and Knorr, 1985). With the increasing need of exploring alternate sources of food, it is necessary to accelerate and expand the production of amaranth.

The grains are consumed in several ways, but the popular way, in the old world and new world, is binding of popped grains in sugar syrup for preparing flat cakes or round balls or *ladoos* of varying sizes, which are given to the school children as diet supplements. Confectionary prepared from amaranth seed is not only cheap, but very tasty and highly nutritive. Recent studies have indicated that tocopherol fraction of amaranth oil contents important cholesterol lowering agents, some of which could be useful in treating cardiovascular disease. Amaranth oil also contains Squalene cosmetic ingredient and skin penetrant, which is also used as a lubricant for computer disc.

Amaranth preferred for both urban and rural population and it's also considered to be the cheapest leafy vegetable in the market and it could be rightly described as a

“poor man’s vegetable” (Varalakshmi, 2004). The amaranth can grow under varied soil and agro-climatic conditions (Katiyar *et al.*, 2000 and, Shukla and Singh, 2000) are tolerant from heat and drought with no major disease problems. Beside its adaptable nature in various climatic conditions, the amaranth plant also has important nutritional and medicinal properties (Lakshmi and Vimala, 2000). Due to use of amaranth as vegetable and grain crop, it can be a cheap alternative rich source of protein and nutrient for poor people in developing countries (Dhan and Pal, 1991; Dhan *et al.*, 1995 and Shukla *et al.*, 2006).

Subramaniam and Srinivasan (1952), while working on vegetable protein, observed that *A. cruentus* L., *A. hypochondriacus* L. contained 14.5 per cent to 16.0 per cent crude protein. Smith *et al.* (1959) have given amino acid profile of *A. hypochondriacus* L. grain, which contain 14.1 per cent crude protein. Other amino acid reported that, arginine 2.9 per cent, histidine 4.7 per cent, phenylalanine 4.3 per cent, threonine 6.9 per cent, soleunine 8.0 per cent, leucine 8.2 per cent, lysine 2.5 per cent, methionine 0.9 per cent, tryptophan and valine 6.0 per cent. They indicate contain highest in lysine and third highest in methionine amongst the various plant species. Leaf parts of amaranth contain 3 per cent protein, 0.3 per cent fat, 3.6 per cent mineral matter and about 8.1 per cent carbohydrates. All the mentioned reason, increases interest in the development of high yielding variety and thus Gujarat Amaranthus-2 has been released during 1998 for general cultivation. So far less breeding work has been done and lack of information on genetics and breeding behavior of this crop.

The collection, maintenance and evaluation of germplasm are most important and primary steps in any crop improvement programme. For formulating a successful breeding programme a better understanding of nature and magnitude of genetic variability present in the breeding materials is important. Yield is a complex character governed by several yield attributing characters. Since, most of the yield attributing character are quantitatively inherited and affected by environment, it is difficult to judge whether the observed variability is heritable or not. The parameters like genotypic and phenotypic coefficient of variation, heritability and genetic advance are useful in understanding the nature of inheritance of different traits.

Correlation coefficient analysis is a handy technique, which elaborates the degree and extent of relationship among important plant characters and it provides basic criteria for selection which leads to directional model based on yield and its

components in the field experiments. Path coefficient analysis, on the other hand, is an efficient statistical technique specially designed to quantify the interrelationship of different components and their direct and indirect effects on yield. Through, this technique yield contributing characters and specific traits producing a given correlation can be categorized (Islam *et al.*, 2010).

The use of molecular markers is more reliable, but morphological descriptors in genetic diversity are still worthwhile and necessary, especially when evaluating and choosing germplasm to be incorporated into breeding programmes (Adebola and Morakinyo, 2006). Phenotypic characterization is a preliminary requirement for the exploitation of useful traits in a breeding programme (Brandolini *et al.*, 2000). Over the past few decades, plant genomics research has been studied extensively bringing about a revolution in the field of plant biotechnology. Molecular markers, useful for plant genome analysis, have now become an important tool in crop improvement. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. The presence of various types of molecular markers, and differences in their principles, methodologies and applications require careful consideration in choosing one or more of such methods. No molecular markers are available yet that fulfill all requirements needed by researchers (Singh and Das, 2009).

The present study was framed with the following objectives.

- [1] To measure the magnitude and extent of genotypic and phenotypic variability
- [2] To estimate correlation and path coefficient between yield and component traits
- [3] To assess variability using molecular markers

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

A comprehensive review of literature is an essential part of any scientific investigation. Review of literature is always necessary to compare the present findings with the previous studies undertaken by the research workers. The literature pertaining to the present investigation entitled, "Variability, correlation and path analysis in grain amaranth (*Amaranthus hypochondriacus* L.)" has been reviewed under following different aspects.

2.1 Variability, heritability and genetic advance

2.2 Correlation and path analysis

2.3 Genetic diversity through ISSR markers

2.1 Variability, heritability and genetic advance

Exploration of genetic variability in the available germplasm is pre-requisite for any successful breeding programme. Distinction between genotype and phenotype was noticed by Johanssen (1909). Interaction between genotype and environment was responsible for variability (Fisher, 1930). The possibility of achieving improvement in any crop plants primarily depends on the magnitude of genetic variability. The statistical methods to calculate genetic component were given by Burton in 1952.

Heritability is an index of the transmission of quantitative characters from parents to their offspring (Falconer, 1989), it has relative role in heredity in the expression of phenotypes (Falconer, 1989 and Allard, 1961). However, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Johnson *et al.*, 1955), genetic advance is the improvement in mean genotypic value of selected plant over parental population. Lush (1949) classified heritability in to broad sense and narrow sense heritability. While, Robinson (1966) classified the heritability in to low (5 to 10 %), medium (10 to 30 %) and high (above 30 %). Both, heritability and genetic advance were considered as important selection parameter.

Joshi (1986) studied twenty promising genotypes of grain amaranth (*A. hypochondriacus* L.), originated from different agro-ecological regions of Himachal Pradesh and Uttar Pradesh hills, showed a wide range of variability for per plant, height, leaves per plant leaf length, leaf width, inflorescence length, spikelets per plant, days to maturity, 1000-seed weight, popping size, percentage of protein and seed yield per plant. Heritability estimates and expected genetic advance

were high for 1000-seed weight, inflorescence length and plant height, followed by seed yield and percentage of protein, indicating usefulness of these traits for the varietal improvement programme of this crop.

Vyas (2000) studied genetic variability in sixty genotypes of grain amaranth. A wide range of variation was observed for seed yield per plant, plant height, panicle length, straw yield per plant, number of branches per plant and harvest index. GCV (%) was high for seed yield per plant, numbers of branches per plant, straw yield per plant and harvest index. The broad sense heritability (h^2_{bs}) estimates were also found to be high for all the characters except panicle length. Expected genetic advance as percent of mean (GA %) was high for seed yield per plant, number of branches per plant and straw yield per plant.

Verma *et al.* (2001) noticed wide range of variability for seed yield per plant, plant height, protein content and phosphorus content in grain amaranth (*A. hypochondriacus* L.). Highest heritability and genetic advance were reported for seed yield per plant and plant height.

Dutta *et al.* (2002) study on grain amaranth (*A. hypochondriacus* L.) revealed presence of genetic variability in seed yield and harvest index. Genetic index flowering time showed high heritability while genetic advance was high for plant height.

Patel (2004) studied genetic variability in fifty genotypes of grain amaranth. A wide range of variation was observed for number of branches per plant, seed yield per plant and plant height. The high heritability estimates were found for 1000-seed weight, days to maturity, protein content and number of branches per plant. Expected genetic advance as percent of mean was high for seed yield per plant, number of branches per plant and protein content.

Patel (2011) studied genetic variability in twenty-eight genotypes of grain amaranth. The GCV, PCV, h^2 and GA as percentage of mean were high for various characters like seed yield per plant, plant height, number of leaves per plant, panicle length, and days to maturity and popping ability. Therefore, more emphasis should be laid on component traits during selection for the improvement of grain yield.

Ahmed *et al.* (2012) genetic variability, heritability analyses were done for yield and its component characters in twenty-two diverse genotypes of stem amaranth. The highest PCV and GCV were observed for primary branches per plant while the lowest PCV was found in plant height and the lowest GCV was found in leaf width. Heritability estimates in broad sense were higher for leaf weight per plant followed by

leaves per plant, primary branches per plant, stem weight per plant and yield per hectare. Leaf weight per plant, stem weight per plant and yield per hectare exhibited high value of heritability along with high genetic advance.

Hasan *et al.* (2012) studied variability, heritability for seventeen genotypes of stem amaranth (*Amaranthus tricolor* L.) were evaluated to determine the genetic variability, degree of association between yield and its component characters. The direct and indirect effects of marketable yield were also evaluated. High heritability with high genetic advance as per cent of mean was registered for number of leaf, leaf weight and marketable yield which in fact demonstrated the presence of additive gene effects.

Akaneme *et al.* (2013) studied of variance five accessions of amaranth (*A. hybridus* L). the species obtained from National Centre for Genetic Resources and Biotechnology Ibadan, Nigeria revealed highly significant differences for leaf width, hypocotyls length, days to 50 per cent flowering, 500 seed weight and leaf length. The range, coefficient of variability, phenotypic and genotypic coefficients of variability also revealed high variability for each of the quantitative traits. The highest broad sense heritability (h^2b), GCV, PCV and GA were obtained for days to 50 per cent flowering which was also positively correlated with leaf length and stem diameter.

Yadav *et al.* (2014) evaluate genotypic variability and character association in grain amaranth genotypes for agronomically useful and yield contributing traits. The characters like leaf blade width, lateral spikelet length and grain yield per plant showed high GCV and PCV values. The highest GCV was recorded with grain yield per plant. High heritability exhibited for all the characters like viz., observed to be high in magnitude for days to 80 per cent maturity, days to 50 per cent flowering, leaf blade width, inflorescence length and plant height. At genotypic level, seed yield per plant showed highly significant positive correlation with days to 80 per cent maturity and plant height and significant positive correlation with days to 50 per cent flowering. Inflorescence length had significant positive correlation with lateral spikelet length.

Venkatesh *et al.* (2014) studies genetic variability, heritability and genetic advance in grain amaranthus. One hundred germplasm accessions of grain amaranth were evaluated assessing the genetic variability for grain yield and yield related traits. Analysis of variance revealed significant differences among the

genotypes for all the characters studied. High PCV and GCV was observed for stem girth, plant height, panicle length and grain yield per plant. On low were observed for days to maturity and grain protein content. All the traits studied exhibited high heritability. High genetic advance as percent of mean was observed and grain yield per plant indicating scope for improvement of the traits of interest through hybridization and selection.

Sheelamary *et al.* (2016) studied genetic variability, heritability and genetic advance in one hundred genotypes of grain amaranth. On the basis of mean performance highest was recorded by days to 80 per cent flowering followed by plant height and days to 50 per cent flowering. The genotypic coefficient of variation (GCV) was observed for seed yield per plant followed by lateral spikelet length, plant height and inflorescence length. The phenotypic coefficient of variation (PCV) was observed for seed yield per plant followed by lateral spikelet length, plant height, inflorescence length, petiole length, stem thickness and days to 50 per cent flowering. The phenotypic coefficient of variation (PCV) is higher than the genotypic coefficient of variation for all the characters. High heritability coupled with high genetic advance was observed for hundred seed weight and days to 80 per cent maturity which indicates the predominance of additive gene action making the selection more effective. The results obtained from this study might be helpful for the breeders trying to develop new varieties with better grain features to improve grain amaranth.

Kumar and Murthy (2017) genetic variability studies for yield component traits in grain amaranth (*Amaranthus* spp.). The investigation on genetic variability and genetic divergence with respect to yield component traits estimated using one hundred five germplasm during *kharif*, 2013 at University of Agricultural Sciences, Bangalore. A high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was observed for all the traits except for, chlorophyll content (SPAD chlorophyll metre reading), panicle width and 1000-seed weight. Heritability estimates were high for all the characters studied. High genetic advance as per cent mean (GAM) was registered for most of the traits except for 50 per cent flowering, 1000-seed weight and days to maturity.

Shrivastav *et al.* (2017) studied genotypic variability of Amaranth (*Amaranthus paniculatus* L.) including three checks (GA 2, BGA 2 and RMA 7). The analysis of variance revealed that mean squares due to treatments were highly significant for all characters except seed volume weight (g/10 ml). The magnitude of

phenotypic coefficient of variation was higher than corresponding genotypic coefficient of variation for all the characters. The high estimates of phenotypic (PCV) and genotypic (GCV) coefficients of variations were found in case of harvest index, biological yield/plant and low estimates of phenotypic and genotypic coefficients of variations were observed for protein content and seed volume weight. High heritability in broad sense was recorded for plant height biological yield per plant, harvest index, days to maturity and inflorescence length. The high estimates of genetic advance in per cent of mean were recorded for harvest index, biological yield per plant, plant height, number of branches per plant and inflorescence length. All the genotypes were grouped into five different non overlapping clusters. The contribution of ten characters towards divergence showed the highest contribution by plant height followed by biological yield per plant.

Gelotar (2018) studied genotypic coefficient of variation, heritability and genetic advance in nineteen germplasm of grain amaranth and she observed highest for the characters viz., number of branches per plant, leaf area, seed yield per plant and plant height. High heritability was observed for plant height, leaf area, days to maturity, seed yield per plant, length of inflorescence, days to flowering and number of branches per plant, while genetic advance was observed for plant height, length of inflorescence, seed yield per plant and days to flowering.

Shah *et al.* (2018) studied twenty-seven diverse genotypes of vegetable amaranth were evaluated for various yield and yield attributing traits. Analysis of variance revealed highly significant differences among all the genotypes under study for all the traits. Range values indicated presence of sufficient genetic variability for all the characters. Phenotypic coefficients of variation were found to be higher than corresponding genotypic coefficients of variation. Most of the traits recorded high phenotypic and genotypic coefficients of variation. All the characters showed the high estimates of heritability coupled with high genetic advance as per cent of mean.

Tiwari (2018) studied twenty-seven genotypes of grain amaranth were used to study genetic variability and heritability which resulted phenotypic variance was higher than the genotypic variance for all the traits. The heritability estimates for all the characters were high and none of the characters showed moderate or low estimates.

Kumar *et al.* (2018) studies on collection and valuation of genetic variability available in amaranthus (*Amaranthus* spp.) twenty-five genotypes of amaranthus were evaluated. High magnitude of phenotypic GCV and PCV was observed for

seed yield plot, followed by test weight, petiole length, number of leaves plant, stem girth, leaf breadth, leaf length, number of branches plant and leaf yield. The heritability estimates recorded to be high for the characters viz., dry matter per cent, fiber content, seed yield plot, stem girth, test weight, leaf yield plot, petiole length, leaf breadth and root length, leaf length, number of branches plant and plant height. Highest estimates of genetic advance as percentage of mean was obtained for characters namely seed yield plot and test weight, petiole length, number of leaves plant, stem girth and leaf breadth.

2.2 Correlation and path analysis

Correlation coefficient measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. Simple correlations are of three type's viz., phenotypic, genotypic and environmental phenotypic correlation. Yield is a final product of interaction of the yield contributing components among themselves and with the environment. Some of the components effect yields directly while others contribute indirectly. To raise the genetic potential of a crop knowledge of the inter relationship of the yield and yield component is very essential for a breeder.

Correlation studies helps to know the magnitude and direction of association of yield with its components and also among various components. Correlated characters are important from three main considerations :

- [i] Correlations due to pleiotropic action of genes and linkage.
- [ii] Changes brought about by selection *i.e.*, correlated response where improvement in one characters will cause simultaneous changes in other characters. The theoretical basis of such genetic correlation.
- [iii] Relationship between metric characters and their fitness in the population.

The original concept of correlation was given by Galton (1988) who suggested the need of coefficient of correlation to describe the degree of association between dependent and independent variables. Later the formula for its quantitative estimation was developed by Pearson (1904) described the mathematical implications of correlation coefficient at phenotypic, genotypic and environmental levels.

The concept of path analysis was originally developed by Sewell Wright (1921), where as the technique was first used for plant selection was demonstrated by Dewey and Lu (1959). The path co-efficient, which simply a standard partial regression co-efficient, which providing a measurement of direct and indirect

effects of independent variables on dependent variables. Therefore, it is important tool for partitioning the correlation co-efficient into components of direct and indirect effects to the yield.

Dutta *et al.* (2002) studied genetic variability, correlation, path analysis on grain amaranth (*A. hypochondriacus* L.). Included characters like viz., harvest index, plant height, inflorescence length, maturity period, flowering time and test weight had significant positive correlation with seed yield. Path analysis revealed highest positive direct effect of harvest index followed by maturity period, basal girth and plant height, inflorescence length and flowering time had substantial indirect contribution through harvest index.

Shukla and Singh (2003) studied correlation coefficient at both genotypic and phenotypic levels in sixty-six genotypes of *Amaranthus* species. They reported that genotypic correlations were higher than their corresponding phenotypic correlations in all the traits, there by suggesting strong inherent association between various characters at genetic level. They further observed that at genotypic level grain yield per plant showed significant positive associations with plant height, number of inflorescence per plant and number of leaf size. It indicated that selection for these traits would lead to improvement in grain yield. They further reported that significant association of leaf size at genotypic levels with grain yield indicating larger leaves were responsible for increasing higher yield; it may be due to high photosynthetic activity.

Prajapati *et al.* (2005) studied the inter relationship between grain yield and eight yield contributing characters days to 50 per cent flowering, plant height, number of branches per plant, panicle length, days to maturity, 1000- seed weight and harvest index in sixty diverse genotypes of amaranth. The grain yield exhibited significant and positive correlation with straw yield per plant, harvest index, 1000-seed weight and number of branches per plant. These four characters accounted for sixty six per cent of total variation in grain yield.

Rana *et al.* (2005) studied correlation analysis using hundred accessions (Fifty from India and fifty from exotic sources) of grain amaranth (*A. hypochondriacus* L.) germplasm were grown in complete Randomised Block Design (RBD). Correlation and path analysis revealed the significance of inflorescence; length, number of leaves and plant height for selecting better yielding genotypes.

Aruna (2009) correlation and path analysis in amaranthus six genotypes of vegetable amaranthus and thirty F₁ hybrids were involved to estimate the correlation and path analysis. The correlation coefficient between yields of stem weight with green weight of leaves was highest both at genotypic level and at phenotypic level. Path analysis indicated that green weight of leaves and stem weight had higher and direct contribution to the yield.

Ahmed *et al.* (2012) correlation studied on leaves per plant, stem diameter, stem weight per plant, leaf weight per plant and plant height exhibited highly significant positive correlation with yield per hectare both at genotypic and phenotypic level. Selection based on these traits would give better response for the improvement in marketable yield in stem amaranth.

Hasan *et al.* (2012) correlation studies using seventeen genotypes of stem amaranthus reported strong positive association of yield with leaf weight, stem weight, stem diameter, dry weight with rind and dry weight without rind. The relationship was non-significant and negative at 1st flowering. The result of path analysis indicated that stem weight had maximum direct effect on marketable yield followed by leaf weight, leaf number and dry weight without rind.

Patil *et al.* (2014) studies on path coefficient analysis in grain amaranth (*Amaranthus* spp.) twenty-two genotypes of amaranth were evaluated for twelve quantitative traits for two years and the pooled data was analyzed. The mean, range, genotypic and phenotypic coefficient of variation, heritability in broad sense and genetic advance were calculated. Path coefficient analysis was carried out using correlation coefficients to know the yield-contributing traits having true associations with seed yield. High heritability coupled with high genetic advance for yield per day to maturity, yield per day to seed fill, harvest index, panicle girth and seed yield per plant was observed. All the traits except days to seed fill possessed positive association with grain yield. Harvest index was positively correlated with days to maturity. Harvest index, aerial biomass per plant and days to maturity also had high phenotypic and genotypic direct effects on seed yield per plant, revealing that indirect selection for these traits would be effective in improving seed yield.

Gerrano *et al.* (2015) thirty-two amaranthus genotypes have been collected and conserved in the gene bank of the ARC-Roodeplaat Vegetable and Ornamental Plant Institute, South Africa. Genetic correlation coefficient for the traits showed that there were significant correlations among some of the phenotypic traits. An extremely

strong correlation was observed between leaf area and leaf area index. A strong correlation was also found between leaf length and leaf width. And moderate correlation with panicle length, fresh biomass, dry biomass and yield per plant. Plant height was also positively correlated with fresh biomass and dry biomass. Yield per plant showed a moderate positive correlation with leaf width, leaf length, leaf area, leaf area index and plant height, and a strong correlation with fresh biomass and dry biomass.

Tejaswini *et al.* (2017) studies correlation and path coefficient analysis using twenty-seven genotypes in grain *Amaranthus* species with nineteen yield and yield attributing characters was carried out in twenty-seven genotypes of vegetable amaranth in Randomized Block Design with three replications. Highly significant and positive correlation with foliage yield was observed with plant height, stem diameter, stem weight per plant, leaf length, leaf width, leaf area index, leaf weight per plant and leaf stem, total chlorophyll content, protein content, ascorbic acid, moisture content and folic acid, whereas number of branches and number of leaves showed negative association with foliage yield. Path coefficient analysis revealed that the characters like plant height, leaf weight per plant, protein content had high to moderate positive direct effect on foliage yield per plant and these traits recorded significant, positive correlation with foliage yield per plant. It clearly indicates that direct selection based on these characters would be effective for an increase in foliage yield.

Jangde *et al.* (2017) studied correlation and path coefficient analysis using twenty-three genotypes of *amaranthus*. The experiment was comprised of twenty-three genotypes of *amaranthus*, laid out in Randomized Block Design (RBD) with three replications was carried out. Analysis of variance revealed that mean sum of squares due to genotypes was highly significant for all the characters. Correlation coefficient correlation studies revealed that leaf yield kilograms per plot showed positive and significant correlation with number of leaves per plant and fresh stem weight for quantitative characters.

Shrivastav *et al.* (2017) studied the seed yield per plant was found highly and positively correlation with inflorescence length, plant height and biological yield per plant. Seed yield per plant showed negative correlation with days to 50 per cent flowering and protein content and non-significant correlation with maturity. The characters showing significant positive correlation among yield and yield

contributing characters would be highly effective and efficient in improving respective traits. Path analysis revealed biological yield per plant and harvest index as important components having high order of direct effect and biological yield per plant via days to maturity followed by harvest index via protein content, inflorescence length via plant height.

Sagar *et al.* (2018) studied genotypic correlation coefficients among growth, yield and quality parameters in twenty-five amaranthus genotypes foliage yield per plant was significantly and positively correlated with stem girth, number of leaves per plant, number of branches per plant, spike length, number of spikes per plant at both phenotypic and genotypic level. Yield per plant was also positively and significantly correlated with leaf area only at genotypic level. Negative and significant correlation was also found for calcium and iron content of leaves with foliage yield per plant at both phenotypic and genotypic level whereas, hypocotyls length exhibited negative and significant association with foliage yield per plant only at genotypic level.

Gelotar (2018) reported that seed yield per plant was significant and positively correlated with days to flowering, plant height and length of inflorescence. Path co-efficient analysis indicated the highest positive direct effect on seed yield per plant by days to flowering, leaf area and 1000-seed weight.

Tiwari (2018) studied twenty-seven genotypes of grain amaranth were used to study correlation and path analysis which resulted days to maturity got significant positive correlation with seed yield, plant height, length of inflorescence and 10 ML seed weight (g). Plant height was also found to have significant positive correlation with seed yield and 10 ML seed weight. Length of inflorescence and 10 ML seed weight had significant and positive correlation with yield. In case of path analysis, days to maturity had positive direct effects on grain yield than 10 ML seed weight, length of inflorescence and plant height.

2.3 Genetic diversity through ISSR markers

Molecular breeding rapid process in molecular biology and genetic engineering, possibility of improving the crop plants with respect to yield and quality. The term molecular breeding was frequently used to represent the breeding methods that are coupled with genetic engineering techniques. Improved agriculture to meet the food demands of the world is a high priority area. For several years, the conventional plant breeding programmes (although time

consuming) have certainly helped to improve grain yield and cereal production. The molecular markers are highly reliable and advantageous in plant breeding programmes. Molecular markers provide true representations of the genetic makeup at the DNA level. They are consistent and not affected by environmental factors. Molecular markers can be detected much before developments of plants occur. A large number of markers can be generated as per the needs.

Molecular phylogenetic studies would enable plant breeder to differentiate among genotypes by estimating the amount of variation within and between species and provide platform for appropriate breeding strategies. Identification of appropriate genetic markers would greatly help in distinguishing for effective conservation of plant material and breeding purposes. Many marker based selection strategies are now available to improve crop plants with speed and precision.

Molecular markers detect DNA sequence variation among genotypes. Since 1980, when restriction fragment length polymorphisms (RFLPs) were first used to construct the first molecular map of the human genome (Botstein *et al.*, 1980). As RFLP have many disadvantages like, it require large amount of high pure DNA, laborious, expensive to identify suitable marker, time consuming and radioactively labeled probes are used. Polymerase chain reaction (PCR) technology has promoted the development of a range of molecular assay systems which detect polymorphism at the DNA level and offer an alternative to the hybridization-based method of RFLP.

Xu and Sun (2001) reported important group of species in the genus amaranth is the *A. hybridus* L. species complex, including three cultivated grain amaranths, *A. cruentus* L., *A. caudatus* L., and *A. hypochondriacus* L. and their putative wild progenitors, *A. hybridus* L., *A. quitensis* L. and *A. powelli* L. Taxonomic confusion exists among these closely related taxa. Internal transcribed spacer (ITS) of nuclear ribosomal DNA, amplified fragment length polymorphism (AFLP), and double primer fluorescent inter-simple sequence repeat (ISSR) were employed to reexamine the taxonomic status and phylogenetic relationships of grain amaranths and their wild relatives. Low ITS divergence in these taxa resulted in poorly resolved phylogeny. However, extensive polymorphisms exist at AFLP and ISSR loci both within and among species. In phylogenetic trees based on either AFLP or ISSR or the combined data sets, nearly all intraspecific accessions can be placed in their corresponding species clades, indicating that these taxa are well-separated species. The AFLP trees share many features in common with the ISSR trees, both showing a

close relationship between *A. caudatus* and *A. quitensis*, placing *A. hybridus* in the same clade as all grain amaranths, and indicating that *A. powellii* is the most divergent taxon in the *A. hybridus* species complex. This study has demonstrated that both AFLP and double-primer fluorescent ISSR have a great potential for generating a large number of informative characters for phylogenetic analysis of closely related species, especially when ITS diversity is insufficient.

Ray and Roy (2007) reported that phylogenetic relationships between some economically important members of *Amaranthaceae* and *Chenopodiaceae* of lower Gangetic plains of India, using RAPD and ISSR markers. A total of 293 RAPD and fragments were generated with 15 (out of 50) decamer arbitrary primers and microsatellite repeat anchored primers. Respectively, mean genetic similarities were estimated at 0.47 and 0.45 using RAPDs and ISSRs, respectively. The members of *Chenopodiaceae* are separated from those of *Amaranthaceae* in both the cases. The mantel test between the two Jaccard's similarity matrices gave $r = 0.83$, showing good correlation between RAPD and ISSR based similarities. However, in both the dendrograms *Celosia* spp. showed distant relationships with other amaranthus.

Singh and Das (2009) ISSR primer involves the amplification of DNA segments present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite directions. The technique uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly ISSR of different sizes. The microsatellite repeats used as primers for ISSRs can be dinucleotide, tri-nucleotide, tetra-nucleotide, or penta-nucleotide. ISSRs use longer primers (15-30 mers) as compared to RAPD primers (10 mers), which permit the subsequent use of a high annealing temperature leading to higher stringency. The annealing temperature depends on the GC content of the primer used and ranges from 45 to 65°C. The technique is simple, quick and the use of radioactivity is not essential. ISSR markers are randomly distributed throughout the genome and usually show high polymorphism although the level of polymorphism has been shown to vary with the detection method used.

Ziarovska *et al.* (2013) studied ISSR markers to analyse Gama ray-irradiated mutants of amaranth Ficha cultivar and K-433 hybrid; tested six microsatellite primed PCR markers. First analysis of gamma γ - irradiated amaranth mutant lines was done using the ISSR. In the study, primers that distinguish the mutant lines from control

plant were reported as well as primer with the differentiation ability for all analysed mutant lines. The very specific changes in the mutant lines non-coding regions based on inter-microsatellite length polymorphism were analysed. Mutant lines of the Ficha cultivar (C-15, C-26, C-27, C-82 and C-236) shared a genetic dissimilarity of 0.16 and their ISSR profiles were more similar to the Ficha than those of K-433 hybrid mutant lines.

Singh *et al.* (2013) Genetic diversity in a set of thirty-one *Amaranthus* accessions was analysed using RAPD, ISSR and SSR markers. Polymorphic Information Content (PIC) values of the polymorphic RAPD, SSR and ISSR markers ranged from 0.067 to 0.471, 0.178 to 0.452 and 0.11 to 0.362 with an average of 0.229, 0.371 and 0.441 per primer, respectively. Gene diversity for RAPD, SSR and ISSR markers, respectively ranged from 0.032 to 0.487, 0.11 to 0.362 and 0.104 to 0.384. The similarity metrics values for the accessions ranged from 0.58 to 0.98 with RAPD, 0.70 to 1.00 with SSR and from 0.44 to 0.88 with ISSR markers. The dendrogram generated by the markers had five major clusters in case of RAPD markers, two distinct clusters in case of SSR markers and six clusters in case of ISSR markers. Amongst all, ISSR markers provided greater confidence for the assessment of genetic diversity in comparison to SSR and RAPD markers. All the accessions studied were found genetically diverse which may be useful in breeding for improving the genotypes of *amaranthus*.

Oduwaye *et al.* (2014) studied effectiveness of two PCR-based molecular techniques, inters simple sequence repeats (ISSRs) and amplified fragment length polymorphisms (AFLPs), for genetic assessment of amaranth. The polymorphic loci ranged from 110 among *A. caudatus* to 228 among *A. cruentus* and 16 among *A. tricolor* to 56 among *A. hypochondriacus* for AFLP primer combinations and ISSR primers, respectively. Among the two marker systems used, ISSR finger-printing detected the highest number of alleles per locus (1.83) compared to AFLPs (1.63). However, the assay efficiency index for AFLP was 14.49, more than five-fold higher than ISSR (1.75). The study also revealed that ISSR primers with di-nucleotide repeats gave a good fingerprint, indicating that di-nucleotide repeats are more frequent in amaranth genome. The reproducibility of the two marker systems was confirmed by the narrow gene diversity (0.03 ± 0.11 to 0.07 ± 0.17) observed between the controls. Bayesian consensus and neighbor-joining trees were constructed to describe the cluster arrangement among the *Amaranthus* spp. The cluster pattern was similar for

both markers, though the cluster order in the trees was slightly different. The results of this study confirm the usefulness of AFLPs and ISSRs for the genetic assessment of amaranth.

Raut *et al.* (2014) studied twenty-eight genotypes of grain amaranth (*Amaranthus hypochondriacus*) were evaluated for their genetic divergence at molecular level. The analysis of morphological variance exhibited significant differences among the genotypes for all the eight characters indicating the presence of substantial degree of variability. Fifteen Inter Simple Sequence Repeat (ISSR) primers amplified a total of 61 bands of which 53 bands (86.88 per cent) were polymorphic. None of the band was unique and 8 bands were monomorphic in their profile. Similarity index ranged from (0.55-0.97) with moderately wide diversity. In dendrogram, all the genotypes studied were distinguished from each other and five broad clusters were observed with Suvarna and SKGPA26 forming most distinct genotype clusters. Though there was considerable inter-group variation. However, extent of variation within the groups was low. When morphological data was correlated with molecular divergence data. Grouping to limited extent was observed with yield per plant, stem girth and inflorescence for traits. Inter-cluster crossing could be used for further widening of genetic variability in grain amaranth breeding programme.

Stefunova *et al.* (2015) studied RAPD and ISSR markers to analyse intra and inter-specific variability of sixteen *A. caudatus* L., eighteen *A. cruentus* L. and twenty-one *A. hypochondriacus* L. accessions. The potential of both approaches was evaluated using three random and three microsatellite primers amplifying in total of 1013 (ISSR) scorable fragments. Similarity values among accessions of three *Amaranthus* species ranged from 0.00 to 1.00 in types of markers. Based on the intraspecific variability the polymorphism percentage varied from 90 to 100 per cent (ISSR), respectively. Resolving power (Rp) of ISSR primers have been higher (5.28 in average) in comparison to RAPD primers (4.84 in average). Percentage of distinguished accessions by ISSR and RAPD primers ranged from 29 to 89 per cent (based on primer's type) and from 19 to 72 per cent, respectively. Cluster analysis based on ISSR data has shown the individual species separation except of three accessions. Two of *A. caudatus* genotypes originated from India clustered with *A. hypochondriacus* accessions and one of *A. hypochondriacus* genotype originated from Nepal clustered with *A. cruentus* accessions. This study has demonstrated that

single primer marker systems as ISSR are able to generate a sufficient level of informative characters for intra and inter-specific analysis of *Amaranthus* genus.

Lymanska *et al.* (2017) studied genetic diversity of the *Amaranthus* species included DNA polymorphism analysis. Using RAPD and ISSR primer, 203 loci have been identified, of which 173 appear to be polymorphic, 30 monomorphic (found in all genotypes analyzed) and 13 unique (found only in one genotype). Unique and monomorphic DNA loci can be used as specific genetic markers, in particular, for the certification of breeds, which is especially important for the identification of plant material and plant genetic variability monitoring. A high level of DNA polymorphism was revealed (about 85 %), a genetic relationship between grain amaranth species established, their monophyletic origin theory verified. *A. mantegazzianus* Passer was proved to be an *A. caudatus* L. sub-species.

Gelotar (2018) studied nineteen germplasms of grain amaranth were evaluated using ISSR markers. Total 11 polymorphic ISSR primers were screened across all the genotypes of grain amaranthus which produced total 114 bands with an average of 10.36. Out of 114 bands, 98 bands were polymorphic. The average per cent polymorphism by ISSR primers was found 87.15 per cent and average Polymorphic Information Content (PIC) value was found 0.853, it indicates study for assessment of genetic diversity was very good. The unique amplicon (marker) producing primers were also found which can be used for identification of genotypes.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigation work on "**Variability, correlation and path analysis in grain amaranth (*Amaranthus hypochondriacus* L.)**" was carried out at Centre for Crop Improvement, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during *Rabi* 2018-19.

The details of the material used, methods adopted and statistical analysis followed during the investigation are described below.

3.1 Experimental materials

The experimental material used for study to be consisted of 46 genotypes of grain amaranth which were obtained from AICRN on potential crops "Center for Crop Improvement" Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, District : Banaskantha (Gujarat State) in Table 3.1.

Table 3.1 : List of genotypes

Sr. No.	Genotypes	Sr. No.	Genotypes	Sr. No.	Genotypes
1.	SKGPA-61	17.	SKNA-1407	32.	KGBA-4
2.	SKGPA-73	18.	SKNA-1414	33.	KGBA-5
3.	SKGPA-150	19.	SKNA-1501	34.	KGBA-7
4.	SKGPA-155	20.	SKNA-1502	35.	RMA-62
5.	SKNA-401	21.	SKNA-1503	36.	RMA-63
6.	SKNA-403	22.	SKNA-1508	37.	RGA-13
7.	SKNA-808	23.	SKNA-1510	38.	RGA-14
8.	SKNA-813	24.	GA-2	39.	RGA-16
9.	SKNA-903	25.	GA-3	40.	RGA-17
10.	SKNA-908	26.	BGA-4-9	41.	RGA-18
11.	SKNA-1201	27.	BGA-7	42.	RGAG-12-22
12.	SKNA-1207	28.	BGA-9	43.	RGAG-14-3
13.	SKNA-1305	29.	BGA-10	44.	RHGA-13-3
14.	SKNA-1313	30.	BGA-14	45.	MGA-502
15.	SKNA-1403	31.	BGA-20	46.	Suvarana
16.	SKNA-1406				

3.2 Experimental details

- [1] Title of experiment : "**Variability, correlation and path analysis in grain amaranth (*Amaranthus hypochondriacus* L.)**"
- [2] Location :
- (a) Field experiment : Center for Crop Improvement, S. D. Agricultural University, Sardarkrushinagar-385 506.

- (b) Lab experiment : Biotechnology Laboratory,
Department of Genetics
and plant Breeding,
C. P. College of Agriculture,
S. D. Agricultural University,
Sardarkrushinagar-385 506.
- [3] Season and year : *Rabi* 2018-19 (Field evaluation)
- [4] Date of sowing : 24th November, 2018
- [5] Number of genotype : 46 (Forty-six)
- [6] Experimental design : RBD (Randomized Block Design)
- [7] Number of replications : 3 (Three)
- [8] Spacing : 45 cm × 15 cm
- [9] Length of row : 3.0 m (Two rows per genotype)
- [10] Plant protection measure : As per requirement and recommendation
- [11] Agronomic practices : As per requirement and recommendation

3.3 Observations recorded

Five competitive plants per genotype were selected at random for recording observations on following characters in each replication and averages were worked out for statistical analysis.

3.3.1 Days to flowering

Number of days from sowing to the date when 50 per cent plants in each plot flowered was recorded as days to flowering.

3.3.2 Leaf area per plant (cm²)

Five plants from each replication were randomly selected and measure leaf area with the help of leaf area metre and then average of leaf area per plant was calculated.

3.3.3 Days to maturity

Number of days taken from the days of sowing to the physiological 80 per cent maturity of crop was recorded as days to maturity.

3.3.4 Length of inflorescence (cm)

The length of inflorescence was measured in centimeter from collar of the ear up to the tip of inflorescence at the time of maturity.

3.3.5 Width of inflorescence (cm)

The width of inflorescence was measured in centimeter from middle of the inflorescence at the time of maturity.



Plate 1. General view of experimental plot of forty-six grain amaranth genotypes

3.3.6 Plant height (cm)

Height of the plant was measured in centimeter from soil surface (ground level) to the tip of main inflorescence at the time of maturity.

3.3.7 Seed yield per plant (g)

Randomly selected five competitive plants was harvested and threshed. Seed will be cleaned and weighted and average on per plant basis were worked out for grain yield per plant in gram.

3.3.8 Stem girth (cm)

The stem girth was measured in centimeter at below inflorescence of stem at the time of maturity.

3.3.9 Harvest index (%)

Harvest index was calculated as per percent basis. It is a ratio of grain yield per plant to biological yield per plant.

$$\text{Harvest Index (\%)} = \frac{\text{Total grain yield per plant}}{\text{Total Biological yield per plant}} \times 100$$

3.3.10 Test weight (g/10 ml)

A random sample of 10 ml bulk grain was taken from each treatment in each replication and weight in grams was recorded on electronic balance.

3.3.11 Protein content (%)

Protein content of the grinded seed sample was determined by Near-infrared (NIR) spectroscopy (Instant analyses) make of Dikey-John, U.S.A.

3.4 Statistical analysis

The mean values of five randomly selected observational plants for 46 different genotypes were used for statistical analysis. The following statistical parameters were worked out for presentation of the data on different quantitative attributes.

3.4.1 Analysis of variance

The data recorded for all the characters were subjected to analysis of variance with the formula suggested by Panse and Sukhatme (1978). The following linear model for the Randomized Block Design (RBD) was used.

$$Y_{ij} = \mu + a_i + b_j + e_{ij}$$

Where,

$$\begin{aligned} Y_{ij} &= \text{Yield of } j^{\text{th}} \text{ genotype in } i^{\text{th}} \text{ replication} \\ \mu &= \text{General mean} \end{aligned}$$

- a_i = Effect of i^{th} replication
 b_j = Effect of j^{th} genotype
 e_{ij} = Uncontrolled variation associated with i^{th} replication and j^{th} genotype

For all the characters under study, the mean values of five randomly selected plants were used for statistical analysis. The data recorded for different characters were subjected to analysis of variance. Different components of variance viz., phenotypic, genotypic and environmental variances were estimated. Different parameters of genetic variability were computed by standard statistical procedures. The phenotypic and genotypic correlations were also estimated. The genotypic correlations were subjected to path coefficient analysis. The analysis of variance was done as suggested by Panse and Sukhatme (1978).

Table 3.2 : Analysis of variance for experimental design

Source of variation	d.f.	MSS	Expected mean square
Replications	(r-1)	MSr	$\sigma_e^2 + t \sigma_r^2$
Genotypes	(t-1)	MSt	$\sigma_e^2 + r \sigma_g^2$
Error	(r-1) (t-1)	MSe	σ_e^2
Total	(rt-1)	-	-

Where,

- r = Number of replications,
 g = Number of genotypes,
 σ_e^2 = Variances due to error,
 σ_r^2 = Variances due to replication,
 σ_g^2 = Variances due to genotypes,
MSr = Mean sum of square for replication,
MSt = Mean sum of square for genotypes, and
MSe = Mean sum of square for error.

The standard error for differences between treatments mean was calculated from ANOVA table.

$$S.E.m.\pm = \sqrt{\frac{\sigma_e^2}{r}}$$

Where,

- S.E.m. = Standard Error of Mean,
 σ_e^2 = Error Mean Square, and
 r = Number of replications.

The critical difference to compare the mean values of various genotypes was calculated by using the following formula.

$$C.D. = \sqrt{2} \times S.E.m. \times t_{0.05 \text{ at error d.f.}}$$

Where,

- C.D. = Critical difference
 S.E.m. = Error Mean Square,
 r = Number of replication, and
 t = Table 't' value at error d.f.

The Coefficient of Variation (C.V. %) was calculated by using the following formula : $x = \frac{-b \pm \sqrt{MSe}}{2a}$

$$C.V. \% = \frac{\sqrt{MSe}}{\bar{X}} \times 100$$

Where,

- C.V. = Coefficient of variation,
 MSe = Mean sum of square of error, and
 $\bar{X}$ = General mean.

3.4.2 Genetic variability parameters

[i] Mean

It is computed by dividing the sum of all observations in a sample by their number.

$$\bar{X} = \frac{\sum X_{ij}}{n}$$

Where,

- $\bar{X}$ = General mean,
 X_{ij} = Any observation in i^{th} replication and j^{th} genotype, and
 n = Number of observations.

[ii] Range

It is the difference between maximum value and minimum value in a particular character. The genotypic, phenotypic and environmental components were estimated as explained by Johnson *et al.* (1955).

$$\text{Range} = \text{Maximum value} - \text{Minimum value}$$

[iii] Genotypic variance (σ^2_g)

The genotypic variance is contributed by genetic cause or the occurrence of difference among individuals due to differences in their genetic make-up. Following formula was used.

$$\text{Genotypic variances } (\sigma^2_g) = \frac{\text{Genotypic M S} - \text{Error M S}}{\text{Number of replications (r)}}$$

[iv] Environmental variance

Following formula was used.

$$\text{Environmental variances } (\sigma^2_e) = \text{Mean square of error (MSe)}$$

[v] Phenotypic variance

It is the sum of the variances contributed by genetically causes and environmental factors and is calculated as under.

$$\text{Phenotypic variance } (\sigma^2_p) = \text{Error variance } (\sigma^2_e) + \text{Genotypic variance } (\sigma^2_g)$$

[vi] Coefficient of variation

The coefficient of phenotypic and genotypic variations was calculated by the formula suggested by Burton (1952).

(a) Phenotypic Coefficient of Variation (PCV)

The phenotypic coefficient of variation, which measures the magnitude of phenotypic variation present in a particular character, was estimated as per the formula suggested by Burton (1952).

$$\text{PCV\%} = \frac{\sqrt{\sigma^2_p}}{\text{Mean } (\bar{X})} \times 100$$

Where,

σ^2_p = Phenotypic variance, and

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

(b) Genotypic Coefficient of Variation (GCV)

The genotypic coefficient of variation, which measures the magnitude of genetic variation present in a particular character, was estimated as per the formula suggested by Burton (1952).

$$\text{GCV\%} = \frac{\sqrt{\sigma^2_g}}{\text{Mean } (\bar{X})} \times 100$$

Where,

σ^2_g = Genotypic variance, and

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

GCV and PCV were categorized as low, moderate and high by following Shivasubramanian and Menon (1973). It is as follows :

0-10 %	=	Low
10-20 %	=	Moderate
20 % and above	=	High

[vii] Heritability (Broad Sense) h^2 (bs)

It is the proportion of phenotypic variability that is due to genetic reasons. It was calculated by using the formula proposed by Allard (1961).

$$h^2(b) \% = \frac{\text{Genotypic variance } (\sigma^2_g)}{\text{Phenotypic variance } (\sigma^2_p)} \times 100$$

Where,

$$\begin{aligned} \sigma^2_g &= \text{Genotypic variance, and} \\ \sigma^2_p &= \text{Phenotypic variance.} \end{aligned}$$

Heritability percentage was categorized as demonstrated by Robinson *et al.* (1966). It is as follows :

0-30 %	=	Low
30-60 %	=	Moderate
60 % and above	=	High

[viii] Expected genetic advance (GA)

Expected genetic advance represents the shift in a population towards superior side under some selection pressure after single generation of selection. It could be calculated by using the methodology suggested by Allard (1961) at 5 per cent selection intensity using the constant 'k' as 2.06.

$$G.A. = h^2(b) \times k \times \sigma_p$$

Where,

$$\begin{aligned} G.A. &= \text{Genetic Advance.} \\ h^2(bs) &= \text{Heritability (Broad sense).} \\ K &= \text{Selection intensity at 5 per cent} = 2.06, \text{ and} \\ \sigma_p &= \text{Phenotypic standard deviation.} \end{aligned}$$

[ix] Genetic advance expressed as per cent of mean (Genetic gain)

The expected genetic advance as expressed in per cent of mean was calculated by the method suggested by Johnson *et al.* (1955).

$$\text{Genetic gain \%} = \frac{\text{Expected genetic advance (G.A.)}}{\text{Mean (}\bar{x}\text{)}} \times 100$$

The genetic advance as per cent mean was categorized as suggested by Johnson *et al.* (1955). It is as follows :

0-10 %	=	Low
10-20 %	=	Moderate
20 % and above	=	High

3.4.3 Correlation coefficient

For this purpose, analysis of covariance for all possible pairs of eleven characters was carried out using the procedure of Panse and Sukhatme (1978).

Table 3.3 : Analysis of variance for correlation coefficient

Source	d.f.	Mean square of products (M.S.)	Expectation of mean square of products
Replications	(r-1)	MSP ₁	Cov _e xy + gCov _r xy
Genotypes	(g-1)	MSP ₂	Cov _e xy + rCov _g xy
Error	(r-1)(g-1)	MSP ₃	Cov _e xy
Total	(rg-1)	-	-

Where,

r	=	Number of replications,
g	=	Number of genotypes,
Cov _e xy	=	Environmental component of covariance,
Cov _r xy	=	Replication component of covariance,
Cov _g xy	=	Genotypes components of covariance,
MSP ₁	=	Mean square products for replication,
MSP ₂	=	Mean square products for genotypes, and
MSP ₃	=	Mean square product for error.

The components of covariance were estimated by equating the observed mean sum of products with their expectations as shown below.

$$\begin{aligned} \text{Cov}_{gxy} &= \frac{(\text{MSP}_2 - \text{MSP}_3)}{r} \\ \text{Cov}_{exy} &= \text{MSP}_3 \\ \text{Cov}_{pxy} &= \text{Cov}_{gxy} + \text{Cov}_{exy} \end{aligned}$$

Where,

Cov _g xy	=	Genotypic components of covariance,
---------------------	---	-------------------------------------

Cov_{exy} = Environmental components of covariance, and

Cov_{pxy} = Phenotypic components of covariance.

The genotypic (r_g) and phenotypic (r_p) correlation coefficient were calculated as under by adopting the procedure explained by Al-Jibouri *et al.* (1958).

$$(a) \quad \text{Genotypic correlation coefficient :} \quad r_g(XY) = \frac{Cov_g(XY)}{\sqrt{\sigma_g^2(X)} \times \sqrt{\sigma_g^2(Y)}}$$

$$(b) \quad \text{Phenotypic correlation coefficient :} \quad r_p(XY) = \frac{Cov_p(XY)}{\sqrt{\sigma_p^2(X)} \times \sqrt{\sigma_p^2(Y)}}$$

Where,

$r_g(xy)$ and $r_p(xy)$ are genotypic and phenotypic correlation coefficient for a pair of trait x and y, respectively.

$Cov_g(xy)$ and $Cov_p(xy)$ are genotypic and phenotypic covariance for a pair of characters x and y, respectively.

The phenotypic correlation was tested using the method suggested by Fisher and Yates (1963).

Test of significance of correlation coefficient

Comparison of sample 'r' with population value.

$H_0 : \rho = 0$ (both the variables are not linearly associated)

$H_a : \rho \neq 0$

$$t_{(n-2)} = \frac{r - \rho}{SE \text{ of } r} \quad SE \text{ of } r = \sqrt{\frac{1-r^2}{n-2}}$$

$$= \frac{r}{\sqrt{1-r^2} \times \sqrt{n-2}} \quad \text{under } H_0 : \rho = 0$$

Where,

r = Correlation coefficient, and

n = Number of pairs of observation.

The genotypic and phenotypic correlation coefficients were worked out of all possible pairs and were tested against standardized tabulated values at 5 and 1 per cent level of significance.

3.4.4 Path coefficient analysis

The cause and effect, interrelationship between two variables cannot be estimated from simple correlation coefficient analysis. Therefore, the path coefficient

analysis was carried-out according to the method suggested by Wright (1921) and used by Dewey and Lu (1959). Genotypic correlation coefficients of twelve variables with seed pod yield per plant were used to estimate the path coefficients for the direct effects of various independent characters on seed yield per plant.

The path coefficients were obtained by solving a set of simultaneous equations as below.

$$r_{ny} = P_{ny} + r_{n2} \cdot P_{2y} + r_{n3} \cdot P_{3y} + r_{n4} \cdot P_{4y} + \dots + r_{nx} \cdot P_{xy}$$

Where,

- r_{ny} = Represents correlation coefficient between one component character and grain yield,
- P_{ny} = Represents the path coefficient between one trait and grain yield, and
- $r_{n2}, r_{n3} \dots r_{nx}$ = Represents correlation coefficient between that character and other yield component in turn.

The following genotypic correlation matrix was formed.

$$[r_{1y} r_{2y} r_{3y} \dots r_{ny}] = [r_{11} r_{12} r_{13} \dots r_{1n} \quad r_{21} r_{22} r_{23} \dots r_{2n} \quad \dots \quad r_{n1} r_{n2} r_{n3} \dots r_{nn}] \times [p_{1y} p_{2y} p_{3y} \dots p_{ny}]$$

$$\begin{array}{c} \textbf{Matrix-A} \\ \left(\begin{array}{c} r_{1y} \\ r_{2y} \\ r_{3y} \\ \vdots \\ \vdots \\ \vdots \\ r_{ny} \end{array} \right) \end{array} = \begin{array}{c} \textbf{Matrix-B} \\ \left(\begin{array}{ccc} r_{11} & r_{12} \dots r_{1n} \\ r_{21} & r_{22} \dots r_{2n} \\ r_{31} & r_{32} \dots r_{3n} \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \\ r_{n1} & r_{n2} & r_{nn} \end{array} \right) \end{array} \times \begin{array}{c} \textbf{Matrix-C} \\ \left(\begin{array}{c} p_{1y} \\ p_{2y} \\ p_{3y} \\ \vdots \\ \vdots \\ \vdots \\ p_{ny} \end{array} \right) \end{array}$$

$$\text{Or } A = B \times C$$

Where,

$r_{11} \dots r_{nn}$

r_{11} = r_{21} and so on, and

r_{1y} = Correlation between first component character and seed yield.

The technique given by Goulden (1962) was followed for inversion of the 'B' matrix using partitioning method of matrix inversion.

Path coefficient (P_{ij}) were obtained as follows.

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

Where,

(B^{-1}) is the inverse of mutual correlation matrix of character.

The indirect effects for a particular character through other characters were obtained by multiplication of direct path and particular correlation coefficient between those two characters, respectively.

$$\text{Indirect effect} = r_{ij} \times P_{ij}$$

Where,

$$i = 1, 2, 3, \dots, n$$

$$j = 1, 2, 3, \dots, n$$

$$P_{ij} = P_{1y} \times P_{2y} \times P_{3y} \times \dots \times P_{ny}$$

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

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

Where,

$$R^2 = (P_{1y} r_{1y} + P_{2y} r_{2y} + \dots + P_{ny} r_{ny}),$$

$$P_{1y}, P_{2y}, \dots, P_{ny} = \text{Direct path values, and}$$

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

Scales for path coefficients (Lenka and Mishra, 1973) :

The direct and indirect effects are rated as follows.

- | | | |
|----|----------------|--------------|
| 1. | 0.00-0.09 | : Negligible |
| 2. | 0.10-0.19 | : Low |
| 3. | 0.20-0.29 | : Moderate |
| 4. | 0.30-1.00 | : High |
| 5. | More than 1.00 | : Very high |

3.5 Molecular diversity analysis by using ISSR markers

ISSR (Inter-Simple Sequence Repeats) primers were utilised to estimate molecular variability of genotypes under present study.

3.5.1 Glasswares, polywares and equipments

All glasswares used which were obtained from Borosil, J-sil, while, the plastic wares from Axygene, Genexy and Tarsons. All the glasswares were thoroughly cleaned with tap and distilled water and dried in oven. All the plastic wares *viz.*, micro tips, 2 ml Eppendorf tubes *etc.*, were autoclaved. The equipments and instrument which used in molecular diversity are given in Table 3.4.

Table 3.4 : Glasswares, polywares and equipments

Sr. No.	Equipments and instruments	Company/manufacture
1.	Weighing balance	Citizen, India.
2.	Hot water bath	Equitron, India.
3.	Gel electrophoresis unit	Bangalore GeNei Ltd., India.
4.	Gel documentation	Uvipro platinum, U.S.A.
5.	pH meter	Electroquip.
6.	Refrigerated centrifuge	CR21GII, Hitachi.
7.	Thermal cycler	Eppendorf, U.S.A.
8.	Microwave oven	Samsung, India.
9.	Micro centrifuge	Bangalore GeNei.
10.	-20 Freezer	Nova India Ltd., India.
11.	Hot air oven	Scientific Instruments Ltd, India.
12.	Nanodrop 2000	Eppendorf.

3.5.2 DNA isolation

The plant genomic DNA was isolated as per CTAB method of Doyle and Doyle (1987).

Table 3.5 : Composition of extraction buffer (CTAB buffer)

Sr. No.	Stocks	Quantity for DNA extraction buffer	Final concentration
1.	CTAB	10 ml	2 % w/v
2.	Tris HCl	1 ml	100 mM
3.	NaCl	10 ml	1.4 M
4.	EDTA	5 ml	20 mM
5.	Polyvinylpyrrolidone (PVP)	5 ml	1 % w/v
6.	D/W	Volume make up to 100 ml	-

Reagents for DNA isolation :

1. Extraction buffer in Table 3.5.
2. β -Mercaptoethanol (20 Micro litter).
3. Chloroform : Isoamyl alcohol (C : I) solution in the ratio of 24:1.
4. Chilled Isopropanol.
5. 1 $\times$ TE (Tris HCL and EDTA) buffer [1M Tris HCL (pH = 8.0) and 0.5 M EDTA (pH = 8.0)].
6. Nuclease free water.
7. RNase A (10 mg/ 10 ml) [10 mM Tris HCL = 10 μ l, 1 mM EDTA = 3 μ l, RNase A = 10 mg and distilled water 700 μ l] for 1 ml.
8. Liquid nitrogen.
9. Ethanol (70 %).

Various reagents and stock solution were prepared for the isolation of genomic DNA. The procedure for the preparation of stock solution of the reagents used in the DNA isolation and agarose gel electrophoresis is given in Table 3.6.

Table 3.6 : Preparation of stock solutions for DNA extraction and agarose gel electrophoresis

Sr. No.	Solution	Method of preparation
1.	1M Tris HCl (pH8.0)	To make 100 ml of 1M Tris (pH8.0), 12.11g Tris base was dissolved in 80 ml distilled water. The pH was adjusted to 8.0 by adding concentrated HCl and the total volume was made 100 ml. Then, it was dispensed in reagent bottles and autoclaved to sterilize.
2.	0.5M EDTA (pH8.0)	To make 100 ml of 0.5M EDTA (pH 8.0), 18.60 g EDTA was dissolved in 80 ml distilled water. Then the pH was adjusted to 8.0 by adding NaOH pellets. The total volume was made up to 100 ml and the reagent was dispensed for autoclaving for sterilizing.
3.	5M NaCl	To make 5M NaCl, 29.22 g of NaCl was dissolved in 50 ml distilled water. After complete dissolution the volume was made up to 100 ml. Then the reagent was dispensed in bottles and sterilized by autoclaving.
4.	70 % Ethanol	To make 70 % ethanol, 70 ml ethanol was taken in a beaker and 30 ml of distilled water was added into it. It was stored in bottles at 4°C.
5.	Chloroform : Isoamyl alcohol (24:1)	To make Chloroform : Isoamyl alcohol (24:1), 96 ml of chloroform and 4 ml of Isoamyl alcohol were mixed thoroughly and stored at room temperature after autoclaving.
6.	Ethidium bromide (10 mg/ml)	To make Etbr (10 mg/ml), 10 mg of Etbr was added to 1 ml of distilled water and the dye was kept on magnetic stirrer for complete dissolution.
7.	5X TBE Buffer (pH 8.0)	To make 5X TBE buffer (pH 8.0), 54.5 g of Tris base, 27.5 g Boric acid and 20 ml of 0.5M EDTA were mixed and pH was adjusted to 8.0. Then final volume was adjusted up to 1 liter.
8.	Gel loading dye (6X)	For preparing (6X) gel-loading dye, 0.25 % of bromophenol blue (BPB), 0.2 5 % of xylene cyanole FF and 30 % of glycerol were added to distilled water.

Protocol for DNA isolation (46 genotypes of grain amaranthus) :

1. Juvenile leaves (1 g) were grind to fine powder with liquid nitrogen in a mortar and pestle.
2. 1 ml of extraction buffer was added with 10 µl β-mecaptoethanol. The tubes were maintained in a water bath and incubate at 65°C for 60 minutes.

3. After incubation, equal volume of chloroform : Isoamyl alcohol (24:1) was added and centrifuged with 10000 rpm for 12 minutes at 37°C.
4. After centrifugation, the supernatant was transferred in fresh micro centrifuge tube and again add equal amount of C : I (24 : 1) was added and centrifuged with 10000 rpm for 12 minutes at 37°C.
5. After centrifugation, the supernatant was transferred in fresh micro-centrifuge tube. In this tube 2-3 µl RNase was added and kept the tubes in hybridization oven at 37° C for 25 minutes.
6. Equal volume of Chloroform : Isoamyl alcohol (24 : 1) was added after treatment of RNase.
7. The tubes were centrifuged at 10000 rpm for 10 minutes at 37°C temperature.
8. After centrifugation, the supernatant was transferred in fresh micro-centrifuge tube and 600 µl of chilled Isopropanol was added and tubes were kept at -20°C over night for better precipitation of DNA.
9. Next day, the pellet of DNA was obtained and tube were centrifuged at 10000 rpm for 10 minutes at 4°C and the pellet was air dried and twice washed with 70 per cent ethanol.
10. After wash with 70 per cent ethanol the tubes were kept in hybridization oven for 15 minutes to remove of ethanol.
11. 100 µl of Tris-EDTA (TE) buffer was added and kept at room temperature.
12. The DNA was dissolved at room temperature and stored at -20°C for further use.

3.5.3 Quantification of extracted genomic DNA by spectrophotometer

The quantity (in term of protein and RNA contamination) of isolated genomic DNA, Spectrophotometric analysis was performed. For this 2 µl DNA was loaded into the well of nanodrop (Eppendorf). The concentration of DNA and absorbance were measured at 260 nm and 280 nm. Pure DNA was considered as the one having A_{260}/A_{280} ratio of 1.8 to 2.0.

3.5.4 Quality check by Agarose Gel Electrophoresis

1. Genomic DNA was resolved by horizontal electrophoresis in 0.8% (W/V) agarose gel.
2. Gel casting plate was washed with distilled water and dried.
3. Plate was wiped with ethanol and air dried.

4. Agarose gel was prepared by dissolving 0.8 % of agarose in 100 ml 0.5 X TBE buffer and heated in a microwave oven.
5. Ethidium bromide (10 mg/ml) 5 µl/100 ml in TBE buffer was added to it after cooling it approximately 35°C.
6. Gel solution was then poured into the gel casting plate inserted with appropriate comb.
7. After solidification of gel, the plate was placed in electrophoresis chamber and sub-merged using 0.5 X TBE buffer and combs were removed gently.
8. Ten µl genomic DNA samples were mixed with 2 µl of 6 X gel loading dye and were loaded in the wells and the standard DNA marker (50 bp/100 bp/500 bp) was included along with the samples.
9. Electrophoresis was carried out at constant voltage at 60 V for 45 minutes. (Primrose and Twyman, 2013). After completion of the run, the gel was viewed under UV light and gel documentation system (Alpha Innotech Corporation, U.S.A.) for visualizing separated bands and photographed.

3.5.5 Dilution of genomic DNA samples

Based on quantification data, a portion of DNA samples were diluted with appropriate quantity of 1 X TE buffer to yield a working concentration of 40 ng/µl and stored at -20°C for further molecular analysis.

3.5.6 PCR based amplification using ISSR primers

PCR reactions for ISSR were carried out in a reaction volume of 15 µl as described in Table 3.7. The PCR mix was prepared with following chemicals.

Table 3.7 : PCR reaction mixture for ISSR primers

Sr. No.	Components	Volume (µl)
1.	Taq buffer B (10x)	1.5
2.	dNTP mix (10 mM)	0.4
3.	Primer (5p mol)	1.5
4.	Taq DNA polymerase (3U/µl)	0.12
5.	Template DNA (50 ng/µl)	1.2
6.	Nuclease Free Water	10.28
Total :		15.0

The isolated and purified genomic DNA was utilised in Polymerase Chain Reaction (PCR) based amplification by using ISSR, dNTPs, *Taq* polymerase, buffers *etc.* The cycles of the PCR master cycler are given below in Table 3.8. The amplified products were analysed by horizontal electrophoresis on 1.5 per cent

agarose gel at 80 V for 2 h 30 minutes and stained with ethidium bromide ($0.5 \mu\text{g ml}^{-1}$). The gel was visualized under UV in gel documentation system.

Table 3.8 : Cycles of PCR for ISSR primers

Sr. No.	Steps	Temperature (°C)	Duration	Number of cycle
1.	Initial denaturation	94	5:00	1
2.	Denaturation	94	1:00	40
3.	Annealing	T _m	1:00	
4.	Extension	72	2:00	
5.	Final extension	72	7:00	1

Table 3.9 : List of ISSR primer used in the present study

Sr. No.	Primer	Sequence	Number of nucleotide	T _m value (°C)
1.	ISSR1	CACACACACACACACAGT	20	57.3
2.	ISSR2	CACACACACACACACAGC	20	59.4
3.	ISSR3	CACACACACACACACAGG	20	59.4
4.	ISSR4	CACACACACACACACAGA	20	57.3
5.	ISSR21	GCCTCTCTCTCTCTCTCT	18	56.0
6.	ISSR22	GACTCTCTCTCTCTCTCT	18	53.7
7.	ISSR23	GTCTCTCTCTCTCTCTCT	18	53.7

3.5.6.1 Resolution of amplified products

All the PCR amplified products were resolved on 1.8 per cent agarose gel for ISSR containing ethidium bromide. Amplified DNA product (20 μl) was mixed with 3 μl of 6X gel loading dye and loaded into the well of 1.5 per cent agarose gel. The standard DNA marker (100 bp or 50 bp) was also run along with the samples. The electrophoresis was carried out at 80 V (constant) for about 3 hours using Genei submerge gel electrophoresis system. After electrophoresis, the gel was carefully taken out of the casting tray and photographed using Fluor Chem FC 2 gel documentation system (Alpha Innotech Corporation, U.S.A.).

3.5.6.2 Analysis of ISSR data

3.5.6.3 Scoring of data

Data were scored for computer analysis on the basis of the presence or absence of the PCR products. If a product was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. The data were entered into MS-Excel data sheet. The data were maintained in the data sheet format for further analysis. The Microsoft Excel, 2010 (Microsoft, U.S.A.) was used for the statistical analysis. In the genotypes where amplification was not observed in the primer, that genotype was scored as a missing value 9.

3.5.7 Construction of dendrogram, Principal Component Analysis (PCA) and Principal Co-ordinate Analysis (PCOA) plots

The data were entered into the binary matrix and subsequently analysed using NTSYSpc (Version 2.20N.) software package (Rohlf, 2000) was used for genetic diversity evaluation. Coefficients of similarity were calculated by using Jaccard's similarity co-efficient (Jaccard, 1908) and cluster analysis was performed by the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) using this software. The Relationship between the grain amaranthus genotypes was graphically represented in the form of dendrograms by using the cluster analysis and the cophenetic correlation analysis was carried out using the CPH function of NTSYSpc (Version 2.20N.). In this method the dendrogram and similarity matrix were correlated to find the goodness-of-fit of the dendrogram constructed based on the similarity coefficients. The marker data were then standardized for Principal Component Analysis (PCA) and Principal Co-ordinate Analysis (PCoA) using PAST (version 3.18) software to highlight the resolving power of ordination (Hammer, 2001). The PCA and PCoA were carried out and scatters were plotted on the first two components and coordinates, respectively.

3.5.7.1 Determination of molecular weight

The software program Alpha Ease FC version 4.0.0 developed by Alpha Innotech Corporation, U.S.A. was used for determining the Molecular Weight (MW) of bands separated on the gel.

3.5.7.2 Determination of Polymorphism Information Content (PIC)

The Polymorphism Information Content (PIC) value for each locus was calculated on the basis of allele frequency by the given formula (Anderson, 1993).

$$PIC = 1 - \sum (P_{ij})^2$$

Where, P_{ij} is the frequency of the j^{th} band for marker i , and summation extends over n^{th} band.

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

The results obtained from the present investigation on "Variability, correlation and path analysis in grain amaranth (*Amaranthus hypochondriacus* L.)" are presented and discussed under the following headings.

4.1 Analysis of variance for experimental design

4.2 Genetic variability parameter

4.3 Correlation coefficient

4.4 Path analysis

4.5 Genetic variability through ISSR markers

4.1 Analysis of variance for experimental design

The analysis of variance depicting mean sum of squares for eleven quantitative traits are given in Table 4.1.

Table 4.1 : Analysis of variance for different characters

Sr. No.	Characters	Mean sum of square		
		Replications	Treatments	Error
	Degree of freedom	2	45	90
1.	Days to flowering	9.66	69.20**	7.81
2.	Leaf area per plant (cm ²)	5867.17	1168839.87**	11103.402
3.	Days to maturity	6.61	138.90**	6.11
4.	Length of inflorescence (cm)	44.22	169.06**	18.94
5.	Width of inflorescence (cm)	5.74	7.35**	1.87
6.	Plant height	182.63	532.724**	63.26
7.	Stem girth (cm)	0.15	0.64**	0.060
8.	Harvest index (%)	1.62	32.87**	2.39
9.	Test weight (g/10 ml)	0.022	0.58**	0.11
10.	Protein content (%)	1.37	5.85**	0.54
11.	Seed yield per plant (g)	3.96	291.48**	1.89

*, ** Significant at 5 and 1 per cent levels of significance, respectively.

The analysis of variance revealed significant differences among the genotypes for eleven characters viz., days to flowering, leaf area per plant, days to maturity, length of inflorescence, width of inflorescence, plant height, stem girth, harvest index, test weight, protein content, seed yield per plant which indicated the existence of variability in the experimental material. The estimates of genotypic (σ^2_g) and phenotypic variances (σ^2_p) of each character were carried out.

4.2 Genetic variability parameters

Knowledge of nature and magnitude of variation present in the base population is a pre-requisite for effective selection of superior genotypes from a

variable population. However, it is essential that the population should possess large amount of heritable variation. Thus, the extent of genetic variability is more important than total variation.

The variability parameters viz., range, genotypic coefficient of variation (GCV %), phenotypic coefficient of variation (PCV %), heritability in broad sense (h^2_{bs}) and genetic advance (GA) as per cent of mean were computed from variance components and mean values. All these estimates are summarised in Table 4.2.

4.2.1 Mean performance of genotypes and range of phenotypic variability

The mean performances of forty-six genotypes of grain amaranth in respect of different traits are given Appendix-II. Character-wise mean performance and range of phenotypic variation are described as under.

4.2.1.1 Days to flowering

There were significantly differed among genotypes for days to flowering. This character showed wide range of variability (37.00 to 64.00) with general mean 54.88 days. The genotype SKNA-903 (37.00) was flowered the earliest followed by genotypes SKNA-813 (47.00) and SKNA-1403 and SKNA-1414 (48.67), RMA-62 (49.33), respectively. While genotypes GA-2 (64.00) took the maximum days for flowering.

4.2.1.2 Leaf area per plant (cm²)

The Genotype SKGPA-61 (2,634.63 cm²) was assessed a highest leaf area per plant followed by genotype GA-2 (2584.63 cm²) and SKNA-908 (2511.33cm²), respectively. The Genotype SKNA-903 (505.39 cm²) recorded lowest leaf area per plant among all the forty-six genotypes.

4.2.1.3 Days to maturity

There were significantly differed among genotypes for days to maturity. The range of phenotypic variation for this trait was high (77.67 to 118.33) with general mean 107.44 days. The genotype SKNA-903 (77.67) was the earliest, while genotypes BGA-14 (118.33) took the maximum days for maturity. The comparison of mean values of genotypes revealed that the genotype SKNA-903 took the minimum days for maturity.

4.2.1.4 Length of inflorescence (cm)

Genotypes exhibited significant differences for this trait. The genotype SKGPA-61 registered the maximum Length of inflorescence (74.33 cm) followed by SKNA-401 (73.13 cm) and SKNA-1313 (69.27 cm). The genotype MGA-502



SKGPA-61



SKGPA- 74



SKGPA-150



SKGPA-155



SKNA-401



SKNA-403



SKNA-808



SKNA-813



SKNA-903



SKNA-908



SKNA-1201



SKNA- 1207

Plate 2. Phenotypic variation observed in inflorescence of grain amaranth genotypes



GA- 2



GA - 3



BGA-4-9



BGA-7



BGA-9



BGA-10



BGA-14



BGA-20



KBGA-4



KBGA-5



KBGA-7



RMA-62

Plate 2. Phenotypic variation observed in inflorescence of grain amaranth genotypes

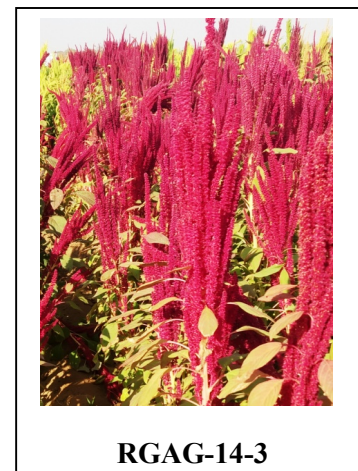
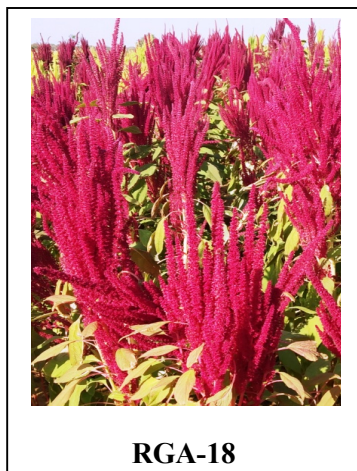


Plate 2. Phenotypic variation observed in inflorescence of grain amaranth genotypes

Table 4.2 : Genetic parameters of variation for seed yield and its contributing characters in grain amaranth

Sr. No.	Characters	Genetic parameters										
		Mean	Range	σ^2_g	σ^2_p	σ^2_e	GCV (%)	PCV (%)	ECV (%)	h^2 (%)	GA	GAM (%)
1.	Days to flowering	54.88	37.00-64.00	20.46	28.27	7.81	8.24	9.69	5.09	72.37	7.928	14.44
2.	Leaf area per plant (cm ²)	1569.17	505.39-2634.63	385912.16	397015.57	11103.40	39.59	40.15	6.71	97.20	1261.68	80.40
3.	Days to maturity	107.44	77.67-118.33	44.26	50.37	6.11	6.19	6.60	2.30	87.86	12.84	11.95
4.	Length of inflorescence (cm)	60.90	39.60-74.33	50.04	68.98	18.94	11.61	13.64	7.14	72.54	12.41	20.38
5.	Width of inflorescence (cm)	19.81	16.33-25.20	1.82	3.70	1.87	6.81	9.71	6.90	49.18	1.94	9.79
6.	Plant height (cm)	150.22	105.20-177.60	156.48	219.75	63.26	8.32	9.86	5.29	71.21	21.74	14.47
7.	Stem girth (cm)	4.05	2.60-5.69	0.19	0.25	0.06	10.76	12.34	6.04	76.00	0.78	19.25
8.	Harvest index (%)	20.36	14.74-27.86	10.15	12.55	2.39	15.64	17.39	7.59	80.87	5.90	28.97
9.	Test weight (g/10 ml)	7.64	6.58-8.48	0.15	0.26	0.11	5.07	6.68	4.34	57.69	0.60	7.94
10.	Protein content (%)	12.88	10.20-15.92	1.77	2.31	0.54	10.32	11.80	5.70	76.62	2.40	18.63
11.	Seed yield per plant (g)	24.26	9.73-44.57	96.53	98.43	1.89	40.49	40.89	5.66	98.06	20.04	82.60

Where, σ^2_g , σ^2_p , σ^2_e are genotypic, phenotypic, environmental variance, respectively;

GCV (%), PCV (%) and ECV (%) are genotypic, phenotypic and environmental coefficient of variance, respectively; and

h^2 (%), GA, GAM (%) are heritability, genetic advance and genetic advance expressed as per cent of mean, respectively.

(39.60 cm) had the shortest inflorescence length. This character showed high range of phenotypic variability (39.60 cm to 74.33 cm) with general mean 60.90 cm.

4.2.1.5 Width of inflorescence (cm)

Genotypes exhibited significant differences for this trait. The genotype SKNA-808 registered the maximum Width of inflorescence (25.20 cm) followed by SKGPA-61 (23.73 cm) and SKNA-1201 (21.80 cm). The genotype SKNA-903 (16.33 cm) had the shortest width of inflorescence.

4.2.1.6 Plant height (cm)

Plant height exhibited high range of variability (105.20 cm to 177.60 cm) with general mean 150.22 cm. The studied under genotypes significantly differed for the character plant height at harvest. The maximum plant height was observed with genotype SKNA-1407 (177.60 cm), while the minimum plant height was recorded with genotype SKNA-903 (105.20 cm).

4.2.1.7 Stem girth (cm)

There were significantly differed among genotypes for stem girth. The genotype SKNA-1313 observed maximum stem girth (5.69 cm) while minimum stem girth recorded with genotype SKNA-903 (2.60 cm).

4.2.1.8 Harvest index (%)

The differences among the genotypes were highly significant for harvest index. The range of phenotypic variation for this trait was high (14.74 % to 27.86 %) with general mean 20.36 per cent. There were significantly differed among genotypes for harvest index. The genotype SKNA-903 observed maximum harvest index (27.86 %) followed by genotype BGA-7 (27.29 %) and BGA-20 (26.56 %), while minimum harvest index recorded with genotype SKGPA-73 (14.74 %).

4.2.1.9 Test weight (g/10 ml)

The highest value of test weight was recorded in genotype SKNA-1508 (8.48 g/10 ml) followed by genotype GA-2 (8.46 g/10 ml), BGA-7 (8.38 g/10 ml). The genotype, BGA-20 (6.58 g/10 ml) had a lowest value for test weight. This character showed moderate range of phenotypic variability (6.58 g/10 ml to 8.48 g/10 ml) with general mean 7.64 g/10 ml.

4.2.1.10 Protein content (%)

Protein exhibited low range of phenotypic variability (10.20 % to 15.92 %) with general mean 12.88 per cent. The maximum protein content was observed in genotype SKNA-1407 (15.92 %) followed by SKNA-1305 (15.71 %),

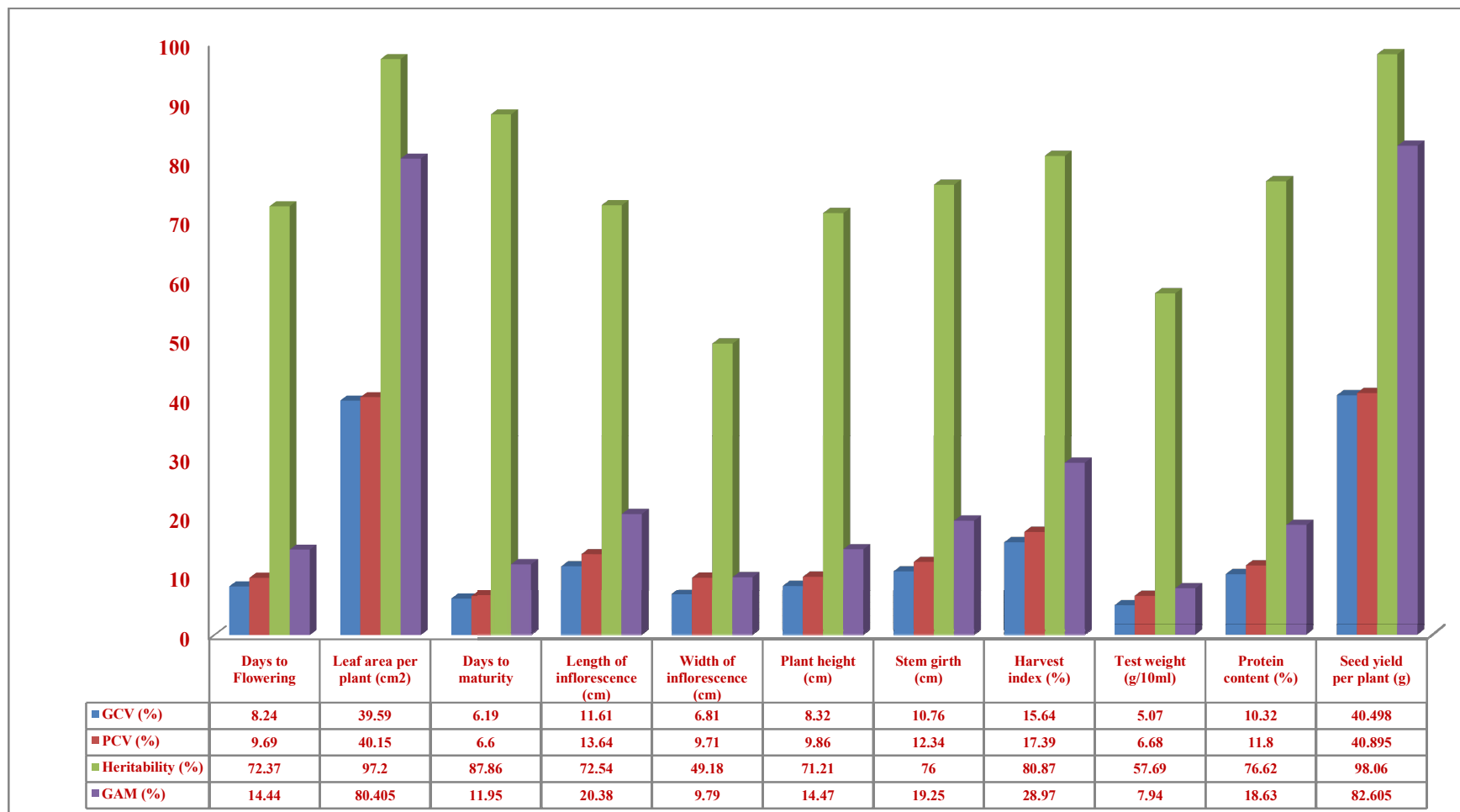


Fig 1. Graphical comparison of GCV (%), PCV (%), Heritability (%) and GAM (%) for eleven characters in grain amaranth

SKNA-1406 (14.92 %) and RGA-13 (14.83 %), while minimum protein content was observed in genotype BGA-9 (10.20%).

4.2.1.11 Seed yield per plant (g)

The variation among genotypes for seed yield per plant was highly significant. This character showed high range of variability (9.73 g to 44.57 g) with general mean 24.26 g. Highest seed yield per plant was recorded for SKNA-1407 (44.57 g) followed by SKGPA-61 (43.13 g), BGA-20 (41.28 g) and SKNA-401 (41.23 g). The genotype SKNA-903 (9.73 g) recorded the lowest seed yield per plant.

4.2.2 Estimation of components of variance

The phenotypic, genotypic and environmental variances calculated for all the eleven characters under present study are presented in Table 4.2 and Fig. 1. The results revealed that magnitude of phenotypic components of variance was higher than genotypic components of variance for all the character studied. The genotypic component of variation was found to be higher than the environmental component of variance for all the character except width of inflorescence, exhibited a close correspondence with phenotypic variance in most characters. This suggest that phenotypic variability was reliable measure of genotypic variability as most of the characters were least influenced by the environment.

The magnitude of genotypic variance was maximum for leaf area per plant (385912.16) followed by plant height (156.48) and seed yield per plant (96.53). The phenotypic variances ranged between 0.25 (stem girth) to 397015.57 (leaf area per plant). A very low genotypic variances coupled with low environmental variances has been observed for test weight (0.15 to 0.11), stem girth (0.19 to 0.06) and protein content (1.77 to 0.54).

The present study showed wide range of phenotypic variability and highly significant varietal differences for all the characters, indicating considerable amount of variability under present investigation. The range of variability was more in case of characters like days to flowering, leaf area per plant, days to maturity, length of inflorescence, plant height, harvest index and seed yield per plant. This naturally suggest that there is a simple scope for improving different characters including seed yield per plant, provided the material is subjected to judicious selection pressure.

4.2.3 Coefficient of variation

The estimates for genotypic coefficient of variation (GCV) were lower than PCV for all characters. The GCV and PCV were highest for seed yield per plant

(40.49 and 40.89) followed by leaf area per plant (39.59 and 40.15). Moderate for harvest index (15.64 and 17.39), length of inflorescence (11.61 and 13.64), stem girth (10.76 and 12.34) and protein content (10.32 and 11.80). Lowest value for days to flowering (8.24 and 9.69), days to maturity (6.19 and 6.60), width of inflorescence (6.81 and 9.71), plant height (8.32 and 9.86), test weight (5.07 and 6.68), respectively.

The results revealed that the magnitude of genetic variability was very close to phenotypic variability for all the characters. This indicates that phenotypic variability has larger due to genetic differences for different traits.

In the present investigation highest estimates of genotypic and phenotypic coefficient of variation were observed for seed yield per plant and leaf area per plant. Moderate estimates of genotypic and phenotypic coefficient of variation were observed for harvest index, length of inflorescence, stem girth and protein content. While lower estimates of genotypic and phenotypic coefficient variation were observed for days to flowering, days to maturity, width of inflorescence, plant height, test weight. Similar results were obtained by Vyas (2000) for seed yield per plant and harvest index. Patel (2004) observed for seed yield per plant and plant height. Patel (2011) for various characters like seed yield per plant, plant height and days to maturity. Yadav *et al.* (2014) for inflorescence length and grain yield per plant. Venkatesh *et al.* (2014) observed for stem girth, plant height, inflorescence length and grain yield per plant. Kumar *et al.* (2018) observed for seed yield per plot, followed by test weight and stem girth.

In the present studies phenotypic coefficient of variation were more than genotypic coefficients of variation in all the traits. This might be due to environmental effect. Similar findings were reported by Venkatesh *et al.* (2014), Sheelamary and Phogat (2016), Kumar and Murthy (2017), Shrivastav *et al.* (2017) and Kumar *et al.* (2018). Phenotypic variation observed in different inflorescence of grain amaranth with particular genotypes present investigation is given in Plate-2.

4.2.4 Heritability and genetic advance

The ratio of genotypic variance to the phenotypic variance is known as broad sense heritability. It is generally expressed in percentage. The heritability is heritable portion of phenotypic variance.

Improvement of mean genotypic value of selected plants over parental population is known as genetic advance. It is measure of genetic gain under selection.

The success of genetic advance under selection depends on three main factors viz., genetic variability, heritability and selection intensity.

4.2.4.1 Heritability percentage (b.s.)

The estimates of heritability as percentage in broad sense for all the characters under present study are presented in Table 4.2.

The heritability (b.s.) estimate ranged from 49.18 per cent (Width of inflorescence) to 98.06 per cent (seed yield per plant). The characters showed high heritability viz., seed yield per plant (98.06 %), leaf area per plant (97.20 %), days to maturity (87.86 %), harvest index (80.87 %), protein content (76.62 %), stem girth (76.00 %), length of inflorescence (72.54 %), days to flowering (72.37 %), plant height (71.21 %) indicates that these characters are least influenced by the environmental effects, the selection for improvement of such characters may not be useful because broad sense heritability is based on total genetic variance which includes both fixable (additive) and non-fixable variance (dominance and epistatic). The lowest heritability not recorded which highly influenced by the environmental effects and genetic improvement through selection will be difficult due to masking effect of the environment on the genotypic effect. The moderate heritability was found in characters number of width of inflorescence (49.18 %) and test weight (57.69 %).

Falconer (1989) suggested that heritability helps in identifying the resemblance between parents and their progeny, while genetic advance provides the knowledge about expected gain for a particular character after selection.

Vyas (2000) the broad sense heritability estimates were also found to be high for all the characters except inflorescence length. Verma *et al.* (2001) highest heritability for seed yield per plant and plant height. Dutta *et al.* (2002) reported for plant height. Patel (2004) reported that high heritability for test weight, days to maturity and protein content. Ahmmmed *et al.* (2012) heritability estimates in broad sense were higher for seed yield per hectare. Shrivastav *et al.* (2017) reported that high heritability in broad sense was recorded for plant height, harvest index, days to maturity and inflorescence length. Gelotar (2018) high heritability was observed for plant height, leaf area, days to maturity, seed yield per plant, length of inflorescence and days to flowering. Kumar *et al.* (2018) the heritability estimates high for seed yield per plot, stem girth, test weight and plant height.

4.2.4.2 Genetic advance

The character leaf area per plant (1261.68) showed highest genetic advance followed by plant height (21.74) and seed yield per plant (20.04). Moderate genetic advance recorded in length of inflorescence (12.42), days to maturity (12.84). The lowest genetic advance observed for test weight (0.60), stem girth (0.78), width of inflorescence (1.94), protein content (2.40), harvest index (5.90) and days to flowering (7.928).

Johnson *et al.* (1955) observed that the genetic gain was low where there was non-additive gene effect. Whereas, the genetic advance would be high, where there was additive gene effect.

4.2.3 Genetic advance as per cent of mean

High value of genetic advance as percent of mean was observed for seed yield per plant (82.60 %) followed by leaf area per plant (80.40 %). Moderate for days to maturity (11.95 %), days to flowering (14.44 %), plant height (14.47 %), protein content (18.63 %) stem girth (19.25 %), length of inflorescence (20.38 %) and harvest index (28.97 %), respectively. Whereas, test weight showed lowest value (7.94 %) followed by width of inflorescence (9.79 %).

In present study high heritability coupled with high genetic advance as per cent mean indicating additive gene action was observed for trait viz., leaf area per plant, length of inflorescence, harvest index, seed yield per plant. Simple selection for such traits should be practiced for improving them. Joshi (1986) reported same results for plant height, harvest index, test weight and seed yield per plant. Verma *et al.* (2001) reported same results for seed yield per plant and plant height. Patel (2011) reported same results for seed yield per plant, plant height, inflorescence length and days to maturity. Hasan *et al.* (2012) reported for seed yield per plant. Akaneme *et al.* (2013) observed same for days to 50 per cent flowering which was also positively correlated with and stem girth. Shah *et al.* (2018) reported that plant height, days to flowering, test weight and inflorescence length. Kumar *et al.* (2018) for the characters viz., seed yield per plot, stem girth, test weight and inflorescence length.

4.3 Correlation coefficient

For estimation of characters association, correlation analysis has been used to determine the type and magnitude of association between a pair of characters. These associations provide a better understanding of the contribution of one trait in building-up the genetic makeup of the other traits of a crop. The knowledge about

correlations between economically important traits and characters contributing to that in all combinations will help to decide the parameters for selection, so that improvement in the associated characters can be brought.

The association between characters, which is directly observed, is the phenotypic correlation. The knowledge about phenotypic correlation between yield contributing characters helps in selection programme for yield improvement of a crop. The genotypic correlation permits the prediction of correlated response as well as evaluation of the relative influence of one character on other and helpful in the construction of selection indices. The phenotypic and genotypic correlation coefficients (Table 4.3) were estimated for eleven characters using forty-six grain amaranth genotypes to find out the association of plant with other yield contributing characters.

4.3.1 Days to flowering

Days to flowering showed significant and positive association with leaf area per plant ($r_g = 0.4256$ and $r_p = 0.3563$), days to maturity ($r_g = 0.4457$ and $r_p = 0.3618$), length of inflorescence ($r_g = 0.4825$ and $r_p = 0.2973$), width of inflorescence ($r_g = 0.4910$ and $r_p = 0.2870$), plant height ($r_g = 0.5910$ and $r_p = 0.4168$), stem girth ($r_g = 0.3758$ and $r_p = 0.2552$) seed yield per plant ($r_g = 0.4094$ and $r_p = 0.3548$) at both genotypic and phenotypic level, respectively. On other hand test weight showed ($r_g = 0.1761$) significant and positive association at genotypic level and as well as non-significant ($r_p = 0.1581$) and positive association at phenotypic levels. Harvest index showed ($r_g = 0.0155$ and $r_p = 0.0026$) and protein content ($r_g = 0.061$ and $r_p = 0.0213$) non-significant with positively correlation both at genotypic and phenotypic level.

4.3.2 Leaf area per plant (cm²)

Leaf area per plant showed significant and positive association with days to maturity ($r_g = 0.3788$ and $r_p = 0.3487$), length of inflorescence ($r_g = 0.5169$ and $r_p = 0.4339$), width of inflorescence ($r_g = 0.3553$ and $r_p = 0.2791$), plant height ($r_g = 0.3397$ and $r_p = 0.0.2908$), harvest index ($r_g = 0.4545$ and $r_p = 0.0.4051$), test weight ($r_g = 0.3895$ and $r_p = 0.3009$), protein content ($r_g = 0.199$ and $r_p = 0.1855$) seed yield per plant ($r_g = 0.7676$ and $r_p = 0.7554$) at both genotypic and phenotypic level, respectively. While stem girth showed ($r_g = 0.1403$ and $r_p = 0.1283$) non-significant and positive association at both genotypic level and phenotypic levels.

Table 4.3 : Genotypic and phenotypic correlation coefficient for different characters in grain amaranth

Characters		01. Days to flowering	02. Leaf area per plant (cm ²)	03. Days to maturity	04. Length of inflorescence (cm)	05. Width of inflorescence (cm)	06. Plant height (cm)	07. Stem girth (cm)	08. Harvest index (%)	09. Test weight (g/10 ml)	10. Protein content (%)	11. Seed yield per plant (g)
01	r _g	1.0000	0.4256**	0.4457**	0.4825**	0.4910**	0.5910**	0.3758**	0.0155	0.1761*	0.061	0.4094**
	r _p	1.0000	0.3563**	0.3618**	0.2973**	0.2870**	0.4168**	0.2552**	0.0026	0.1581	0.0213	0.3548**
02	r _g		1.0000	0.3788**	0.5169**	0.3553**	0.3397**	0.1403	0.4545**	0.3895**	0.199*	0.7676**
	r _p		1.0000	0.3487**	0.4339**	0.2791**	0.2908**	0.1283	0.4051**	0.3009**	0.1855*	0.7554**
03	r _g			1.0000	0.5140**	0.4047**	0.5914**	0.4373**	-0.1218	0.0817	-0.006	0.3090**
	r _p			1.0000	0.3721**	0.2498**	0.4933**	0.3817**	-0.1047	0.0200	-0.0053	0.2822**
04	r _g				1.0000	0.3936**	0.6104**	0.2563**	0.3600**	0.0858	0.168*	0.6043**
	r _p				1.0000	0.2733**	0.4879**	0.2094*	0.2812**	0.0885	0.1330	0.5208**
05	r _g					1.0000	0.4049**	0.2848**	0.0816	0.4784**	-0.035	0.4437**
	r _p					1.0000	0.3000**	0.2326**	0.0766	0.2404**	0.0028	0.3201**
06	r _g						1.0000	0.6202**	0.1700*	0.0780	0.126	0.5034**
	r _p						1.0000	0.4885**	0.1430	0.0845	0.0895	0.4273**
07	r _g							1.0000	0.0502	0.0241	0.035	0.3449**
	r _p							1.0000	0.0290	0.0721	0.0648	0.3135**
08	r _g								1.0000	0.2588**	0.334**	0.7822**
	r _p								1.0000	0.1634	0.2306**	0.7114**
09	r _g									1.0000	0.167	0.3715**
	r _p									1.0000	0.0966	0.2875**
10	r _g										1.0000	0.2397**
	r _p										1.0000	0.2038*
11	r _g											1.0000
	r _p											1.0000

*, ** Significant at 5 and 1 per cent level of significance, respectively.

4.3.3 Days to maturity

The results indicated that the characters recorded viz., length of inflorescence ($r_g = 0.5140$ and $r_p = 0.3721$), width of inflorescence ($r_g = 0.4047$ and $r_p = 0.2498$), plant height ($r_g = 0.5914$ and $r_p = 0.4933$), stem girth ($r_g = 0.4373$ and $r_p = 0.3817$) and seed yield per plant ($r_g = 0.3090$ and $r_p = 0.2822$) showed significant with positively correlation both genotypic and phenotypic level. Harvest index ($r_g = -0.1218$ and $r_p = -0.1047$) and protein content ($r_g = -0.006$ and $r_p = -0.0053$) indicate non-significant with negatively correlation at both genotypic and phenotypic level. Test weight ($r_g = 0.817$ and $r_p = 0.0200$) showed positively non-significant with both genotypic and phenotypic level.

4.3.4 Length of inflorescence (cm)

This character length of inflorescence showed significant and positive correlations with width of inflorescence ($r_g = 0.3936$ and $r_p = 0.2733$), plant height ($r_g = 0.6104$ and $r_p = 0.4879$), stem girth ($r_g = 0.2563$ and $r_p = 0.2094$), harvest index ($r_g = 0.3600$ and $r_p = 0.2812$) and seed yield per plant ($r_g = 0.6043$ and $r_p = 0.5208$) both genotypic and phenotypic level, respectively. Protein content showed ($r_g = 0.168$ and $r_p = 0.1330$) positively significant correlation at genotypic level whereas, non-significant correlation at phenotypic level. Test weight showed ($r_g = 0.0858$ and $r_p = 0.0885$) positively non-significant at both genotypic and phenotype level.

4.3.5 Width of inflorescence (cm)

The results indicated that the character width of inflorescence showed highly significant and positive correlation with plant height ($r_g = 0.4049$ and $r_p = 0.3000$), stem girth ($r_g = 0.2848$ and $r_p = 0.2326$), test weight ($r_g = 0.4784$ and $r_p = 0.2404$) and seed yield per plant ($r_g = 0.4437$ and $r_p = 0.3201$) at both genotypic and phenotypic levels, respectively.

4.3.6 Plant height (cm)

The characters showed highly significant and positively association with stem girth ($r_g = 0.6202$ and $r_p = 0.4885$), seed yield per plant ($r_g = 0.5034$ and $r_p = 0.4273$) at both genotypic and phenotypic levels, respectively. On other hand test weight ($r_g = 0.0780$ and $r_p = 0.0845$) and protein content ($r_g = 0.126$ and $r_p = 0.0895$) showed non-significant positively correlated with both genotypic and phenotypic level.

4.3.7 Stem girth (cm)

This trait had non-significant and positive association with harvest index ($r_g = 0.0502$ and $r_p = 0.0290$), test weight ($r_g = 0.0241$ and $r_p = 0.0721$), protein content

($r_g = 0.035$ and $r_p = 0.0648$) at both genotypic level and phenotypic levels, respectively. On other hand seed yield per plant showed ($r_g = 0.3449$ and $r_p = 0.3135$) highly significant and positive association with both genotypic level and phenotypic levels of the trait.

4.3.8 Harvest index (%)

Harvest index had highly significant and positive association with test weight ($r_g = 0.2588$ and $r_p = 0.1634$) at the genotypic level and non-significant positive association at phenotypic level. Protein content ($r_g = 0.334$ and $r_p = 0.2306$) and seed yield per plant ($r_g = 0.7822$ and $r_p = 0.7114$) showed highly significant and positive correlation at both genotypic and phenotypic levels with harvest index, respectively.

4.3.9 Test weight (g/10 ml)

The character test weight showed highly significant and positive correlation with seed yield per plant ($r_p = 0.3715$ and $r_g = 0.2875$) at both genotypic and phenotypic levels.

4.3.10 Protein content (%)

Protein content had significant and positive correlation with seed yield per plant ($r_g = 0.2397$ and $r_p = 0.2038$) at both genotypic and phenotypic levels.

Overview :

In plant breeding programmes, several characters are often to be handled together by a breeder as most of the characters might be interrelated with each other. Thus, the different components of yield exhibit considerable degree of association among themselves as well as with yield. Therefore, the understanding of correlations has been observed very useful for rationale improvement of yield and its components.

It is imperative to obtain information regarding the interrelationships of different plant characters with yield, because, it facilitates the quicker assessment of high yielding genotypes in selection programme. An estimate of total correlation is not sufficient to fully evaluate the correlation between two variables, since it is the result of interaction between genotypes and environment.

The study of genotypic correlation gives an idea of the extent of relationship between different variables. This relationship among yield contributing characters as well as their association with the yield provides information for exercising selection pressure for bringing genetic improvement in grain amaranth.

In the present study the positive and highly significant correlation was observed between seed yield per plant with days to flowering, leaf area per plant, length of inflorescence and harvest index. Whereas, positive non-significant correlation was observed between seed yield per plant with stem girth and plant height.

Dutta *et al.* (2002) observed in his study that the recorded for harvest index, plant height, inflorescence length, days to maturity, days to flowering and test weight had significant positive correlation with seed yield per plant. Shukla and Singh (2003) reported that plant height had significant and positively correlation with seed yield per plant. Prajapati *et al.* (2005) found that harvest index and test weight had significant positive correlation with seed yield per plant. Shrivastav *et al.* (2017) found that inflorescence length and plant height had significant positive correlation with seed yield per plant. Sagar *et al.* (2018) reported that stem girth had significant and positive correlation with seed yield per plant. Gelotar (2018) reported that seed yield per plant was significant and positively correlated with days to flowering, plant height and length of inflorescence.

Present study, genotypic correlations were higher than corresponding phenotypic correlations in all the traits, therefore strong inherent association between various characters at genetic level. Positive and highly significant correlations were found more in days to maturity than leaf area per plant at genotypic level with seed yield per plant. That indicate days to maturity take longer duration plant stand at field level so plant take photosynthesis rate more which contributed towards higher seed yield.

From overall view of point, the correlation study revealed that the character like leaf area per plant, length of inflorescence and width of inflorescence were important attributes which contributed towards higher seed yield. Therefore, more emphasis should be given to these components during selection for higher yield per plant.

4.4 Path coefficient analysis

Path coefficient analysis is a tool to partition the observed correlation coefficient into direct and indirect effect of yield components on seed yield. Path analysis differ from simple correlation in the way, it points out the cause and relative importance, whereas, the latter measure simply the mutual association ignoring the causation.

Seed yield per plant was considered as the resultant variable; while the remaining ten yield contributing characters are as the causal variables. The genotypic correlation coefficients were worked out between seed yield per plant and each of the ten causal variables and among themselves to study the direct and indirect effects on seed yield per plant. The data on the direct and indirect effects of these variables on seed yield per plant are presented in Table 4.4.

4.4.1 Days to flowering vs. seed yield per plant (g)

The correlation coefficient between days to flowering and seed yield per plant was positive ($r_g = 0.4094$). However, the direct effect of this character on seed yield per plant was high and positive (0.0579). Days to flowering exerted negligible and positive indirect effects on seed yield per plant *via* days to maturity (0.025), length of inflorescence (0.0116), width of inflorescence (0.0732), plant height (0.0359). Stem girth (0.0503), harvest index (0.0094), respectively. Days to flowering had moderate and positive indirect effects on seed yield per plant *via* leaf area per plant (0.1508) while negligible and negative indirect effect on seed yield per plant *via* test weight per plant (-0.0022) and protein content (-0.0028), respectively.

4.4.2 Leaf area per plant (cm²) vs. seed yield per plant (g)

The correlation coefficient between leaf area per plant and seed yield per plant was significant and positive ($r_g = 0.7676$). However, the direct effect of this character on seed yield per plant was high and positive (0.3544). Leaf area per plant negligible and positive indirect effects on seed yield per plant *via* length of inflorescence (0.0124), stem girth (0.0188), plant height (0.0207), days to maturity (0.0214), days to flowering (0.0247), width of inflorescence (0.0529). This character showed negligible and negative indirect effect through test weight (-0.0048) and protein content (-0.0091). While, it showed moderate and positive indirect effect through harvest index (0.2760).

4.4.3 Days to maturity vs. seed yield per plant (g)

Days to maturity had significant and positive association with seed yield per plant ($r_g = 0.3090$). However, its direct effect was positive and negligible (0.0565). It exhibited low and positive indirect effect *via* leaf area per plant (0.1343), while exerted negligible and positive indirect effect of this character *via* other characters viz., days to flowering (0.0258), length of inflorescence (0.0124), width of inflorescence (0.0603), plant height (0.0360), stem girth (0.0585), protein content

Table 4.4 : Direct and indirect effects of yield components on seed yield in grain amaranth

Sr. No.	Characters	Days to flowering	Leaf area per plant (cm ²)	Days to maturity	Length of inflorescence (cm)	Width of inflorescence (cm)	Plant height (cm)	Stem girth (cm)	Harvest index (%)	Test weight (g/10 ml)	Protein content (%)	Genotypic correlation with seed yield per plant (g)
1.	Days to flowering	0.0579	0.1508	0.0252	0.0116	0.0732	0.0359	0.0503	0.0094	-0.0022	-0.0028	0.4094
2.	Leaf area per plant (cm ²)	0.0247	0.3544	0.0214	0.0124	0.0529	0.0207	0.0188	0.2762	-0.0048	-0.0091	0.7676
3.	Days to maturity	0.0258	0.1343	0.0565	0.0124	0.0603	0.0360	0.0585	-0.0740	-0.0010	0.0003	0.3090
4.	Length of inflorescence (cm)	0.0279	0.1832	0.0290	0.0240	0.0586	0.0371	0.0343	0.2188	-0.0059	-0.0077	0.6043
5.	Width of inflorescence (cm)	0.0284	0.1259	0.0229	0.0095	0.1490	0.0246	0.0381	0.0496	-0.0059	0.0016	0.4437
6.	Plant height (cm)	0.0342	0.1204	0.0334	0.0147	0.0603	0.0608	0.0830	0.1033	-0.0010	-0.0058	0.5034
7.	Stem girth (cm)	0.0218	0.0497	0.0247	0.0062	0.0424	0.0377	0.1338	0.0305	-0.0003	-0.0016	0.3449
8.	Harvest index (%)	0.0009	0.1611	-0.0069	0.0087	0.0122	0.0103	0.0067	0.6077	-0.0032	-0.0153	0.7822
9.	Test weight (g/10 ml)	0.0102	0.1380	0.0046	0.0021	0.0713	0.0047	0.0032	0.1573	-0.0123	-0.0077	0.3715
10.	Protein content (%)	0.0036	0.0706	-0.0004	0.0041	-0.0052	0.0077	0.0046	0.2027	-0.0020	-0.0459	0.2397

*, ** Significant at 5 and 1 per cent level of significance, respectively.

Residual effect = 0.06964

(0.0003). This character showed negligible negative indirect effect through harvest index (-0.0740) and test weight (-0.0010).

4.4.4 Length of inflorescence (cm) vs. seed yield per plant (g)

Length of inflorescence showed positive correlation with seed yield per plant ($r_g = 0.6043$) and its direct effect was also negligible and positive (0.0240). However, length of inflorescence had low and positive indirect effect through leaf area per plant (0.1832). While, it exerted negligible positive indirect effect *via* days to flowering (0.0279), days to maturity (0.0290), width of inflorescence (0.0586), plant height (0.0371) and stem girth (0.0343), respectively. The character showed moderate and positive indirect effect through harvest index (0.2188). While this trait showed negligible negative indirect effect through test weight (-0.0059) and protein content (-0.0077).

4.4.5 Width of inflorescence (cm) vs. seed yield per plant (g)

There was a positive association between number width of inflorescence and seed yield per plant ($r_g = 0.4437$). The direct effect of width of inflorescence was positive and low (0.1490). The very low and positive indirect effect of this character on seed yield per plant was observed through leaf area per plant (0.1259). This trait showed negligible negative indirect effect through test weight (-0.0059). While, it showed negligible and positive indirect effects through days to flowering (0.0284), days to maturity (0.0229), length of inflorescence (0.0095), plant height (0.0246), stem girth (0.0381), harvest index (0.0496) and protein content (0.0016).

4.4.6 Plant height (cm) vs. seed yield per plant (g)

Positive correlation was observed between plant height and seed yield per plant ($r_g = 0.5034$). The direct effect of plant height on seed yield per plant was negligible and positive (0.0608). This trait exhibited low and positive indirect effect through leaf area per plant (0.1204) and harvest index (0.1033). While, this trait showed negligible and negative indirect effects through test weight (-0.0010) and protein content (-0.0058). This character showed negligible positive indirect effects through days to flowering (0.0342), day to maturity (0.0334), length of inflorescence (0.0147), width of inflorescence (0.0603) and stem girth (0.0830).

4.4.7 Stem girth (cm) vs. seed yield per plant (g)

Stem girth had a positive association with seed yield per plant ($r_g = 0.3449$). However, its direct effect was positive and low (0.1338). It exhibited negligible and positive indirect effects *via* days to flowering (0.0218), leaf area per plant (0.0497),

days to maturity (0.0247), length of inflorescence (0.0062), width of inflorescence (0.0424), plant height (0.0377) and harvest index (0.0305). While, it showed negligible negative indirect effect through test weight (-0.0003) and protein content (-0.0016).

4.4.8 Harvest index (%) vs. seed yield per plant (g)

There was positive association between harvest index and seed yield per plant ($r_g = 0.7822$). The direct effect of harvest index was positive and high (0.6077). This trait showed negligible and positive indirect effect through days to flowering (0.0009), length of inflorescence (0.0087), width of inflorescence (0.0122), plant height (0.0103, stem girth (0.0067). It showed negligible and negative indirect effects through test weight (-0.0032) and protein content (-0.0153).

4.4.9 Test weight (g/10 ml) vs. seed yield per plant (g)

Test weight showed significant and positive correlation with seed yield per plant ($r_g = 0.3715$) and its direct effect was negligible and negative (-0.0123). However, test weight had moderate and positive indirect effects through leaf area per plant (0.1380) and harvest index (0.1573). While, it exerted negligible negative indirect effect *via* protein content (-0.0077). This trait showed negligible positive indirect effect through days to flowering (0.0102), day to maturity (0.0046), length of inflorescence (0.0021), width of inflorescence (0.0713), plant height (0.0047) and stem girth (0.0032).

4.4.10 Protein content (%) vs. seed yield per plant (g)

Protein content showed positive correlation with seed yield per plant ($r_g = 0.2397$) and its direct effect was negligible and negative (-0.0459). However, protein content had negligible and negative indirect effects through days to maturity (-0.0004), width of inflorescence (-0.0052) and test weight (-0.0020). It exhibited negligible and positive indirect effects through days to flowering (0.0036), leaf area per plant (0.0706), length of inflorescence (0.0041), plant height (0.0077) and stem girth (0.0046). This trait showed moderate and positive indirect effect through harvest index (0.2027).

Overview :

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

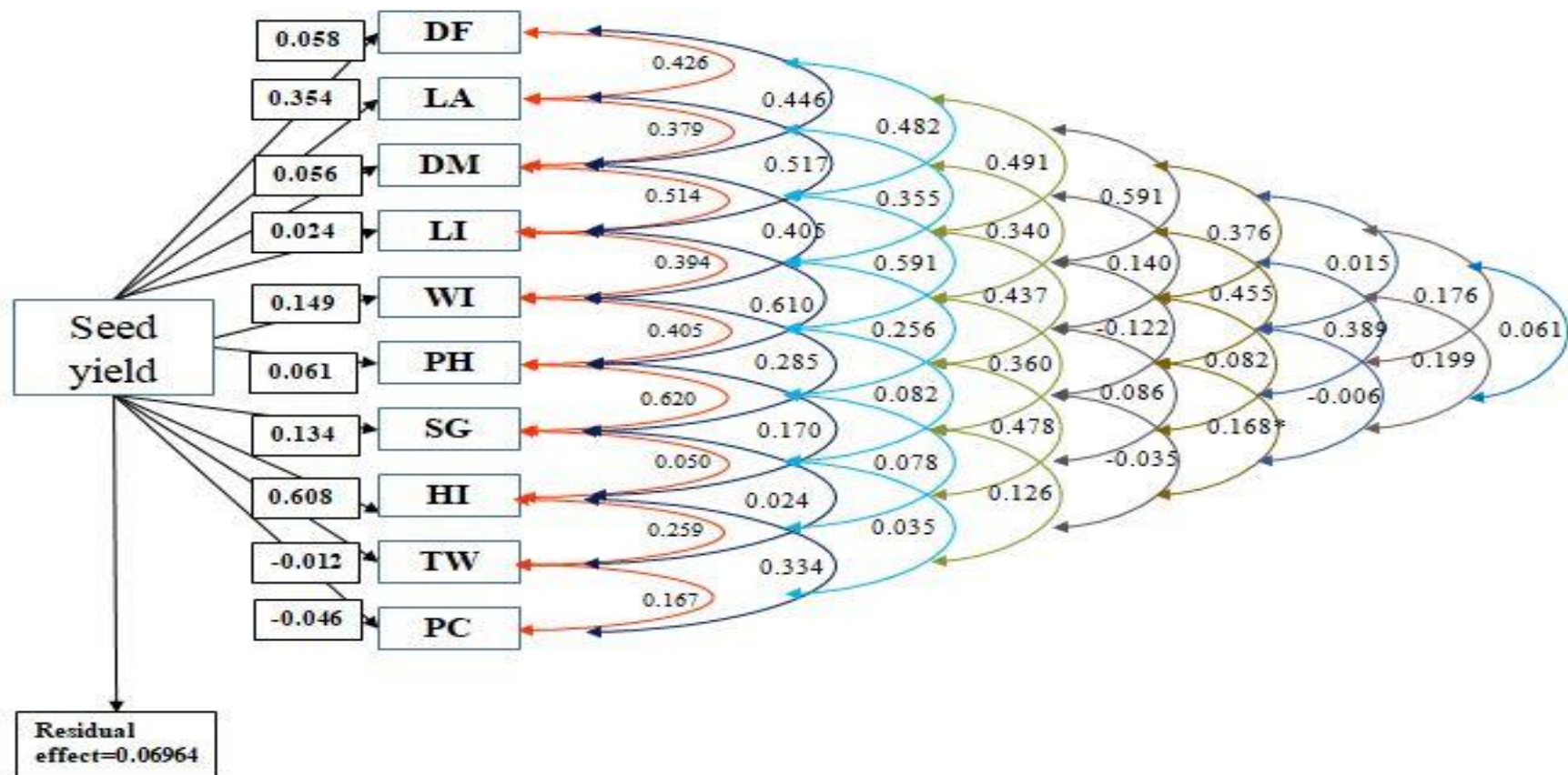


Fig. 2 : Genotypic path diagram for seed yield per plant (g)

Where,

DF = Days to flowering, **LA** = Leaf area per plant (cm²), **DM** = Days to maturity, **LI** = Length of inflorescence (cm), **WI** = Width of inflorescence (cm), **PH** = Plant height (cm), **SG** = Stem girth (cm), **HI** = Harvest index, **TW** = Test weight (g/10 ml) and **PC** = Protein content (%).

Direct effect of any component characters on seed yield gives an idea about reliability of indirect selection to be made through that character to bring the improvement in yield. If both the correlation coefficient and the direct effect are high and positive, then correlation explains its true relationship and a selection for that character will be effective. If the correlation coefficient is positive, but the direct effect is negative or negligible, in such relations the indirect causal factors are to be considered simultaneously for selection. When correlation coefficient is negative but the direct effect is positive and high in such cases direct selection for such traits should be practiced to reduce the undesirable direct effect.

The residual effect determines how best the causal factors account for the variability of the dependent factors, the seed yield in this case if the value of residual effect is moderate or high, it indicates that besides the character studied are some other attributes which contribute for yield.

In the present study, harvest index (0.6077) recorded the highest positive direct effect on seed yield per plant followed by leaf area per plant (0.3544). The trait showed low positive direct effect on seed yield per plant by width of inflorescence (0.1490) and stem girth (0.1338), respectively. While, days to flowering (0.0579), days to maturity (0.0565), length of inflorescence (0.0240) and plant height (0.0608) had negligible positive direct effect on seed yield per plant. On other hand, test weight (-0.0123) and protein content showed (-0.0459) negligible negative effect on seed yield per plant.

Based on findings of the present investigation the most desirable ideotype of grain amaranth for seed yield should possess early to mature, a large leaf area per plant, more harvest index and thicker width of inflorescence. Selection on the basis of these criteria would improve the efficiency of selection programme.

Dutta *et al.* (2002) reported that harvest index, days to maturity, plant height, inflorescence length and days to flowering had highest positive direct effect on seed yield per plant. Shukla and Singh (2003) reported that days to flowering, days to maturity and plant height had positive direct effect on seed yield per plant. Patial *et al.* (2014) reported that harvest index and days to maturity had high phenotypic and genotypic direct effects on seed yield per plant. Gelotar (2018) reported that highest positive direct effect on seed yield per plant by days to flowering, leaf area and 1000-seed weight. Tiwari (2018) reported that days to maturity, 10 ML seed weight, length of inflorescence and plant height had positive direct effects on grain yield.

4.5 Genetic variability through ISSR marker

In this study, forty-six grain amaranth genotypes were subjected to amplification by Inter Simple Sequence Repeats (ISSR) to access genetic diversity. The banding pattern thus obtained by ISSR primers clearly distinguished into different clusters showing diversity.

4.5.1 Isolation of genomic DNA

The genomic DNA from forty-six genotypes of grain amaranth was isolated from tender fresh leaves of each genotype by using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method described by Doyle and Doyle, (1987) with modifications. Qualitative and quantitative analysis of each extracted genomic DNA samples were done by Spectrophotometric method and agarose gel electrophoresis.

4.5.2 Purity check of extracted genomic DNA by agarose gel electrophoresis

Purity of extracted genomic DNA samples was confirmed by agarose gel electrophoresis using 0.8 % agarose gel. Result of the separation of genomic DNA on 0.8 % agarose gel exhibited intact band of genomic DNA which proves good quality of DNA (Genomic DNA Plate-3).

4.5.3 Quantification of genomic DNA isolated from grain amaranth genotypes using spectrophotometer

For the assessment of genetic diversity among forty-six grain amaranth genotypes, genomic DNA was extracted from the young leaves of all the grain amaranth genotypes. The Spectrophotometric analysis of the extracted genomic DNA samples was done to know the quantity as well as quality which are prerequisite step before amplification by PCR.

As the results of Spectrophotometric analysis showed, an average concentration of genomic DNA was 797.70 ng/μl. The concentration of genomic DNA of all the forty-six genotypes of grain amaranth ranged from 170.6-1473.2 ng/μl in Table 4.5. The purity or quality of genomic DNA was also assessed by the Spectrophotometric analysis which was represented as O.D. (A_{260}/A_{280}). All the samples showed A_{260}/A_{280} ratio between 1.70-1.91 and average ratio was 1.

Table 4.5 : Quantification of extracted DNA obtained from grain amaranth genotypes

Sr. No.	Germplasm	Nucleic acid concentration (ng/μl)	A₂₆₀/A₂₈₀
1.	SKGPA-61	805.5	1.82
2.	SKGPA-74	1217.9	1.78
3.	SKGPA-150	373.5	1.73
4.	SKGPA-155	987.1	1.70
5.	SKNA-401	470.0	1.74
6.	SKNA-403	1078.0	1.79
7.	SKNA-808	1285.1	1.81
8.	SKNA-813	739.1	1.78
9.	SKNA-903	879.0	1.82
10.	SKNA-908	1382.9	1.75
11.	SKNA-1201	365.6	1.77
12.	SKNA-1207	806.4	1.83
13.	SKNA-1305	1130.7	1.80
14.	SKNA-1313	446.2	1.88
15.	SKNA-1403	971.0	1.85
16.	SKNA-1406	369.2	1.73
17.	SKNA-1407	346.7	1.81
18.	SKNA-1414	587.5	1.77
19.	SKNA-1501	1012.2	1.91
20.	SKNA-1502	813.8	1.71
21.	SKNA-1503	684.5	1.75
22.	SKNA-1508	1137.6	1.82
23.	SKNA-1510	1066.7	1.76
24.	GA-2	561.5	1.87
25.	GA-3	170.6	1.84
26.	BGA-4-9	487.9	1.72
27.	BGA-7	1168.7	1.83
28.	BGA-9	843.9	1.72
29.	BGA-10	732.6	1.84
30.	BGA-14	983.2	1.82
31.	BGA-20	1473.2	1.83
32.	KBGA-4	1046.4	1.84
33.	KBGA-5	1279.7	1.84
34.	KBGA-7	1244.1	1.83
35.	RMA-62	1156.7	1.86
36.	RMA-63	1216.3	1.83
37.	RGA-13	482.7	1.78
38.	RGA-14	909.7	1.74
39.	RGA-16	621.4	1.86
40.	RGA-17	713.6	1.74
41.	RGA-18	516.8	1.89
42.	RGAG-12-22	622.0	1.75
43.	RGAG-14-3	495.4	1.77
44.	RHGA-13-3	187.7	1.90
45.	MGA-502	591.2	1.71
46.	Suvarna	232.9	1.83
Average :		797.70	1.79
Range :		170.6-1473.2	1.70-1.91

4.5.4 Amplification by ISSR primers

Total 7ISSR primers were used for screening of 46 grain amaranth genotypes. All the 7 ISSR primers were found polymorphic. The result obtained using 7 polymorphic primers have been presented in Table 4.6.

4.5.5 Pooled ISSR Results

ISSR markers analysis of all seven primers series viz., ISSR 1, ISSR 2, ISSR 3, ISSR 4, ISSR 21, ISSR22 and ISSR 23 generated total number of 46 loci (bands). Out of 46, 40 polymorphic bands and 6 bands monomorphic. Number of alleles ranged from 6 to 8 with an average 6.57 in present study. Ray and Roy (2007) and Singh *et al.* (2013) reported 11.36 and 8.5 average bands per ISSR primers in amaranth genotype. This is higher than the findings of the present investigation. The highest total number of amplified (bands) loci (8) was exhibited by ISSR 1 and ISSR 2. The lowest number of amplified (bands) loci (4) detected in ISSR 4.

The mean performance of polymorphic as well as monomorphic band was 5.71 and 0.85, respectively. The highest polymorphism (100 %) found in ISSR 1 primer whereas the lowest polymorphism (75 %) observed in ISSR 4. The average percent polymorphism detected by ISSR primers in the present investigation was 85.79. Gelotar (2018) reported ISSR primers were 87.15 per cent polymorphism in different species of grain amaranth. Singh *et al.* (2013) reported 77.15 per cent polymorphism in amaranth genotype. Stefunova *et al.* (2015) 83 to 100 per cent polymorphism in amaranth genotype. Total number of lowest fragment amplified (100) in ISSR 4, When highest number of fragment amplified (235) in ISSR 21. In present investigation PIC value (polymorphic information content) of different ISSR primers showed ranged from 0.456 to 0.641 with an average of 0.572. Singh *et al.* (2013) reported PIC value range 0.203 to 0.742 with an average of 0.229 per primer. Gelotar (2018) detected range of PIC value 0.683 to 0.893 and average value was 0.853. The size of PCR amplified DNA fragments varied from 125 to 2429 bp (Table 4.6).

The amplification profiles obtained for grain amaranth genotypes with ISSR primers ISSR 1, ISSR 2, ISSR 3, ISSR 4, ISSR 21, ISSR22 and ISSR 23 are shown in Plate-4, Plate-5, Plate-6, Plate-7, Plate-8, Plate-9 and Plate- 10.

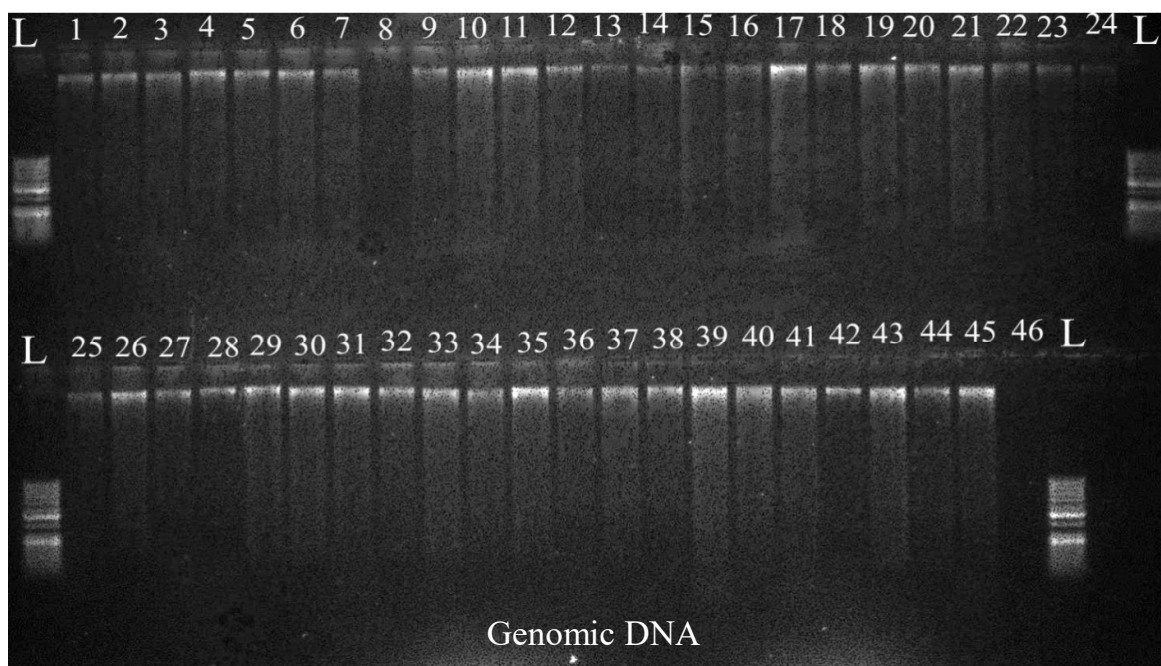


Plate 3. Separation of genomic DNA of grain amaranth by agarose gel electrophoresis (after dilution)

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406				

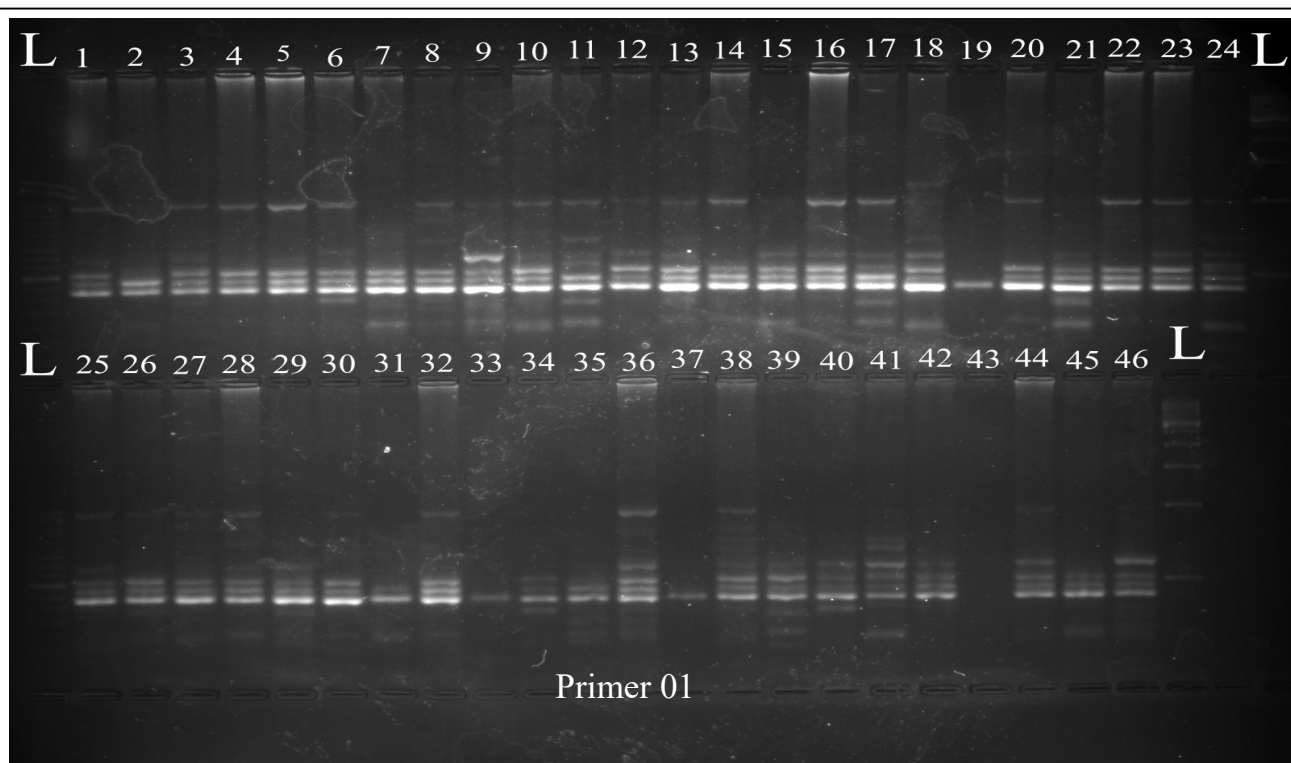


Plate 4. Banding pattern of ISSR 01 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406	Leader left side : 100 bp, Right side : 500 bp			

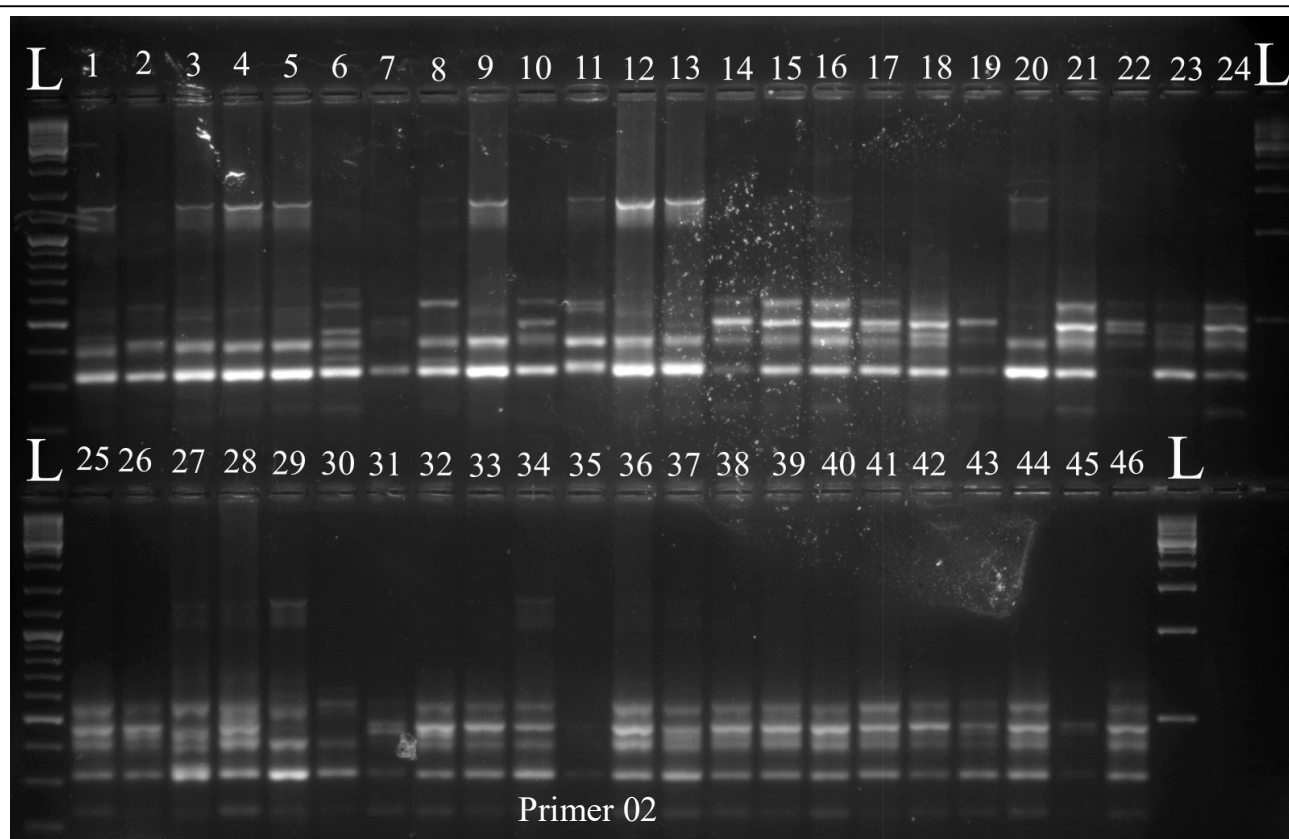


Plate 5. Banding pattern of ISSR 02 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406	Leader left side : 100 bp, Right side : 500 bp			

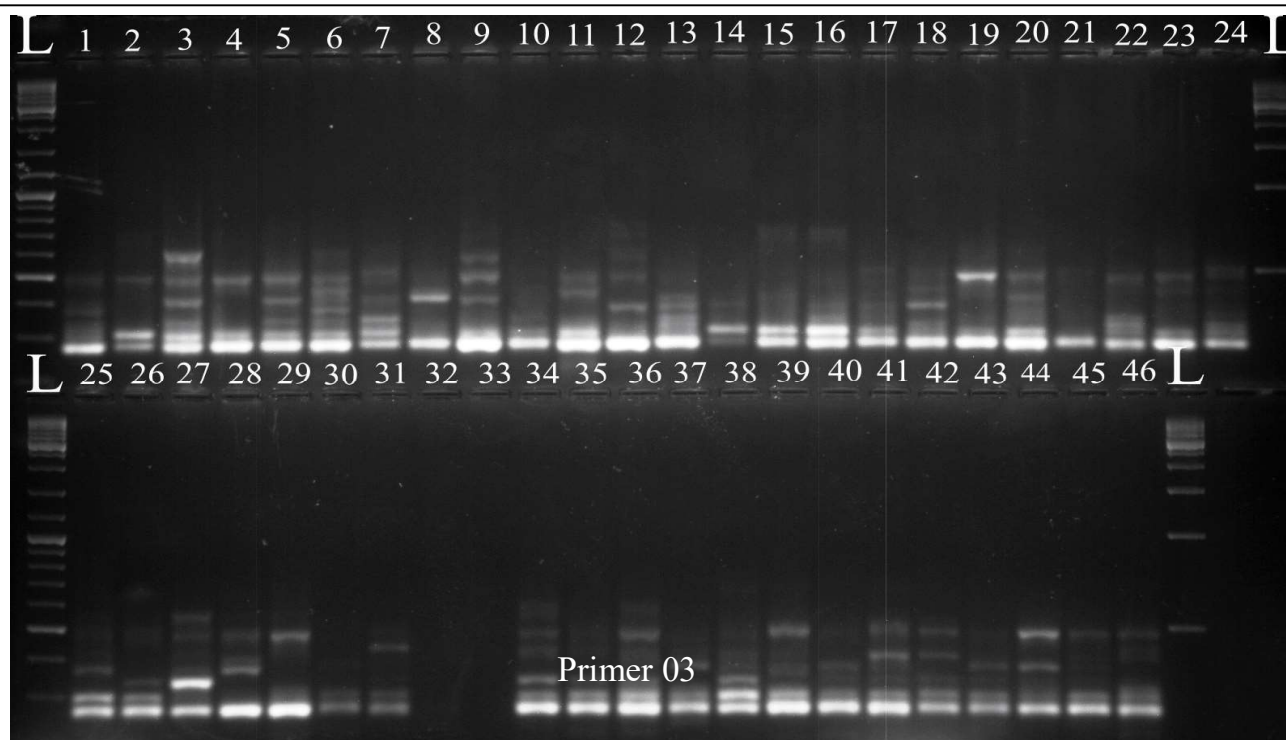


Plate 6. Banding pattern of ISSR 03 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406	Leader left side : 100 bp, Right side : 500 bp			

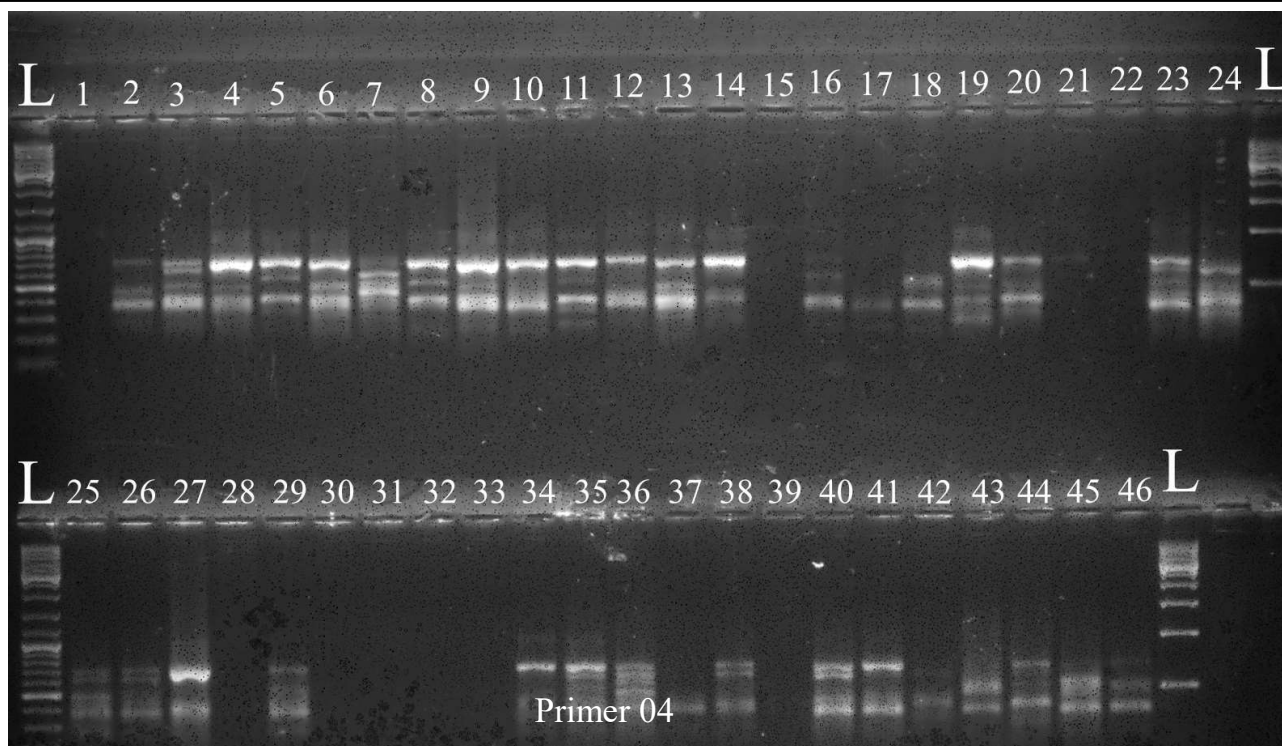


Plate 7. Banding pattern of ISSR 04 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406				

Leader left side : 100 bp, Right side : 500 bp

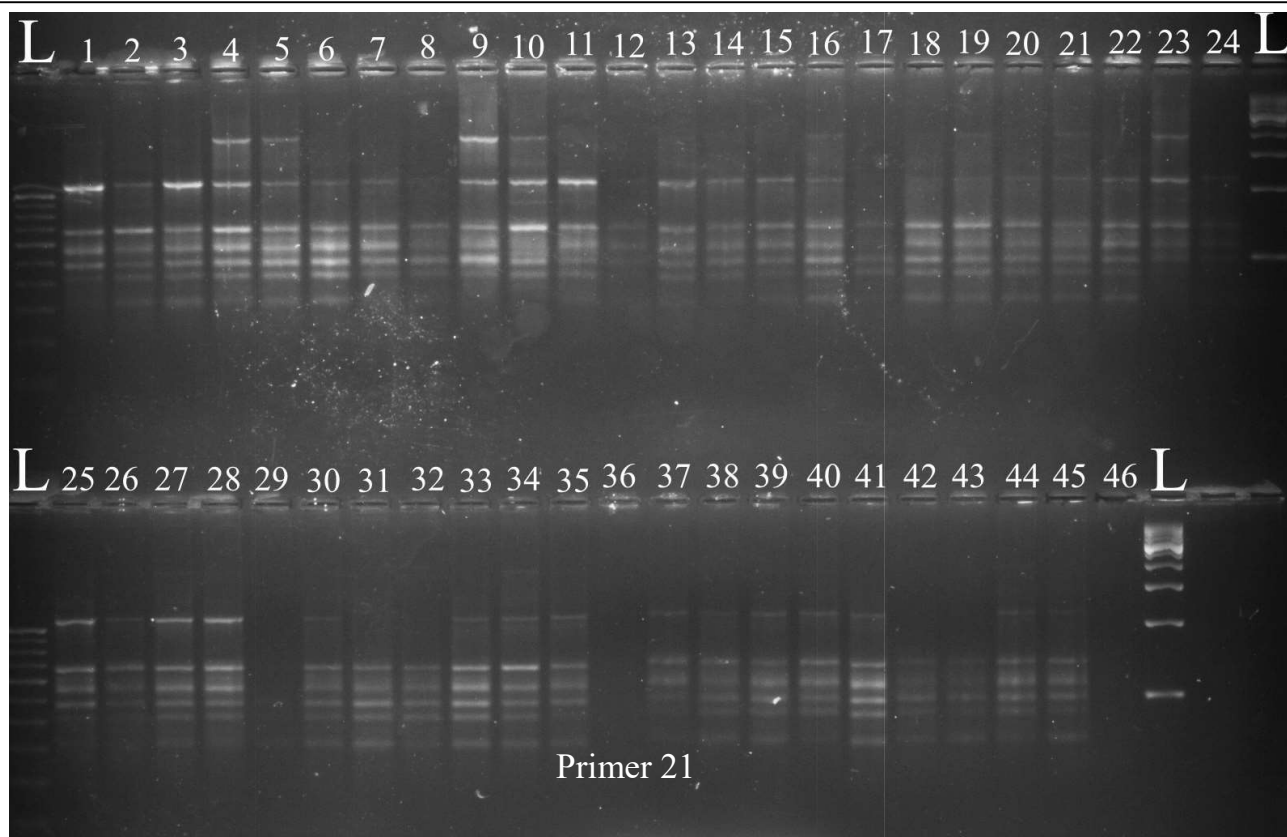


Plate 8. Banding pattern of ISSR 21 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406	Leader left side : 100 bp, Right side : 500 bp			

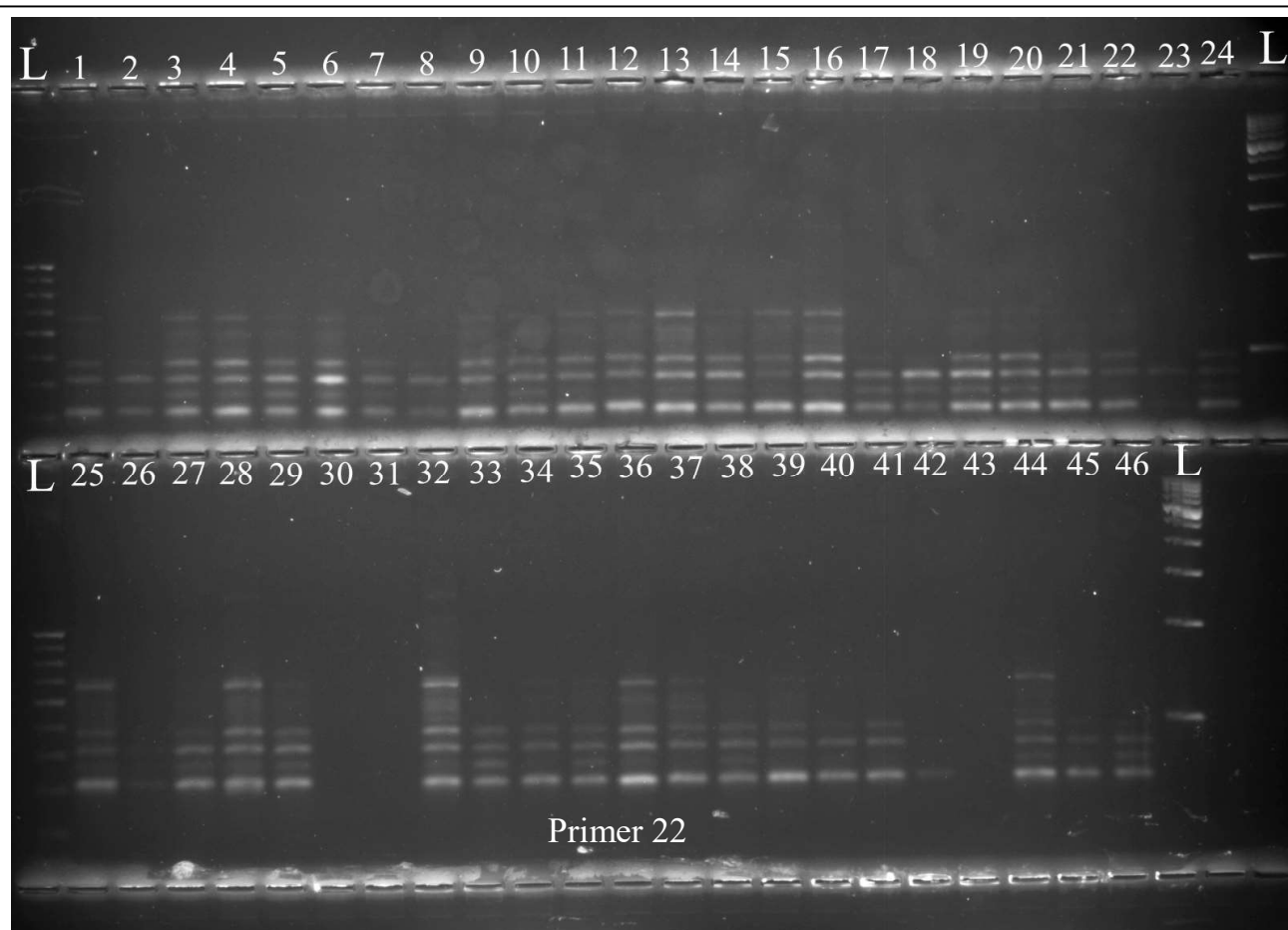


Plate 9. Banding pattern of ISSR 22 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406				

Leader left side : 100 bp, Right side : 500 bp

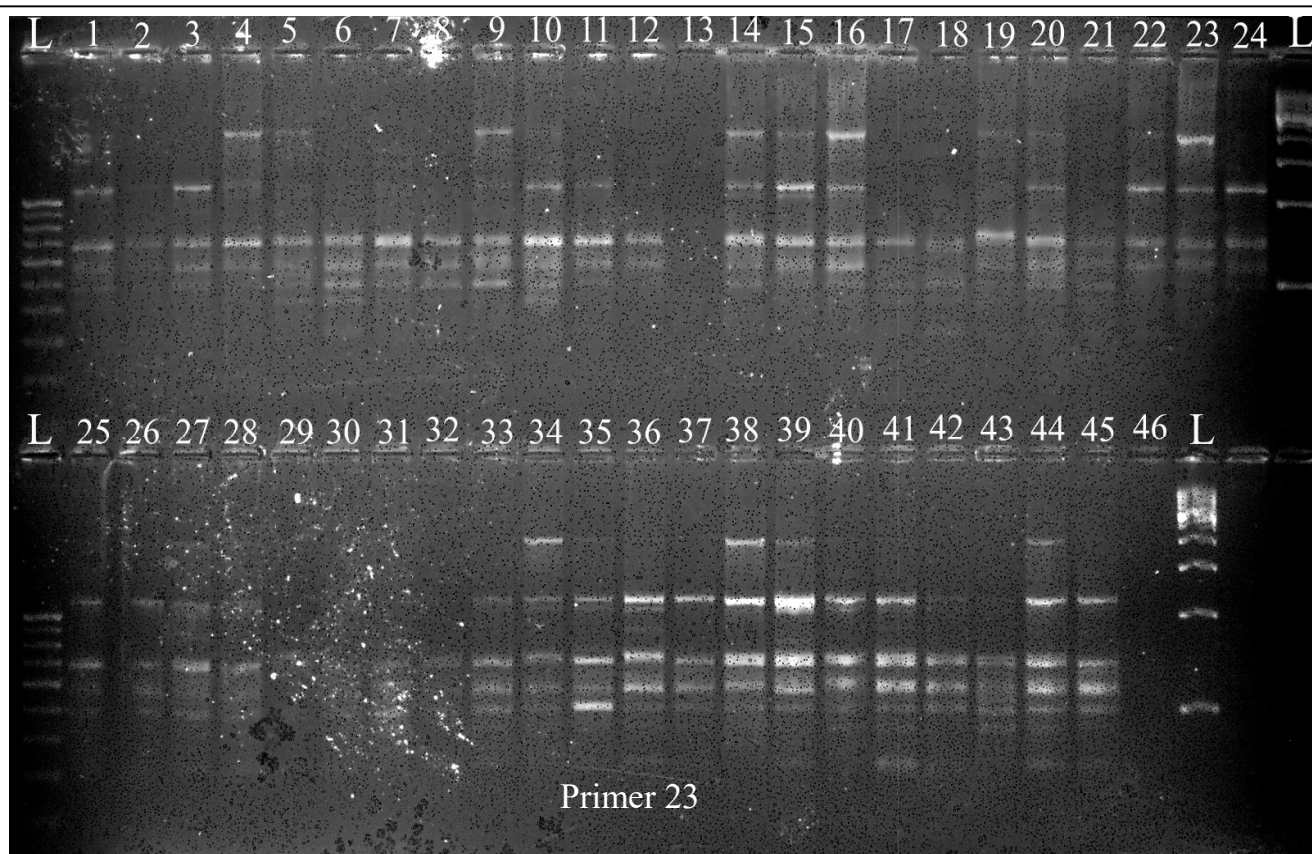


Plate 10. Banding pattern of ISSR 23 primer among grain amaranth genotypes

1	SKGPA-61	17	SKNA-1407	32	KGBA-4
2	SKGPA-73	18	SKNA-1414	33	KGBA-5
3	SKGPA-150	19	SKNA-1501	34	KGBA-7
4	SKGPA-155	20	SKNA-1502	35	RMA-62
5	SKNA-401	21	SKNA-1503	36	RMA-63
6	SKNA-403	22	SKNA-1508	37	RGA-13
7	SKNA-808	23	SKNA-1510	38	RGA-14
8	SKNA-813	24	GA-2	39	RGA-16
9	SKNA-903	25	GA-3	40	RGA-17
10	SKNA-908	26	BGA-4-9	41	RGA-18
11	SKNA-1201	27	BGA-7	42	RGAG-12-22
12	SKNA-1207	28	BGA-9	43	RGAG-14-3
13	SKNA-1305	29	BGA-10	44	RHGA-13-3
14	SKNA-1313	30	BGA-14	45	MGA-502
15	SKNA-1403	31	BGA-20	46	Suvarana
16	SKNA-1406	Leader left side : 100 bp, Right side : 500 bp			

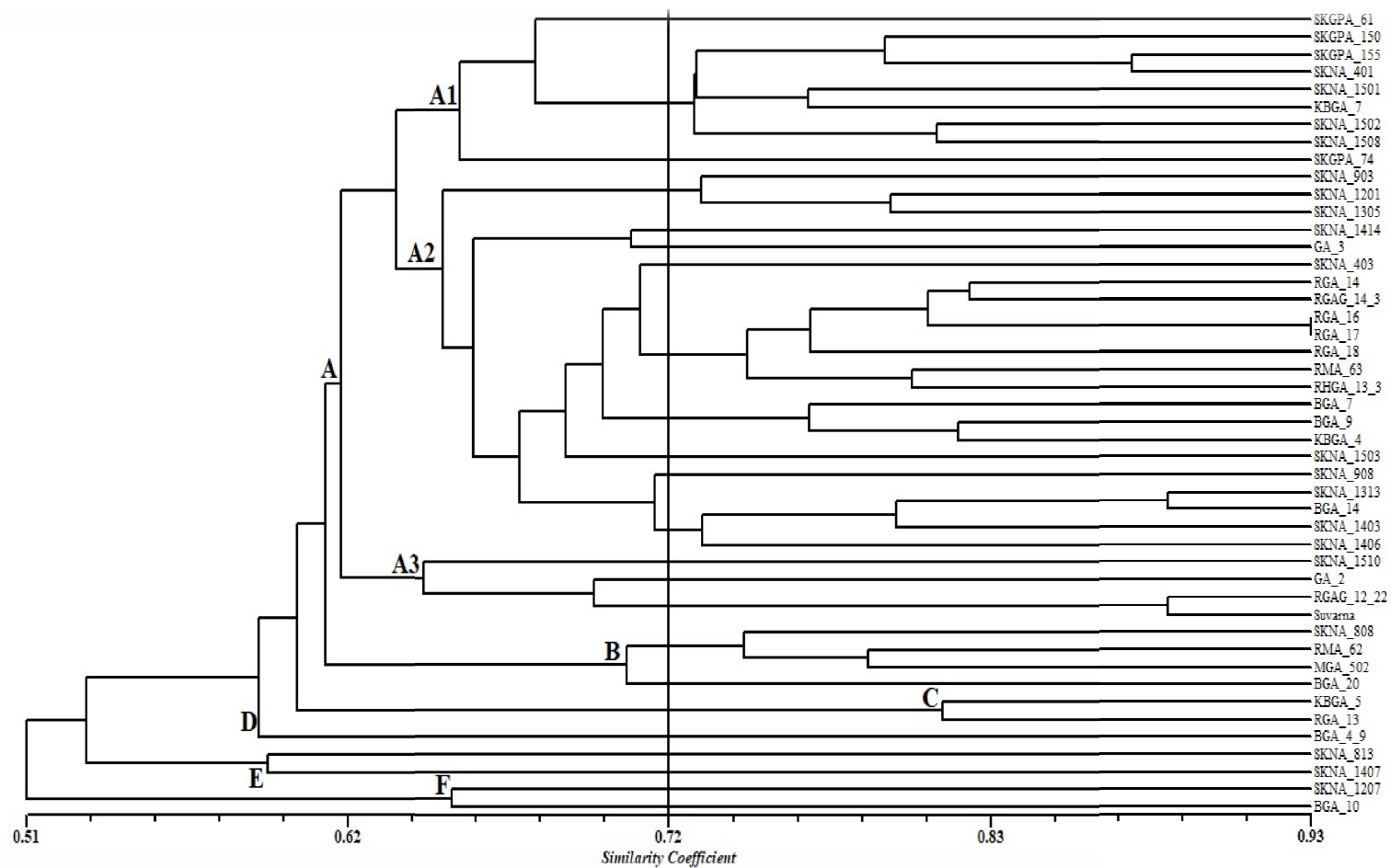


Fig. 3. Dendrogram (UPGMA based) of grain amaranth genotypes

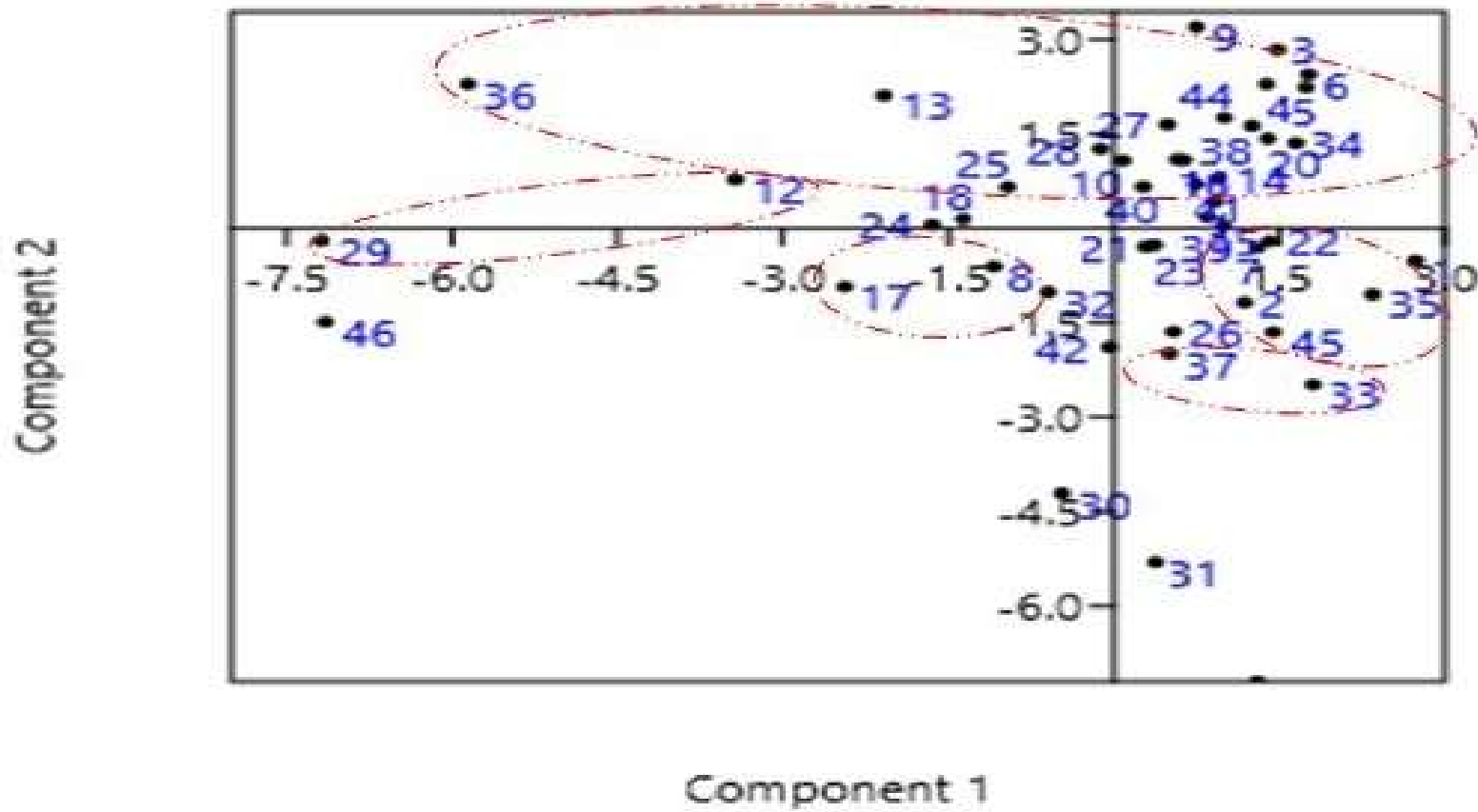


Fig. 4. Principle Component Analysis (PCA) of grain amaranth genotypes

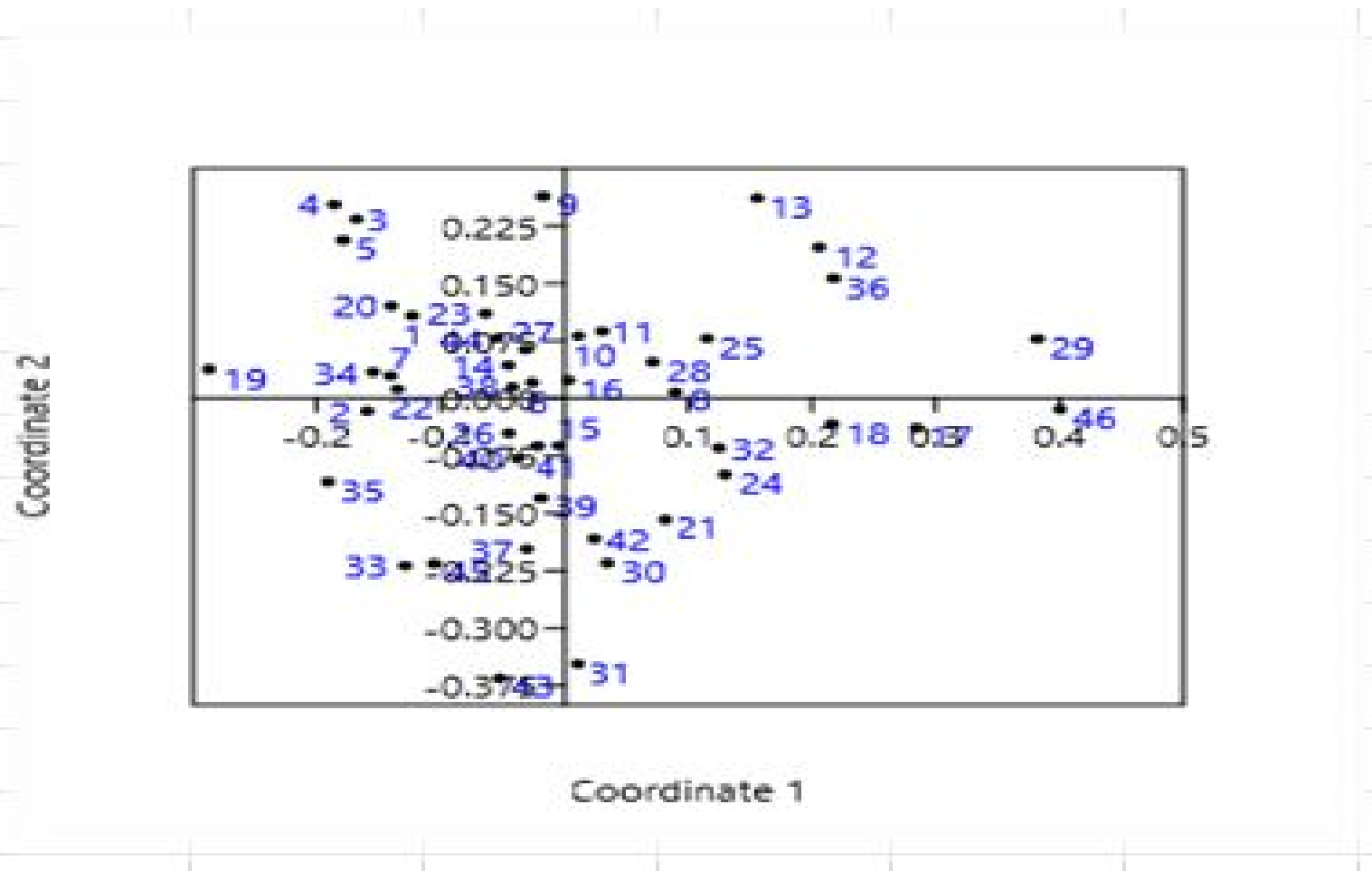


Fig. 5. Principle Co-ordinate Analysis (PCoA) of grain amaranth genotypes

Table 4.6 : Polymorphism study used with 7 ISSR primers for forty-six genotypes of grain amaranth

Sr. No.	Primer name	Total number of loci	Number of polymorphic loci	Number of monomorphic loci	Per cent of polymorphic loci	Total number of fragment amplified	Polymorphism Information Content (PIC) value	Range of molecular weight (bp)
1.	ISSR1	8	8	0	100.00	209	0.592	141 to 2429
2.	ISSR2	8	7	1	87.50	174	0.641	125 to 1626
3.	ISSR3	7	6	1	85.71	139	0.589	168 to 761
4.	ISSR4	4	3	1	75.00	100	0.558	170 to 854
5.	ISSR21	7	6	1	85.71	235	0.542	308 to 1970
6.	ISSR22	6	5	1	83.33	166	0.456	232 to 934
7.	ISSR23	6	5	1	83.33	150	0.624	308 to 2395
Total		46	40	6	-	-	-	-
Mean		6.57	5.71	0.85	85.79	-	0.572	-
Range		-	-	-	75.00 to 100.00	-	0.456 to 0.641	-

4.5.6 Construction of dendrogram and classification of genotypes based on ISSR

Jaccard's co-efficient were used to compare set of variables and to generate similarity matrix. Jaccard's co-efficient for all genotypes are shown in Table 4.7. Similarity indices were estimated on the basis of seven primers ranged from 0.320 (between BGA-10 and BGA-4-9) to 0.930 (between RGA-16 and RGA-17), indicating the presence of genetic diversity among the genotypes of grain amaranth.

The dendrogram was prepared based on UPGMA (Unweighted pair-group method with arithmetic mean) by using Jaccard's similarity co-efficient through NTSYSpc (Version 2.20N). These results indicate that the two genotypes, were found to be the most BGA-10 and BGA-4-9, RGA-16 and RGA-17 diverse type. Cultivars showing the lowest genetic similarity are of great concern to plant breeders to be further selected as parents. Weising *et al.* (2005) mentioned that, it is mandatory that genetically divergent parents could be chosen which exhibit sufficient polymorphism, but are not so distant as to cause sterility of the progeny.

In the present investigation, the results revealed that different types of profiles expressed different levels of genetic similarity among the forty-six cultivars. This could be due to the different mechanisms of polymorphism detection by the different bands type. The accuracy of genetic similarity estimates based on molecular data depends on several variable factors such as the number of bands analyzed, their distribution over the genomes and the accuracy in scoring them Table 4.7. Dendrogram clustered with the data generated by all the primers and their amplicons grouped the forty six genotypes into six clusters *i.e.*, Clusters A, B, C, D, E and F in Fig. 3. The detailed distribution of genotypes based on the cluster from UPGMA dendrogram is showed below in Table 4.8.

Dendrogram showed major clusters cophenetic similarity coefficient was 0.720 in Fig. 3. Mainly grouping of Clusters A divided into A1, A2, A3 and B, C, D, E and F. The cluster A divided into A1, A2 and A3. A1 contains 9 genotypes, A2 cluster contains 22 genotypes, A3 cluster contains 4 genotypes, cluster B contains 4 genotypes, cluster C contains 2 genotypes, cluster D contain 1 genotype, cluster E contains 2 genotypes cluster F contains 2 genotypes, genotypes RGA-16 and RGA-17 had highest similarity.

Table 4.7 : Similarity matrix from ISSR analysis

	SKGPA-61	SKGPA-74	SKGPA-150	SKGPA-155	SKNA-401	SKNA-403	SKNA-808	SKNA-813	SKNA-903	SKNA-908	SKNA-1201	SKNA-1207	SKNA-1305	SKNA-1313	SKNA-1403	SKNA-1406	SKNA-1407	SKNA-1414	SKNA-1501	SKNA-1502	SKNA-1503
SKGPA-61	1																				
SKGPA-74	0.63	1																			
SKGPA-150	0.75	0.68	1																		
SKGPA-155	0.7	0.63	0.79	1																	
SKNA-401	0.66	0.59	0.79	0.87	1																
SKNA-403	0.58	0.57	0.71	0.63	0.68	1															
SKNA-808	0.71	0.63	0.69	0.65	0.66	0.63	1														
SKNA-813	0.6	0.48	0.56	0.52	0.53	0.56	0.55	1													
SKNA-903	0.67	0.56	0.7	0.71	0.72	0.61	0.67	0.59	1												
SKNA-908	0.64	0.55	0.66	0.62	0.58	0.65	0.61	0.63	0.69	1											
SKNA-1201	0.66	0.59	0.65	0.57	0.54	0.64	0.61	0.63	0.72	0.73	1										
SKNA-1207	0.6	0.5	0.53	0.53	0.5	0.53	0.52	0.48	0.61	0.5	0.55	1									
SKNA-1305	0.67	0.55	0.71	0.65	0.61	0.63	0.64	0.55	0.74	0.63	0.79	0.61	1								
SKNA-1313	0.7	0.6	0.66	0.62	0.63	0.65	0.67	0.58	0.74	0.8	0.73	0.55	0.71	1							
SKNA-1403	0.57	0.6	0.57	0.57	0.58	0.61	0.56	0.48	0.64	0.69	0.68	0.55	0.63	0.8	1						
SKNA-1406	0.53	0.56	0.62	0.68	0.64	0.62	0.44	0.5	0.61	0.7	0.59	0.47	0.59	0.7	0.75	1					
SKNA-1407	0.46	0.45	0.44	0.48	0.46	0.53	0.47	0.59	0.51	0.66	0.59	0.45	0.59	0.55	0.55	0.61	1				
SKNA-1414	0.47	0.53	0.55	0.56	0.57	0.63	0.5	0.52	0.58	0.57	0.61	0.53	0.63	0.62	0.62	0.63	0.63	1			
SKNA-1501	0.62	0.73	0.67	0.79	0.74	0.57	0.62	0.44	0.6	0.59	0.54	0.45	0.48	0.59	0.59	0.61	0.41	0.49	1		
SKNA-1502	0.68	0.67	0.76	0.78	0.79	0.66	0.68	0.59	0.74	0.7	0.64	0.52	0.65	0.75	0.7	0.66	0.56	0.63	0.77	1	
SKNA-1503	0.57	0.47	0.54	0.59	0.61	0.68	0.59	0.55	0.62	0.7	0.66	0.5	0.64	0.65	0.73	0.69	0.64	0.63	0.57	0.63	1
SKNA-1508	0.75	0.7	0.69	0.7	0.66	0.63	0.64	0.54	0.61	0.64	0.6	0.54	0.58	0.7	0.68	0.63	0.52	0.57	0.68	0.81	0.57
SKNA-1510	0.65	0.59	0.6	0.66	0.62	0.55	0.6	0.57	0.73	0.63	0.62	0.53	0.59	0.68	0.58	0.64	0.48	0.47	0.63	0.69	0.55
GA-2	0.55	0.47	0.54	0.5	0.51	0.58	0.58	0.55	0.57	0.66	0.65	0.47	0.5	0.61	0.61	0.57	0.57	0.59	0.56	0.62	0.67
GA-3	0.63	0.59	0.65	0.56	0.53	0.64	0.55	0.52	0.63	0.68	0.67	0.59	0.66	0.68	0.58	0.64	0.59	0.71	0.53	0.64	0.58
BGA-4-9	0.67	0.62	0.63	0.53	0.55	0.57	0.63	0.59	0.56	0.6	0.59	0.4	0.63	0.66	0.5	0.56	0.56	0.53	0.5	0.61	0.5
BGA-7	0.71	0.56	0.71	0.63	0.69	0.7	0.58	0.5	0.61	0.6	0.55	0.56	0.63	0.65	0.56	0.57	0.47	0.58	0.56	0.66	0.61
BGA-9	0.63	0.63	0.72	0.59	0.65	0.71	0.59	0.56	0.7	0.66	0.65	0.58	0.69	0.71	0.66	0.62	0.53	0.74	0.57	0.76	0.69
BGA-10	0.48	0.41	0.48	0.52	0.53	0.47	0.44	0.41	0.52	0.5	0.45	0.65	0.5	0.52	0.56	0.47	0.5	0.55	0.44	0.57	0.6
BGA-14	0.67	0.71	0.7	0.74	0.71	0.63	0.67	0.5	0.61	0.68	0.59	0.38	0.62	0.88	0.79	0.75	0.6	0.75	0.61	0.79	0.67
BGA-20	0.46	0.65	0.55	0.54	0.53	0.63	0.67	0.54	0.55	0.56	0.59	0.42	0.5	0.59	0.59	0.52	0.52	0.59	0.54	0.61	0.63
KBGA-4	0.6	0.54	0.66	0.57	0.62	0.7	0.58	0.56	0.63	0.68	0.56	0.54	0.63	0.74	0.68	0.58	0.58	0.74	0.48	0.68	0.7
KBGA-5	0.65	0.58	0.64	0.55	0.61	0.63	0.56	0.48	0.52	0.61	0.55	0.46	0.48	0.67	0.67	0.57	0.39	0.5	0.71	0.67	0.69
KBGA-7	0.6	0.61	0.67	0.73	0.79	0.7	0.63	0.46	0.61	0.65	0.55	0.52	0.59	0.7	0.7	0.71	0.52	0.54	0.77	0.76	0.71
RMA-62	0.59	0.64	0.7	0.61	0.62	0.64	0.78	0.52	0.54	0.68	0.67	0.39	0.57	0.63	0.63	0.54	0.48	0.51	0.69	0.69	0.66
RMA-63	0.6	0.54	0.77	0.63	0.65	0.7	0.64	0.52	0.69	0.72	0.66	0.57	0.73	0.72	0.63	0.67	0.53	0.65	0.55	0.7	0.65
RGA-13	0.58	0.5	0.53	0.48	0.5	0.57	0.52	0.43	0.47	0.6	0.55	0.5	0.43	0.66	0.55	0.56	0.45	0.53	0.61	0.56	0.59
RGA-14	0.59	0.54	0.68	0.65	0.7	0.76	0.6	0.57	0.63	0.62	0.7	0.5	0.69	0.71	0.67	0.68	0.54	0.69	0.63	0.72	0.7
RGA-16	0.63	0.59	0.6	0.61	0.62	0.69	0.66	0.47	0.68	0.61	0.67	0.57	0.64	0.77	0.8	0.65	0.55	0.64	0.63	0.74	0.74
RGA-17	0.68	0.65	0.65	0.61	0.62	0.73	0.66	0.53	0.63	0.58	0.67	0.59	0.63	0.68	0.68	0.59	0.5	0.61	0.64	0.69	0.73
RGA-18	0.63	0.63	0.68	0.59	0.65	0.63	0.69	0.61	0.7	0.61	0.69	0.53	0.63	0.71	0.71	0.58	0.49	0.69	0.62	0.76	0.69
RGAG-12-22	0.57	0.59	0.65	0.56	0.62	0.59	0.55	0.57	0.58	0.53	0.53	0.48	0.47	0.58	0.58	0.64	0.53	0.61	0.58	0.69	0.63
RGAG-14-3	0.53	0.62	0.63	0.61	0.6	0.75	0.62	0.65	0.6	0.59	0.68	0.55	0.61	0.71	0.64	0.61	0.53	0.79	0.61	0.65	0.72
RHGA-13-3	0.67	0.66	0.75	0.71	0.72	0.74	0.62	0.54	0.73	0.69	0.68	0.56	0.68	0.79	0.74	0.74	0.51	0.71	0.7	0.79	0.67
MGA-502	0.63	0.64	0.65	0.61	0.62	0.64	0.71	0.52	0.54	0.63	0.57	0.39	0.47	0.63	0.63	0.64	0.53	0.56	0.63	0.69	0.69
Suvarna	0.53	0.61	0.54	0.62	0.64	0.58	0.55	0.52	0.58	0.55	0.48	0.57	0.48	0.57	0.57	0.7	0.65	0.68	0.61	0.67	0.67

Table 4.7 Continued....

	SKNA-1508	SKNA-1510	GA-2	GA-3	BGA-4-9	BGA-7	BGA-9	BGA-10	BGA-14	BGA-20	KBGA-4	KBGA-5	KBGA-7	RMA-62	RMA-63	RGA-13	RGA-14	RGA-16	RGA-17	RGA-18	RGAG-12-22	RGAG-14-3	RHGA-13-3	MGA-502	Suvarna
SKGPA-61																									
SKGPA-74																									
SKGPA-150																									
SKGPA-155																									
SKNA-401																									
SKNA-403																									
SKNA-808																									
SKNA-813																									
SKNA-903																									
SKNA-908																									
SKNA-1201																									
SKNA-1207																									
SKNA-1305																									
SKNA-1313																									
SKNA-1403																									
SKNA-1406																									
SKNA-1407																									
SKNA-1414																									
SKNA-1501																									
SKNA-1502																									
SKNA-1503																									
SKNA-1508	1																								
SKNA-1510	0.65	1																							
GA-2	0.55	0.59	1																						
GA-3	0.63	0.67	0.65	1																					
BGA-4-9	0.67	0.64	0.52	0.7	1																				
BGA-7	0.66	0.54	0.53	0.64	0.61	1																			
BGA-9	0.63	0.6	0.63	0.75	0.58	0.76	1																		
BGA-10	0.46	0.44	0.58	0.46	0.32	0.61	0.62	1																	
BGA-14	0.72	0.6	0.52	0.63	0.72	0.64	0.71	0.45	1																
BGA-20	0.52	0.52	0.56	0.48	0.54	0.55	0.64	0.5	0.63	1															
KBGA-4	0.63	0.47	0.5	0.63	0.54	0.77	0.81	0.61	0.87	0.7	1														
KBGA-5	0.68	0.5	0.59	0.56	0.52	0.76	0.68	0.5	0.67	0.57	0.68	1													
KBGA-7	0.71	0.64	0.57	0.54	0.56	0.71	0.62	0.57	0.67	0.55	0.62	0.73	1												
RMA-62	0.59	0.52	0.59	0.56	0.59	0.59	0.6	0.43	0.67	0.71	0.57	0.68	0.64	1											
RMA-63	0.63	0.66	0.7	0.69	0.57	0.65	0.77	0.68	0.71	0.61	0.71	0.58	0.67	0.62	1										
RGA-13	0.58	0.53	0.68	0.7	0.56	0.61	0.58	0.44	0.56	0.48	0.54	0.81	0.61	0.59	0.55	1									
RGA-14	0.65	0.66	0.64	0.71	0.64	0.72	0.73	0.52	0.68	0.53	0.65	0.69	0.77	0.66	0.72	0.64	1								
RGA-16	0.69	0.67	0.68	0.65	0.57	0.67	0.75	0.6	0.71	0.62	0.67	0.72	0.77	0.61	0.7	0.66	0.81	1							
RGA-17	0.74	0.67	0.65	0.67	0.59	0.69	0.74	0.55	0.67	0.62	0.6	0.7	0.74	0.62	0.73	0.65	0.8	0.93	1						
RGA-18	0.69	0.65	0.73	0.6	0.58	0.62	0.77	0.61	0.71	0.66	0.63	0.68	0.67	0.65	0.71	0.58	0.73	0.81	0.79	1					
RGAG-12-22	0.63	0.61	0.65	0.61	0.64	0.59	0.7	0.54	0.65	0.65	0.59	0.64	0.64	0.56	0.66	0.59	0.66	0.7	0.72	0.75	1				
RGAG-14-3	0.61	0.61	0.59	0.65	0.62	0.63	0.73	0.47	0.77	0.75	0.8	0.76	0.63	0.64	0.71	0.7	0.82	0.79	0.82	0.74	0.76	1			
RHGA-13-3	0.72	0.73	0.66	0.73	0.61	0.69	0.8	0.61	0.79	0.62	0.7	0.69	0.74	0.63	0.8	0.61	0.81	0.78	0.77	0.8	0.73	0.74	1		
MGA-502	0.63	0.56	0.7	0.56	0.59	0.59	0.65	0.48	0.78	0.75	0.57	0.62	0.64	0.79	0.62	0.59	0.61	0.7	0.67	0.7	0.72	0.64	0.68	1	
Suvarna	0.63	0.72	0.75	0.7	0.61	0.57	0.65	0.7	0.62	0.63	0.56	0.53	0.7	0.48	0.67	0.61	0.65	0.72	0.71	0.71	0.88	0.73	0.8	0.72	1

Table 4.8 : Distribution of genotypes based on the clusters from UPGMA dendrogram from ISSR primer

Cluster	Sub-cluster	Genotypes	Number of genotypes
A	A1	SKGPA-61, SKGPA-150, SKGPA-155, SKNA-401, SKNA-1501, KBGA-7, SKNA-1502, SKNA-1508 and SKGPA-74.	09
	A2	SKNA-903, SKNA-1201, SKNA-1305, SKNA-1414, GA-3, SKNA-403, RGA-14, RGAG-14-3, RGA-16, RGA-17, RGA-18, RMA-63, RHGA-13-3, BGA-9, BGA-7, KBGA-4, SKNA-1503, SKNA-1313, SKNA-908, BGA-14, SKNA-1414 and SKNA-1406.	22
	A3	SKNA-1510, GA-2, RGAG-12-22 and Suvarna.	04
B		SKNA-808, RMA-62, BGA-20 and MGA-502.	04
C		KBGA-5 and RGA-13.	02
D		BGA-4-9.	01
E		SKNA-813 and SKNA-1407.	02
F		SKNA-1207 and BGA-10.	02

Principal Component Analysis (PCA) and Principal Co-ordinate Analysis (PCoA) carried out using software PAST version 3.18. In the PCA (Principle Component analysis) plot derived from the ISSR genotyping data, (which indicates that two principle co-ordinates), it can be observed that Suvarna is placed farthest in the 1st co-ordinate (X-axis), while BGA-20 placed farthest in the 2nd co-ordinate (Y-axis) in Fig. 4. In the PCA plot total five clusters have been formed while other genotypes are scattered on PCA plot. Genotypes in each cluster were found in same cluster in dendrogram also. So, it represents the efficiency to correlate the results of PCA plot with result of dendrogram.

On the basis of result found in PCA study :

- [1]** RMA-62, SKNA-1305, RHGA-13-3, GA-3, BGA-7, BGA-9 and RGA-14 falls under same cluster which also showed similarity among different morphological characters like plant height, inflorescence width and inflorescence length.
- [2]** SKNA-808, RMA-62 and MGA-502 falls under same cluster which also showed similarity at morphological level for the characters like test weight and stem girth.

- [3] Inflorescence width and length values were found similar for KBGA-5 and RGA-13 which also falls under same cluster in PCA.
- [4] SKNA-813 and SKNA-1407 falls under same cluster which also shows similarity among different morphological characters like days to maturity, leaf area index and inflorescence length.
- [5] BGA-10 and SKNA-1207 similar for characters like days to maturity and inflorescence width which are found in one cluster in PCA as well as same cluster in dendrogram.

From the PCoA (Principle Co-ordinate analysis) plot all genotype have been distributed in four groups, it can be observed that genotype RGAG-14-3 placed farthest in the 2nd coordinate (Y-axis), while genotype Suvarna placed farthest in the 1st coordinate (X-axis) in Fig. 5. However, in PCA plot total five clusters have been formed while other genotypes are scattered on PCoA plot.

In grain amaranth, various studies have been reported at molecular level deciphering variation across accessions and varieties. Ray and Roy (2007) and Gelotar (2018) used Principal Component Analysis (PCA) to support cluster analysis for ISSR primers.

In present study PCoA and scatter plot analysis distinct that all genotypes were scattered in all direction around axis indicating highly diverse origin of genotype which again concentrating our pattern of dendrogram and fulfilling pre-requisite of our diverse population selection for molecular characterisation. It can be observed that genotype RGAG-14-3 placed farthest in the 2nd co-ordinate (Y-axis), while genotype Suvarna placed farthest in the 1st co-ordinate (X-axis). Which, can be used in the breeding programme for the broadening of gene pool and to increase the diversity of grain amaranth.

The present study indicates that ISSR markers are suitable to assess genetic diversity in grain amaranth and able to individually distinguish all the forty-six genotypes. Such molecular data would also be useful for detecting DNA patterns unique for a given genotype or set of genotypes. Overall, it is concluded that the genetic diversity obtained in this study might be useful in future grain amaranth improvement programmes and selection in grain amaranth should be based on the phenotypic traits as well as based on the molecular markers. Hence, studies on morphological and molecular markers are quite useful in analysis the genetic diversity in grain amaranth.

SUMMARY AND CONCLUSIONS

V. SUMMARY AND CONCLUSIONS

The present investigation work on "**Variability, correlation and path analysis in grain amaranth (*Amaranthus hypochondriacus* L.)**" was conducted at Centre for Crop Improvement, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during *Rabi* 2018-19 with the following objectives :

- [1] To measure the magnitude and extent of genotypic and phenotypic variability
- [2] To estimate correlation and path coefficient between yield and component traits
- [3] To assess variability using molecular markers

The experimental materials used for study to be consisted of forty-six genotypes of grain amaranth which was obtained from Centre for Crop Improvement Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, District : Banaskantha (Gujarat). The date of sowing was 24th November 2018 Randomized Block Design (RBD) with the three replications. The method prescribed by Panse and Sukhatme (1978) was followed to determine variability and correlation coefficient by Al-Jibouri (1958) and path analysis as per method suggested by Deway and Lu (1959) and molecular diversity analysis by using ISSR markers by Doyle and Doyle (1987). The genotypes were evaluated for eleven characters to study the extent of genetic variability viz., days to flowering, leaf area per plant (cm²), days to maturity, length of inflorescence (cm), width of inflorescence (cm), plant height (cm), stem girth (cm), harvest index (%), test weight (g/10ml), protein content (%) and seed yield per plant (g).

- [1] *Per se* performance of genotypes for days to flowering (Range : 37.00 to 64.00, Mean : 54.88 days), leaf area per plant (Range : 505.39 cm² to 2634.63 cm², Mean : 1569.17 cm²), days to maturity (Range : 77.67 to 118.33, Mean : 107.44 days), length of inflorescence (Range : 39.60 cm to 74.33 cm, Mean : 60.90 cm), width of inflorescence (Range : 16.33 cm to 25.20 cm, Mean : 19.81 cm), plant height (Range : 105.20 cm to 177.60 cm, Mean : 150.22 cm), stem girth (Range : 2.60 cm to 5.69 cm, Mean : 4.05), harvest index (Range : 14.74 % to 27.86 %, Mean: 20.36 %), test weight (Range : 6.58 g/10 ml to 8.48 g/10 ml, Mean : 7.64 g/10 ml),

protein content (Range : 10.20 % to 15.92 %, Mean : 12.88 %) and seed yield per plant (Range : 9.73 g to 44.57 g, Mean : 24.26 g).

- [2] The analysis of variance revealed that mean sum of squares due to genotypes was found significant for all the character studied. This indicated the presence of adequate amount of variability in the experimental material and the genotypes under study were genetically diversified.
- [3] A broad range of phenotypic variability was recorded for seed yield per plant and its components traits. The values of phenotypic coefficient of variation (PCV) were slightly higher than that of genotypic coefficient of variation (GCV) for all the characters under studied, denoting less influence of environment on the expression of traits.
- [4] The GCV and PCV were measured highest for seed yield per plant (40.498 and 40.895) followed by leaf area per plant (39.59 and 40.15), harvest index (15.64 and 17.39) and lowest value in test weight (5.07 and 6.68), respectively. The results revealed that the magnitude of genetic variability was very close to phenotypic variability for all the characters. This indicates that phenotypic variability has larger due to genetic differences for different traits.
- [5] Heritability (bs) was recorded highest for seed yield per plant (98.06 %), leaf area per plant (97.20 %), days to maturity (87.86 %), harvest index (80.87 %), protein content (76.62 %), stem girth (76.00 %), length of inflorescence (72.54 %), days to flowering (72.37 %), plant height (71.21 %), respectively. This suggested that heritability may be due to higher contribution of the genotypic component in these characters. Heritability (bs) was recorded moderate for number of width of inflorescence (49.18 %) and test weight (57.69 %).
- [6] The genetic advance expressed as percentage of mean values were observed high for seed yield per plant (82.605 %) followed by leaf area per plant (80.405 %). Whereas, test weight showed lowest value (7.945 %) followed by width of inflorescence (9.79 %), days to maturity (11.95 %), days to flowering (14.44 %), plant height (14.47 %), protein content (18.63 %), stem girth (19.25 %), length of inflorescence (20.38 %) and harvest index (28.97 %), respectively.
- [7] The high heritability coupled with high genetic advance as percent of mean indicating additive gene action was observed for trait viz., leaf area per plant,

length of inflorescence, harvest index, seed yield per plant. Simple selection for such traits should be practiced for improving them. Which may be attributed to the prevalence of additive gene action which is fixable for governing the character and acquired high selective value and thus, selection pressure could profitably be applied on these traits for their judicious improvement by following different breeding methods viz., pedigree, bulk, single seed descent method, backcross and bi parental mating design along with advance breeding method to speed-up the improvement for yield and its contributing traits. The selection may be effective for improvement of the traits. High heritability exhibited that larger portion of variation for the character in the material was due to additive gene action.

- [8] Correlation studies revealed the importance of characters viz., leaf area per plant, length of inflorescence, width of inflorescence and harvest index for seed yield in grain amaranth.
- [9] In the present study, seed yield per plant showed highly significant and positive association with days to flowering ($r_g = 0.4094$), leaf area per plant ($r_g = 0.7676$), length of inflorescence ($r_g = 0.6043$), plant height ($r_g = 0.5034$) and harvest index ($r_g = 0.7822$) at genotypic level respectively. Which indicate the dependency of these characters with each other. While seed yield per plant showed positive association with leaf area per plant ($r_p = 0.7554$), length of inflorescence ($r_p = 0.5208$), width of inflorescence ($r_p = 0.3201$), harvest index ($r_p = 0.7114$) and plant height ($r_p = 0.4273$) at phenotypic level.
- [10] Path coefficient analysis indicated the positive direct effect on seed yield per plant by harvest index followed by leaf area per plant, width of inflorescence, stem girth, days to flowering, days to maturity, length of inflorescence and plant height. On the basis of path analysis, it can be concluded that improving seed yield per plant in grain amaranth due consideration should be harvest index, leaf area per plant, width of inflorescence and stem girth while making selection.
- [11] The path coefficient analysis revealed that the character harvest index (0.6077) recorded the highest positive direct effect on seed yield per plant followed by leaf area per plant (0.3544). The trait showed low positive direct effect on seed yield per plant by width of inflorescence (0.1490) and stem girth (0.1338). While, days to flowering (0.0579), days to maturity (0.0565), length of

inflorescence (0.0240) and plant height (0.0608) had negligible positive direct effect on seed yield per plant. On other hand, 1000-seed weight (-0.0123) and protein content showed (-0.0459) negligible negative effect on seed yield per plant. Thus, path analysis and correlation studies revealed that based on findings of the present investigation the most desirable ideotype of grain amaranth for seed yield should possess early to mature, a larger or broad leaf area per plant, harvest index, width of inflorescence are good indicator of seed yield per plant in grain amaranth.

- [12]** The DNA extraction protocol for Doyle and Doyle (1987) by CTAB method adopted in the present investigation was appropriate for good quality and quantity of genomic DNA of grain amaranth. Range of OD A_{260}/A_{280} ratios for forty-six genotypes were 1.70-1.91. Average DNA concentration was obtained as 797.70 (ng/ μ l). Total 7 ISSR primers were used for screening of forty-six grain amaranth genotypes. Amplified ISSR primers generated total number of 46 loci (bands). Out of 46, 40 polymorphic bands and six bands monomorphic were appeared. The average percent polymorphism detected by ISSR primers in the present investigation was 85.79. In present investigation PIC value (polymorphic information content) of different ISSR primers showed ranged from 0.456 to 0.641 with an average of 0.572. PCA plot deciphering all the genotype have been distributed in four groups while PCoA plot analysis indicating scattered distribution of all the genotype in all the direction around axis indicating wide genetic origin of all the genotypes.
- [13]** Molecular characterization of grain amaranth : (1) The ISSR marker system revealed high level of polymorphism among the grain amaranth genotypes, indicating its effectiveness for evaluating genetic diversity in grain amaranth. (2) The dendrogram, based on Jaccard's similarity co-efficient was able to classify all the genotypes of grain amaranth in six clusters. (3) Among the grain amaranth genotypes RGA-16 and RGA-17 evident maximum similarity; while the genotypes BGA-10 and BGA-4-9 shown minimum similarity on the basis of Jaccard's co-efficient result. On the basis of result found in PCA study. (1) RHGA-13-3, GA-3, BGA-7, BGA-9, SKNA- 403, KBGA-7 falls under same cluster which also showed similarity among different morphological characters liked days to flowering, inflorescence width, stem girth, test weight. (2) SKNA-808, RMA-62, MGA-502 falls under same

cluster which also showed similarity at morphological level for the characters like test weight, harvest index and stem girth. (3) Inflorescence width, inflorescence length, stem girth and test weight values were found similar for KBGA-5, RGA-13 which also falls under same cluster in PCA. (4) SKNA-813, SKNA-1407 falls under same cluster which also shows similarity among different morphological characters like leaf area, inflorescence length and inflorescence width. (5) BGA-10, SKNA-1207 similar for characters like days to maturity, inflorescence width, test weight, protein content and seed yield per plant which are found in one cluster in PCA as well as same cluster in dendrogram.

- [14]** In view of all the obtained data, it is evident that ISSR molecular markers are a good tool in assessing genetic variation and germplasm identification among the forty-six grain amaranth cultivars. These markers provide interesting tool for breeding new varieties in grain amaranth and crop improvement programmes.

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APPENDICES

Appendix-A : Weekly meteorological data recorded during the crop season (November-2018 to March-2019)

Month	Standard weeks	Temperature (°C)		Relative Humidity (%)		Wind velocity (km/h)	Bright Sunshine (hrs/day)	Rainfall (mm)	Rainy Day
		Maximum	Minimum	RH-I	RH-II				
November-2018	44	36.1	15.1	59.0	33.3	1.9	9.4	0.0	0
	45	34.9	13.6	58.3	33.2	1.8	8.5	0.0	
	46	33.3	13.3	59.6	31.6	2.5	9.6	0.0	
	47	32.7	12.3	53.2	40.7	2.4	9.5	0.0	
December-2018	48	30.7	10.2	51.2	39.0	2.9	6.9	0.0	0
	49	29.8	10.4	54.4	35.8	2.5	9.3	0.0	
	50	27.4	9.9	53.6	40.0	3.9	8.5	0.0	
	51	25.6	6.6	42.4	46.2	3.6	9.1	0.0	
	52	25.3	5.7	42.1	40.1	4.2	9.6	0.0	
January-2019	1	26.7	7.2	44	40	3.5	9.3	0.0	0
	2	27.1	7.4	40	35	3.7	9.3	0.0	
	3	29.1	8.9	45	26	2.5	9.4	0.0	
	4	24.0	6.9	54	48	5.8	9.4	0.0	
	5	23.8	9.3	60	41	7.3	8.9	0.0	
February-2019	6	24.9	6.6	55	46	3.7	9.2	0.0	0
	7	27.7	10.2	62	32	4.3	9.4	0.0	
	8	29.4	12.0	63	42	5.1	8.2	0.0	
	9	29.1	10.7	59	49	6.0	8.0	2.2	
March-2019	10	31.2	12.7	61	43	4.9	9.1	0.0	0
	11	32.4	13.1	62	40	6.3	9.2	0.0	
	12	34.3	14.2	66	34	4.5	9.5	0.0	
	13	39.5	18.1	66	26	4.2	9.6	0.0	
	14	41.4	20.1	64	27	4.8	9.5	0.0	

Source : Agricultural Meteorology Department, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Appendix-B : Mean values of different characters in grain amaranth

Genotypes	Days to flowering	Leaf area per Plant (cm ²)	Days to maturity	Length of inflorescence (cm)	Width of inflorescence (cm)	Plant height (cm)	Stem girth (cm)	Harvest index (%)	Test weight (g/10 ml)	Protein content (%)	Seed yield per Plant (g)
SKGPA-61	58.33	2634.63	115.33	74.33	23.73	170.87	4.40	23.65	8.23	12.41	43.13
SKGPA-73	51.67	1373.50	108.00	46.93	18.40	125.93	3.13	14.74	7.49	12.22	14.07
SKGPA-150	50.00	784.50	100.67	60.40	20.47	142.53	3.77	17.28	7.46	14.24	12.93
SKGPA-155	57.67	1303.13	100.67	59.93	19.33	143.00	4.11	16.11	7.55	10.29	13.20
SKNA-401	59.33	1857.40	108.67	73.13	21.27	158.13	4.08	25.52	7.60	13.70	41.23
SKNA-403	55.33	1898.10	104.00	67.67	19.80	155.20	3.91	18.30	7.31	13.47	21.53
SKNA-808	62.67	1848.00	106.33	53.67	25.20	144.20	3.82	17.37	7.40	11.34	20.80
SKNA-813	47.00	2491.47	112.33	69.20	20.73	124.07	3.99	20.71	7.63	12.49	29.53
SKNA-903	37.00	505.39	77.67	43.73	16.33	105.20	2.60	27.86	7.49	13.95	9.73
SKNA-908	58.00	2511.33	107.33	55.40	20.27	161.80	4.46	21.49	8.35	13.48	35.10
SKNA-1201	52.33	1285.13	109.00	63.13	21.80	163.87	3.91	18.69	7.68	10.46	21.20
SKNA-1207	52.67	1392.13	107.33	53.20	19.13	145.20	4.71	21.36	7.38	12.50	18.53
SKNA-1305	58.00	1370.47	117.33	66.60	20.13	160.07	4.21	19.68	7.61	15.71	18.20
SKNA-1313	56.67	1759.27	110.33	69.27	20.53	158.93	5.69	23.54	7.30	13.67	38.40
SKNA-1403	48.67	2342.27	112.00	60.00	20.00	144.80	3.75	21.86	8.33	10.36	26.53
SKNA-1406	53.33	1886.57	104.67	63.93	19.40	153.33	3.79	20.75	7.43	14.92	22.27
SKNA-1407	58.00	2314.77	110.67	68.53	21.73	177.60	4.51	25.85	8.20	15.92	44.57
SKNA-1414	48.67	1529.20	114.33	61.13	17.13	152.60	3.91	16.46	7.46	12.11	16.00
SKNA-1501	59.00	1351.67	104.00	60.07	19.60	139.07	3.63	17.85	8.38	14.59	15.67
SKNA-1502	60.00	1474.70	106.00	61.60	19.80	148.33	3.63	17.02	7.51	14.43	15.73
SKNA-1503	55.33	1875.10	99.67	60.73	19.13	149.60	4.16	18.85	8.10	11.84	23.07
SKNA-1508	48.67	634.07	101.33	47.73	20.20	132.53	3.72	19.67	8.48	12.01	22.20
SKNA-1510	58.00	1981.27	114.00	67.33	22.80	167.73	4.13	25.28	8.33	12.98	38.28
GA-2	64.00	2584.63	118.00	68.47	20.60	164.27	4.63	21.00	8.46	13.50	36.67
GA-3	55.67	2355.87	108.67	62.87	19.73	154.73	3.76	22.11	7.26	12.57	35.45
BGA-4-9	50.33	677.14	105.67	57.73	18.67	151.47	4.15	18.78	6.67	12.57	18.60
BGA-7	56.00	1670.53	100.67	61.20	20.00	150.93	4.14	27.29	8.38	14.04	37.75
BGA-9	56.67	568.93	101.67	67.60	19.87	149.27	4.09	18.12	7.39	10.20	15.33
BGA-10	55.00	1086.43	106.67	57.80	20.73	133.73	3.81	18.44	7.64	13.87	16.40
BGA-14	56.00	1675.17	118.33	62.27	19.27	130.60	3.69	17.48	7.56	12.53	19.13

Appendix-B Continue...

Appendix-B Continue...

Genotypes	Days to flowering	Leaf area per Plant (cm ²)	Days to maturity	Length of inflorescence (cm)	Width of inflorescence (cm)	Plant height (cm)	Stem girth (cm)	Harvest index (%)	Test weight (g/10 ml)	Protein content (%)	Seed yield per Plant (g)
BGA-20	55.67	2357.77	112.33	68.47	18.53	155.13	3.70	26.56	6.58	12.69	41.28
KGBA-4	55.00	2243.73	108.67	65.80	18.87	156.67	3.90	24.22	7.45	14.49	32.28
KGBA-5	53.67	911.77	102.00	58.53	18.40	148.87	4.27	17.42	7.33	12.72	18.93
KGBA-7	57.67	1134.50	112.00	64.13	19.40	170.40	3.77	17.71	7.38	12.50	15.40
RMA-62	49.33	837.80	110.00	55.67	19.73	151.07	4.15	17.86	7.36	13.80	16.33
RMA-63	60.33	1630.27	104.00	63.60	17.47	155.67	4.07	19.61	7.33	13.05	22.60
RGA-13	48.67	1383.93	109.00	57.40	18.60	157.07	4.67	19.63	7.59	14.83	24.13
RGA-14	59.33	685.97	118.00	62.60	18.47	156.07	4.11	20.61	7.32	11.80	17.40
RGA-16	54.00	1066.30	109.33	60.80	18.87	155.27	4.41	20.19	7.42	10.40	18.40
RGA-17	58.67	2348.63	104.33	63.33	19.07	156.27	3.91	25.09	7.51	12.57	37.72
RGA-18	51.33	655.10	109.33	61.67	19.93	161.93	4.14	17.09	7.41	10.99	15.67
RGAG-12-22	54.67	2270.13	111.00	59.20	19.67	152.67	4.00	17.92	7.98	13.96	19.07
RGAG-14-3	59.67	2089.33	106.33	67.87	19.67	141.60	3.81	24.75	8.22	13.05	39.07
RHGA-13-3	54.33	1285.60	102.00	62.80	20.33	142.47	4.17	18.95	7.54	13.04	21.87
MGA-502	58.67	1459.47	100.33	39.60	17.93	137.47	4.16	18.53	7.37	11.66	19.53
Suvarana	53.33	798.53	112.00	44.27	20.47	151.80	4.86	17.54	7.41	12.65	15.20
General mean	54.88	1569.17	107.44	60.90	19.81	150.22	4.05	20.36	7.64	12.88	24.26
Range	37.00-64.00	505.39-2,634.63	77.67-118.33	39.60-74.33	16.33-25.20	105.20-177.60	2.60-5.69	14.74-27.86	6.58-8.48	10.20-15.92	9.73-44.57
S. Em.±	2.28	86.04	2.02	3.55	1.12	6.50	0.20	1.26	0.27	0.60	1.12
C.D. at 5 %	4.53	170.93	4.01	7.06	2.22	12.90	0.40	2.51	0.54	1.20	2.23
C.V. %	5.09	6.72	2.30	7.15	6.92	5.29	6.05	7.60	4.35	5.73	5.68

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