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In situ-collection and Morphological Characterization of Chilli (*Capsicum annuum* L.) Genotypes

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A B S T R A C T

A study was conducted on exploration, collection and characterization of some endangered genotypes of chilli (*Capsicum annuum* L.). These genotypes/ landraces are collected from the hilly area of Suding, Muskuti and Kundeijharan village of Kalahandi district, Odisha which are the good genetic resources for future exploration. 6 endangered landraces are identified, collected and characterized with different parameters (as per IPGRI, 1995 guidelines) like conserver details, conservation needs, passport data of the cultivar, qualitative characters, quantitative characters etc. Cluster analysis was made using Ward's Minimum Variance Cluster Analysis using the SAS system for understanding the similarity and diversity among the studied genotypes with Root-Mean-Square Distance between Observations. From the analysis of the qualitative traits, it was found that clear division of the 6 landraces into three distinct clusters. In cluster 1, three genotypes CV1, CV2 and CV4 fall, in cluster 2, CV5 and CV6 and in cluster 3 comes CV3 indicating that genotype and CV3 is distinctly different from other 5 genotypes. And when studied the quantitative parameters, 6 landraces were clearly divided into three distinct clusters. In cluster 1, three genotypes CV2, CV3 and CV4 fall, in cluster 2, CV1 and CV6 and in cluster 3 comes CV5 indicating that genotype CV5 is distinctly different from other 5 genotypes. Landraces shown substantial differences in clustering pattern using both qualitative and quantitative characters giving opportunity to breeders intended to work with either of the characters.

Introduction

Capsicum is a genus of the highly diverse Solanaceae family with origins in South and Central America and thought to be domesticated around 7000 BC and spread throughout the world, including tropical, subtropical, and temperate regions

(Pickersgill, 1997). Hot pepper is widely used in local medicine. Pungent peppers cause strong salivation, aid digestion and are laxative. Capsaicin, the active ingredient, stimulates the mucous membrane of the mouth, stomach and bowels, causing strong peristalsis. Hot pepper has often been ascribed antibiotic properties. It is recommended for people suffering from amoeba infection and intestinal worms. The leaves are used as a dressing for wounds and sores.

The wild species of chilli are potentially important as genitors of valuable disease resistance genes even though most landraces have low productivity. Most farmers still save and use their own seed. In the future, many landraces and local cultivars are likely to disappear as growers will switch to improved cultivars, and hence there is a need for collecting the traditional capsicum material and conserving it in gene banks and other analyses. The characterization and the evaluation of the Capsicum domesticated species is important, since a wide variability, not yet fully known and exploited, is available in these species (Sudré *et al.,* 2006; Ince *et al.,* 2010). Landraces of chilli in tribal dominated area are being conserved by women in their own homestead or garden due to their liking for specific local types and a potential vitamin provider along with as an appetizer for taking with deficient diets. Perennial type of chilli has more preference for them due to less maintenance and less frequent multiplication. Countless potentially very useful local forms exist within these tribal areas. Tribal farming in the current scenario is largely influenced by their growing non-farming type of livelihood due to ecological degradation, high migration, mining or extremist activities, displacement for industrialisation and energy projects. These cultivars were suitable for cultivation in the respective areas and fulfilled the needs of farmers. However, with the release and adoption of new improved varieties, the cultivation of the landraces are either lost or confined to certain small pockets. In this context conservation of local land races is not given priority. Hence ex-situ conservation is essential. A growing concern is being raised about the rapid loss of the conventional farmers' varieties or land races due to lack of their conservation practices and are very prone to climate change. So, ex-situ conservation practice may not give a good result. Therefore a combination of in-situ and ex situ conservation has a great impact which can be used as a method to conserve the degrading conventional varieties of the tribal. Each pepper variety/group/type has specific qualities unique to that variety/group/type e.g. colour, aroma, pungency, size, thickness of flesh etc.

So, thorough characterization and evaluation of all the available regional landrace material is imperative so as to ensure the propagation of commercially worthwhile material, while still conserving the available diversity. This study focuses on collection of different chilli genotypes from remote areas of Kalahandi district which is 80 km away from district headquarter having very poor transport system.

Materials and Methods

An erroneous identification of species can lead to losses ranging from the propagation and inadequate conservation of accessions to the use of misidentified genetic material in breeding, resulting in a waste of time and financial resources.

Capsicum species are traditionally identified by morphological descriptors or related traits. Flower morphology, including flower colour, calyx constriction and the number of flowers per axil, is most used in taxonomic descriptions. The descriptors are considered essential for a more accurate germplasm

characterization, such as the ones indicated by the IPGRI (1995). And these are documented by following steps including

Conserver details

Conserver details of the land races including the type of the farm families i.e. tribal or non-tribal or migrated and conserved by whom i.e. by the husband or wife or both are surveyed along with their place and type of conservation. It is also documented that from how long they are conserving the land races and from whom they have learnt the conservation practices and whether they will continue to conserve the land races for future or not.

Conservation needs

It is based on the different types of varietal preference, seed storage container for long term storage and different preventive measures taken for conservation of that land races.

Passport data of the cultivar

These provide the basic information used for the general management of the accession (including the registration at the gene bank and other identification information) and describe parameters that should be observed when the accession is originally collected.

Qualitative characters

It is based on life cycle of plant along with different plant, stem, leaf, flower and fruit parameters etc.

Quantitative characters

It is based on different quantitative parameters of plant, stem, leaf, flower and fruit and are expressed with an unit

Results and Discussion

Conserver details

6 land races of chilli (*Capsicum annum* L.) have been collected from different villages of Kalahandi District of Odisha. All most all the conservers are tribal who are continuing the conservation of these land races (Table 1).

Among these 2 land races are conserved only by the women and other 4 are by both farmer and farmwomen. They have self-saved seed and also some are collected from their neighbour. 2 land races are cultivated at Kitchen garden and other 2 land races each are cultivated at backyard and field. All 6 landraces are conserved in-situ since some years and they will continue to conserve. Two of the conserver have learnt the conservation practices from their ancestors while 3 of them from community where they are residing and rest 1 is from their in-laws and all the conservers want to continue the conservation of all these landraces.

Conserver needs

The conservers conserve the landraces due to different needs like specific varietal preference, seed storage practices and conservation measures. Among 6 landraces, two landraces are preferred due to its widely adoption, pungency, good storage capability and suitability for consumption whereas, other two land races are preferred for its pungency and suitable for consumption (Table 2).

From rest two landraces, one is preferred for widely adoption, pungency and suitable for consumption and another one is preferred for widely adoption and suitable for consumption. When we consider different storage structures, the conservers conserve 2 landraces in mud bin, 2 land races in wooden bin, 4 landraces in poly bags and 2 landraces in other storage

structures. When conservation measures were taken in to consideration, 4 landraces are conserved by series cultivation and proper storage methods while 2 landraces are conserved by only series cultivation.

Passport data

Passport data are collected for all the 6 landraces. Different data like cultivar name, breeding method, acquisition date, type of material, collecting source, status of sample, number of plant sampled, general appearance of population, population state, cropping system, plant population density, population isolation, genetic erosion, relative competition with associated flora and use of the accession etc. (Table 3). Among 6 landraces 2 are propagated by mass selection method whereas other 4 are by single plant maintenance. We have collected the sample from the source i.e. 1 in wild habitat, 2 are from farm land and other 3 are backyard. Three are of wild type and 3 are of landraces. The appearance of the population is very poor to medium and is of primitive cultivated material and some are in spontaneous state. Almost all landraces are grown in monoculture cropping system having very low to intermediate population density and the all the plant population are grown in isolation because there is a very sparse population of these land races are almost all location of our collection is at the foot of the hill, so barrier isolation as well as space isolation is prevailing among all the landraces. So relative competition with associated flora is very less and somewhere it is intermediate.

The genetic erosion of those landraces is very rapid somewhere intermediate, so it is a great concern to conserve the landraces for future use as these are the very good genetic material for better hybridisation programme. All most all the landraces are used as vegetable or spice purposes by the cultivator rather conserver.

Qualitative characters

Different quality parameters are recorded in-situ such as plant, leaf, stem, flower and fruit characteristics (Table 4). When plant parameters are evaluated we found that 1 landrace is annual, 2 are biennial and 3 are of perennial life cycle. All the 6 landraces have same stem colour i.e. green and same lamina margin i.e. entire. Besides these, nodal anthocyanin, stem shape, stem pubescence, plant growth habit, branching habit, tillering, leaf density, leaf colour, leaf shape and leaf pubescence data of all landraces are recorded (Table 4). It was also found that corolla colour was white, corolla spot colour was white and male sterility was absent in all 6 landraces when flower parameters were evaluated.

Besides, sufficient differences in flower position, corolla colour, anther colour, filament colour, stigma exertion, calyx pigmentation, calyx annular constriction, anthocyanin spot/ stripes was exhibited. When fruit characteristics were evaluated it was found that fruit blossom appendages was absent in all 6 landraces whereas, different types of characters found in different fruit parameters like fruit colour at intermediate stage, fruit set, fruit colour at mature stage, fruit shape, fruit shape at pedicel attachment, neck at base of the fruit, fruit shape at blossom end, fruit cross-sectional corrugation, fruit surface, pedicel with fruit, pedicel with stem etc. exhibited diversity among all 6 genotypes.

Cluster analysis among 6 landraces using qualitative characters

Cluster analysis was made using Ward's Minimum Variance Cluster Analysis using the SAS system for understanding the similarity and diversity among the studied genotypes with Root-Mean-Square Distance between Observations (Fig. 1 and 2).

Table.1 Conserver details

			1	2	3	4	5	6	7	8
Sl. No.	Name	Address	Type	Conserved by	Seeds collected from	Where cultivating	Conservation	Conserved since	Learned from	Continue to conserve
C1	KailashSahoo	At- Suding, PO- Takarla Dist- Kalahandi, Odisha	1	2	3	3	1	12	2	1
C2	Postak Ch. Bhoi	At- Kundeijharan PO- Takarla Dist- Kalahandi, Odisha	1	12	2	1	1	12	1	1
C3	Deepak Tripathy	At- Muskuti Po- Muskuti Dist- Kalahandi, Odisha	1	12	3	3	1	12	1	1
C4	KapilaChhatar	At- Kundeijharan PO- Takarla Dist- Kalahandi, Odisha	1	12	2	2	1	12	1	1
C5	SarbePatra	At- Suding PO- Takarla Dist- Kalahandi, Odisha	1	2	3	1	1	12	3	1
C6	Jayant Dang	At- Suding, PO- Takarla Dist- Kalahandi, Odisha	1	12	3	2	1	12	2	1

1. Type		**2. Conserved by**		**3. Seeds collected from**		**4. Where cultivating**		**5.Conservation**		**6. Conserved since**		**7. Learned from**		**8. Continue to Conserve**	
Tribal	1	Husband	1	Seed store	1	Kitchen garden	1	In-situ	1	Recent	1	Community	1	Yes	1
Non-tribal	2	Wife	2	Neighbour	2	Backyard	2	Ex-situ	2	Long time	2	Ancestors	2	No	2
Migrated	12	Both	12	Self-saved	3	Field	3	Both	12	Some years	12	In-laws	3	Not decided	12

Table.2 Conservation needs

	Varietal preference						Seed Storage practices						Conservation measures				
	1	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**
C1	√	×	√	×	×	√	×	×	×	√	√	×	√	×	×	×	×
C2	×	√	√	×	√	√	×	×	√	×	×	√	√	×	√	×	×
C3	×	√	√	×	×	√	×	×	×	×	√	×	√	×	√	×	×
C4	×	√	√	×	√	√	×	×	×	×	√	×	√	×	×	×	×
C5	×	√	×	×	×	√	×	×	√	×	√	×	√	×	√	×	×
C6	√	×	√	×	×	√	×	×	×	√	×	√	√	×	√	×	×

Varietal Preference		**Storage of seeds in**		**Measures taken for conservation**	
Preference	Code	Container	Code	Practices	Code
Easy to cultivate	**1**	Cloth bag	**1**	Series Cultivation	**1**
Widely adopted	**2**	Tin box	**2**	Mass Multiplication	**2**
Pungent	**3**	Mud bin	**3**	Proper Storage	**3**
Marketability	**4**	Wooden bin	**4**	Community cultivation	**4**
Storage	**5**	Poly bag	**5**	Seed bank	**5**
Suitable for eating	**6**	Other	**6**		

Table.3 Passport data

Respondent	Cultivar Name	Breeding method	Acquisition date	Type of material received	Collecting source	Status of sample	Number of Plant Sampled	General appearance of population	Population state	Cropping system	Plant population density	Population isolation	Genetic erosion	Relative competition with associated flora	Use of the accession
Kailash Sahoo	Sudingmircha	Mass selection	27.9.15	fruit	Farm land	Landrace	4	Medium	Primitive cultivated material	monoculture	Intermediate		Intermediate	Intermediate	Vegetable
Postak Ch. Bhoi	Suryamukhi	Single plant maintenance	28.9.16	fruit	Backyard	Wild	1	Poor	Spontaneous	monoculture	Low		Rapid	Low	Spice & vegetable
Deepak Tripathy		Mass selection	28.9.16	fruit	Farm land	Landrace	4	Medium	Primitive cultivated material	monoculture	Intermeediate		Intermediate	Low	Spice & vegetable
KapilaChhatar		Single plant maintenance	28.9.16	fruit	Wild habitat	Wild	1	Poor	Spontaneous	monoculture	Low		Rapid	Low	Spice & vegetable
SarbePatra		Single plant maintenance	27.9.16	fruit	Backyard	Wild	1	Poor	Spontaneous	monoculture	Low		Rapid	Low	Spice & vegetable
Jayant Dang		Single plant maintenance	27.9.16	fruit	Backyard	Landrace	2	medium	Primitive cultivated material	monoculture	Intermediate		Intermediate	Low	Spice & vegetable

Table.4 Qualitative characters

Sl. No.	Descriptors	CV1	CV2	CV3	CV4	CV5	CV6
Plant parameters							
1	Life cycle	2 Biennial	3 Perennial	1 Annual	2 Biennial	3 Perennial	3 Perennial
2	Stem colour	1 Green	1 Green	1 Green	1 Green	1 Green	1 Green
3	Nodal anthocyanin	2 Light Purple	2 Light Purple	2 Light Purple	2 Light Purple	1 Green	1 Green
4	Stem shape	2 Angled	1Cyllindrical	2 Angled	1 Cyllindrical	3 Flattened	3 Flattened
5	Stem pubescence	3 Dense	3 Dense	1 Sparse	1 Sparse	3 Dense	2 Intermediate
6	Plant growth habit	3 Erect	3 Erect	2 Intermediate	1 Prostrate	1 Prostrate	3 Erect
7	Branching habit	3 Dense	3 Dense	1 Sparse	3 Dense	3 Dense	2 Intermediate
8	Tillering	1 Sparse	1 Sparse	2 Intermediate	1 Sparse	1 Sparse	2 Intermediate
9	Leaf density	3 Dense	2 Intermediate	2 Intermediate	3 Dense	2 Intermediate	2 Intermediate
10	Leaf colour	4Dark Green	4Dark Green	3 Green	3Green	2Light Green	4Dark Green
11	Leaf shape	3 Lanceolate	1 Deltoid	3 Lanceolate	1 Deltoid	1 Deltoid	3 Lanceolate
12	Lamina margin	1 Entire	1 Entire	1 Entire	1 Entire	1 Entire	1 Entire
13	Leaf pubescence	1 Sparse	1 Sparse	1 Sparse	1 Sparse	2 Intermediate	1 Sparse
Flower Parameters							
1	Flower position	1 Pendant	3 Erect	1 Pendant	3 Erect	1 Pendant	1 Pendant
2	Corolla colour	1 White	1 White	1 White	1 White	1 White	1 White
3	Corolla spot colour	1 White	1 White	1 White	1 White	1 White	1 White
4	Corolla shape	1 Rotate	1 Rotate	1 Rotate	2 Campanulate	1 Rotate	1 Rotate
5	Anther colour	3 Pale Blue	3 Pale Blue	3 Pale Blue	2 Yellow	2 Yellow	2 Yellow
6	Filament colour	1 White	1 White	1 White	5 Purple	1 White	1 White
7	Stigma exertion	3 Exserted	1 Inserted	1 Inserted	1 Inserted	2 Same level	2 Same level
8	Male sterility	0 Absent	0 Absent	0 Absent	0 Absent	0 Absent	0 Absent
9	Calyx pigmentation	0 Absent	1 Present	0 Absent	1 Present	0 Absent	0 Absent
10	Calyx margin	3 Dentate	2 Intermediate	3 Dentate	1 Entire	1 Entire	1 Entire
11	Calyx annular constriction	0 Absent	0 Absent	1 Present	0 Absent	0 Absent	0 Absent
12	Anthocyanin spot/ stripes	0 Absent	0 Absent	0 Absent	1 Present	0 Absent	0 Absent

Fruit Parameters							
1	Fruit colour at intermediate stage	3 Green	5 Purple	3 Green	5 Purple	5 Purple	1 White
2	Fruit set	3 High	3 High	3 High	3 High	1 Low	2 Intermediate
3	Fruit colour at mature stage	7 Light Red	8 Red	3 Pale Orange-yellow	4 Orange-yellow	3 Pale Orange-yellow	8 Red
4	Fruit shape	1 Elongate	1 Elongate	4Campanulate	1 Elongate	1 Elongate	2 Almost Round
5	Fruit shape at pedicel attachment	2 Obtuse	2 Obtuse	4 Cordate	3 Truncate	1 Acute	4 Cordate
6	Neck at base fruit	0 Absent	1 Present	0 Absent	0 Absent	0 Absent	1 Present
7	Fruit shape at blossom end	1 Pointed	1 Pointed	1 Pointed	1 Pointed	1 Pointed	2 Blunt
8	Fruit blossom end appendage	0 Absent	0 Absent	0 Absent	0 Absent	0 Absent	0 Absent
9	Fruit cross-sectional corrugation	1 Slightly Corrugated	2 Intermediate	2 Intermediate	1 Slightly Corrugated	1 Slightly Corrugated	2 Intermediate
10	Fruit surface	2 Semi wrinkled	2 Semi wrinkled	1 Smooth	2 Semi wrinkled	1 Smooth	1 Smooth
11	Pedicel with fruit	1 Slight	1 Slight	1 Slight	1 Slight	1 Slight	2 Intermediate
12	Pedicel with stem	1 Slight	1 Slight	2 Intermediate	2 Intermediate	2 Intermediate	2 Intermediate

Table.5 The CLUSTER Procedure (Ward's Minimum Variance Cluster Analysis) using the SAS System for qualitative characters

Cluster History									
No. of Clusters	Clusters Joined		Freq	Semi partial R-Square	R-Square	Pseudo F Statistic	Pseudo t-Squared	Tie	Root-Mean-Square Distance Between Observations
5	V1	V2	2	0.0865	.913	2.6	.		0.552825
4	V5	V6	2	0.1636	.750	2.0	.		
3	CL5	V4	3	0.1719	.578	2.1	2.0		
2	V3	CL4	3	0.2291	.349	2.1	1.4		
1	CL3	CL2	6	0.3489	.000	.	2.1		

Table.6 Quantitative characters

	PH	PCW	SL	SD	MLL	MLW	NFPA	CL	AL	FL	FW	FWt	FPL	NL
CV1	>85	27.6	17.2	1.3	4.2	1.6	3	<1.5	0.3	5.2	1.2	1.4	2.1	6
CV2	>85	17.8	8.3	0.5	7.8	4.2	2	<1.5	0.3	7.2	1.2	1.3	2.9	5
CV3	46-65	22.1	14.2	1.2	7.6	3.4	2	<1.5	0.3	6.9	1.3	3.2	3.6	5
CV4	46-65	21.4	11	0.9	8.2	4.4	4	<1.5	0.2	5.3	0.8	1.3	3.4	5
CV5	46-65	42	6.4	0.9	3.7	2	2	<1.5	0.2	2.5	0.8	0.76	1.8	4
CV6	46-65	31.3	19.2	1.1	7.4	3.2	2	<1.5	0.2	1.2	2.1	2.4	1.8	7

Plant height (in cm)	**PH**	**Mature leaf width (in cm)**	**MLW**	**Fruit length (in cm)**	**FL**
Plant canopy width (in cm)	**PCW**	Number of flower per axil	**NFPA**	Fruit width (in cm)	**FW**
Stem length (in cm)	**SL**	Corolla length (in cm)	**CL**	Fruit weight (in g)	**FWt**
Stem diameter (in cm)	**SD**	Anther length (in cm)	**AL**	Fruit pedicel length (in cm)	**FPL**
Mature leaf length (in cm)	**MLL**			Number of locules	**NL**

Table.7 The CLUSTER procedure (Ward's minimum variance cluster analysis) using the SAS System for quantitative characters

Cluster History								
Number of Clusters	Clusters Joined		Freq	Semi partial R-Square	R-Square	Pseudo F Statistic	Pseudo t-Squared	Tie	Root-Mean-Square Distance Between Observations
5	V3	V4	2	0.0192	.981	12.8	.		15.35176
4	V1	V6	2	0.0428	.938	10.1	.		
3	V2	CL5	3	0.0475	.890	12.2	2.5		
2	CL4	CL3	5	0.3148	.576	5.4	8.6		
1	CL2	V5	6	0.5757	.000	.	5.4		

Fig.1

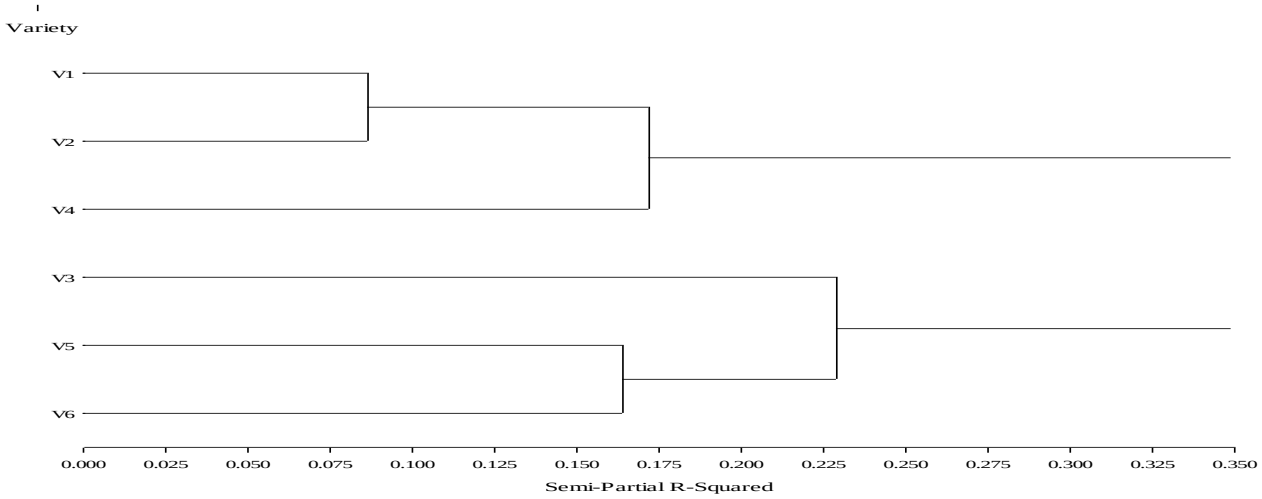
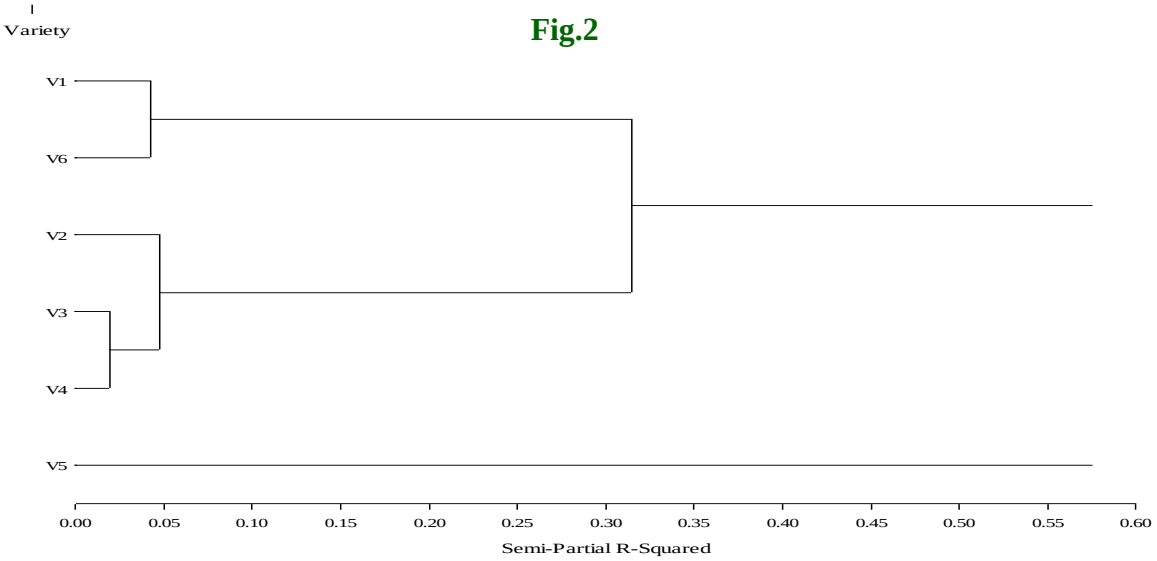


Fig.2



From Table-5, it is observed that it could clearly divide the 6 genotypes into three distinct clusters. In cluster 1, three genotypes V1, V2 and V4 fall, in cluster 2, V5 and V6 and in cluster 3 comes V3 indicating that genotype V3 is distinctly different from other 5 genotypes. Genotypes V1 and V2 are genetically close enough so also V5 and V6.

However sufficient diversity was noticed among the genotypes indicating its importance as useful genetic material.

Quantitative characters

Different quantitative characters like plant height (in cm), plant canopy width (in cm), stem length (in cm), stem diameter (in cm), mature leaf length (in cm), mature leaf width (in cm), number of flower per axil, corolla length (in cm), anther length (in cm), fruit length (in cm), fruit width (in cm), fruit weight (in g), fruit pedicel length (in cm), number of locules were measured and documented (Table-6). Highest plant height was found in 2 landraces i.e. more than 85cm followed by

other 3 i.e. in between 46 to 65 cm. Plant canopy width found highest in CV5 i.e. 42cm followed by CV6 i.e. 31.3cm and lowest in CV2 i.e. 17.8cm. The stem length was highest in CV6 i.e. 19.2cm followed by CV1 i.e. 17.2cm. Highest stem diameter found in CV1 i.e. 1.3cm followed by CV6 i.e. 1.1 cm. and lowest in CV2 i.e. 0.5cm. Highest length of mature leaf was found in CV4 i.e. 8.2cm followed by CV2 and CV3 i.e. 7.8 and 7.6cm respectively. Mature leaf width found highest in CV4 i.e 4.4cm followed by CV2 i.e. 4.2cm. Number of flower per axil was highest in CV4 i.e. 4 and lowest in CV2, CV3, CV5 and CV6 i.e. 2. Corolla length was same for all i.e. less than 1.5cm. Anther length was same for CV1, CV2, CV3 i.e. 0.3cm or 3mm and 0.2cm or 2 mm in CV4, CV5 and CV6. Fruit length found highest in CV2 i.e. 7.2cm followed by CV3 i.e. 6.9cm and lowest in CV6 i.e. 1.2cm. Fruit width found highest in CV4 and CV5 i.e. 0.8cm and fruit weight found highest in CV3 i.e. 3.2g. Fruit pedicel length found highest in CV3 i.e. 3.6cm followed by CV4 i.e. 3.4cm. Number of locules found highest in CV6 i.e. 7 followed by CV1 i.e. 6.

Cluster analysis among 6 landraces using quantitative characters

Cluster analysis was made using Ward's Minimum Variance Cluster Analysis using the SAS system for understanding the similarity and diversity among the studied genotypes using quantitative characters with Root-Mean-Square Distance between Observations (Table 7). This could clearly divide the 6 genotypes into three distinct clusters. In cluster 1, three genotypes V2, V3 and V4 fall, in cluster 2, V1 and V6 and in cluster 3 comes V5 indicating that genotype V5 is distinctly different from other 5 genotypes. Genotypes V1 and V6 are genetically close enough so also V3 and V4. However sufficient diversity was noticed among the genotypes indicating its importance as useful genetic material.

Genotypes shown substantial differences in clustering pattern using both qualitative and quantitative characters giving opportunity to breeders intended to work with either of the characters. However for characterizing genotypes, both these characters are of equal importance.

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