# Managing and Enhancing the Use of Germplasm – Strategies and Methodologies





International Crops Research Institute for the Semi-Arid Tropics



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Hari D Upadhyaya and CL Laxmipathi Gowda



**AT** International Crops Research Institute for the Semi-Arid Tropics

Patancheru 502 324, Andhra Pradesh, India 2009

# **Characterization and Preliminary Evaluation**

Characterization and preliminary evaluation of germplasm are the prerequisites for utilization in crop improvement.

## **10A.** Phenotypic characterization and evaluation

- Characterization involves recording characters, which are
  - highly heritable,
  - easily seen by the eye, and
  - > are expressed in all environments.
- Preliminary evaluation consists of recording a limited number of additional agronomic traits considered to be desirable by users of the crop.

Follow the same sowing and cultural practices for the field grow-out, as described under regeneration (see section 9). Grow the accessions in 1-3 rows of 4 m each. Maintain the row to row distance at 60 cm (chickpea) or 75 cm (other crops) and plant-to-plant distance at 10 cm (50 cm in pigeonpea). Evaluate the accessions in an augmented block design. Plant standard check cultivars at every 10 or 20 accessions. Use the descriptors developed by ICRISAT and IBPGR (now Bioversity International) for characterization and preliminary evaluation (ICRISAT/IBPGR 1992a,b and 1993a,b; ICRISAT/IBPGR/ICARDA 1993).

### **10A.1.** Descriptors for characterization of sorghum

#### Vegetative phase

<u>Plant height (cm)</u>: Height of the main axis from ground to the top of inflorescence at 50% flowering. Mean of 5 randomly selected plants (Fig. 10A.1.1).



Figure 10A.1.1. Plant height in sorghum.

Plant pigmentation: Stem and plant pigmentation at maturity.

- P Pigmented
- T Tan

Basal tillers number: Number of basal tillers, main plant as 1.

Nodal tillers number: Presence or absence of nodal tillers.

- P Present
- A Absent

Midrib color: Color of the midrib.

- W White
- D Dull green
- Y Yellow
- B Brown

## **Reproductive phase**

<u>Days to flowering</u>: Number of days from the day of first irrigation to the date when 50% of plants started flowering within an accession.

<u>Panicle exsertion</u>: Length of peduncle from ligule flag leaf to base of inflorescence (Fig. 10A.1.2).



Figure 10A.1.2. Panicle exsertion in sorghum.

- 1 Slightly exserted
- 2 Exserted
- 3 Well-exserted
- 4 Peduncle recurved

Panicle length (cm): Length of panicle from base to the tip. Mean from five representative plants.

Panicle width (cm): In natural position at the widest portion. Mean from five representative plants.

Panicle compactness and shape (Fig. 10A.1.3 and 1.4)



Figure 10A.1.3 Inflorescence compactness and shape in sorghum.

- VLSB Very loose stiff branches
- VLDB Very loose drooping branches
- LSB Loose stiff branches
- LDB Loose drooping branches

- SLSB Semi-loose stiff branches
- SLDB Semi-loose drooping branches
- SCE Semi-compact elliptic
- CE Compact elliptic
- CO Compact oval
- SCO Semi-compact oval



Figure 10A.1.4. Diversity for panicle traits in sorghum germplasm assembled at ICRISAT.

<u>Glume color</u>: Color of the seed covering structures.

W	White	R	Red
S	Straw	DR	Dark red
Υ	Yellow	Р	Purple
LB	Light brown	В	Black
В	Brown	G	Grey
RB	Reddish brown	PSB	Partly straw and brown
LR	Light red	PSP	Partly straw and purple

<u>Glume covering</u>: Extent of seed covered by glumes at maturity (Fig. 10A.1.5).

- 1 25% seed covered
- 2 50% seed covered

- 3 75% seed covered
- 4 Seed fully covered
- 5 Glumes longer than seed



Figure 10A.1.5. Seed covering in sorghum.

Seed color: Color of freshly harvested seeds.

CW	Chalky white	RB	Reddish brown
W	White	LR	Light red
S	Straw	R	Red
Y	Yellow	G	Grey
LB	Light brown	Ρ	Purple
В	Brown	WR	White and red mixed

Seed lustre: Shininess of seed.

- L Lustrous
- NL Nonlustrous

<u>Seed sub-coat</u>: Presence or absence of black layer below the testa.

- P Present
- A Absent

Seed size (mm): Width of the seed at the broadest point.

Seed weight (g): Weight of 100 seeds at 12% moisture content.

Endosperm texture: Nature of endosperm (Fig. 10A.1.6).

- 1 Completely corneous
- 2 Almost corneous
- 3 Partly corneous
- 4 Almost starchy
- 5 Completely starchy



Figure 10A.1.6. Endosperm texture in sorghum seed.

<u>Threshability</u>: Ease with which seeds can be separated from the panicle.

- FT Freely threshable
- PT Partly threshable
- DT Difficult to thresh

### **10A.2.** Descriptors for characterization of pearl millet

#### **Vegetative phase**

<u>Plant height</u> (cm): Mean height of five plants measured from ground level to the tip of the panicle at dough stage (Fig.10A.2.1).

<u>Productive tillers number</u>: Number of tillers bearing panicles, counted at dough stage. Recorded as mean of five plants.

<u>Nodal tillers</u>: Visual score on 1–9 scale for number of nodal tillers at dough stage.

- 3 Few
- 5 Intermediate
- 7 Many

<u>Total tillers number</u>: Total number of tillers including main stem, counted at dough stage. Recorded as mean of five plants.

<u>Photoperiod sensitivity</u>: Visual score on 1–9 scale for sensitivity to photoperiod.

- 3 Insensitive
- 5 Partly sensitive
- 7 Highly sensitive



Figure 10A.2.1. Recording plant height in pearl millet germplasm.

<u>Fodder yield potential</u>: Visual score on 1-9 scale for green fodder yield potential considering tillering, leafiness and bulk at flowering.

- 3 Poor
- 5 Intermediate
- 7 Good

#### **Reproductive phase**

<u>Days to 50% flowering</u>: Number of days from first irrigation after sowing to when 50% of plants flower in the accession. Stigma emergence on the main panicle is considered as flowering.

Panicle exsertion (cm): Distance between ligule of the flag leaf and the base of the panicle on main plant.

<u>Panicle length</u> (cm): Mean length of five panicles on main axis of five representative plants, measured at dough stage.

<u>Panicle thickness</u> (mm): Mean thickness of five panicles at widest portion on main tiller of five plants, measured at dough stage.

Panicle shape: Shape of panicle at dough stage (Fig. 10A.2.2 and 3).



Figure 10A.2.2. Panicle shapes in pearl millet.



Figure 10A.2.3 Diversity for panicle traits in pearl millet germplasm.

<u>Spikelet density</u>: Density of spikelets, visually scored on 1–9 scale at maturity. Also referred to as compactness of panicle.

- 3 Loose
- 5 Intermediate
- 7 Compact

Synchrony of panicle maturity: Uniformity for maturity, visually scored on 1–9 scale at dough stage.

- 3 Non-synchronous
- 5 Intermediate
- 7 Synchronous

Bristle length: Length of bristles, visually scored on 1–9 scale at dough stage.

- 3 Short (bristles below the level of apex of the seed)
- 5 Medium (bristle length between 0 and 2 cm above the seed)
- 7 Long (bristles longer than 2 cm above the seed)

Seed color: Color of freshly harvested seeds recorded after threshing.

1	lvory	6	Grey brown
2	Cream	7	Brown
3	Yellow	8	Purple
4	Grey	9	Purplish black
5	Deep grey	10	Mixture of white and grey

<u>Seed weight (g)</u>: Weight of 1,000 seeds drawn randomly from plot yield, at 12% moisture content.

<u>Seed shape</u>: Shape of seed after drying (Fig. 10A.2.4).

<u>Seed yield potential</u>: Seed yield potential of the accession, visually scored on 1 - 9 scale considering panicle number, size and density.

- 3 Low
- 5 Intermediate
- 7 High

Endosperm texture: Texture of endosperm visually scored on 1–9 scale.

- 3 Mostly corneous
- 5 Partly corneous
- 7 Mostly starchy



Figure. 10A.2.4. Seed shapes in pearl millet.

# 10A.3. Descriptors for characterization of chickpea

### **Vegetative phase**

Growth habit: Angle of primary branches, recorded at mid-pod filling stage (Fig. 10A.3.1).

- E Erect; 0–15° from vertical
- SE Semi-erect; 16–25° from vertical
- SS Semi-spreading; 26–60° from vertical
- S Spreading; 61–80° from vertical
- P Prostrate, branches flat on the ground



Figure 10A.3.1. Growth habit in chickpea.

<u>Plant height</u> (cm): Mean canopy height of five representative plants, measured from soil surface at the end of flowering.

<u>Plant width</u> (cm): Mean canopy spread of five representative plants, measured at the time of flower ending.

Plant pigmentation: Presence of anthocyanin pigment in plant parts.

- NA No anthocyanin
- LA Low anthocyanin
- HA High anthocyanin

<u>Basal primary branches number</u>: Branches emerging from the axils on the lower half of the main stem, average of five representative plants from each accession at the time of harvest.

<u>Apical primary branches number</u>: Number of branches emerging from the leaf axils on the upper half of the main stem, average of 5 representative plants from each accession at the time of harvest.

<u>Basal secondary branches number</u>: Number of branches emerging from the leaf axils of basal primary branches, average of 5 representative plants from each accession at the time of harvest.

<u>Apical secondary branches number</u>: Number of branches emerging from the leaf axils of apical primary branches, average of 5 representative plants from each accession at the time of harvest.

<u>Tertiary branches number</u>: Number of branches emerging from the leaf axils of basal and apical secondary branches, average of 3–5 representative plants from each accession at the time of harvest.

#### Reproductive phase

<u>Days to 50% flowering</u>: Number of days from sowing (first irrigation) to the stage when 50% of plants have begun to flower in an accession.

<u>Flowering duration</u>: Number of days from 50% flowering to the date when 50% of the plants of an accession stops flowering.

Flower color: Color of standard petal.

В	Blue	VLP	Very light pink
LB	Light blue	W	White
DP	Dark pink	WBS	White with blue streaks
Ρ	Pink	WPS	White with pink streaks
LP	Light pink		

<u>Days to maturity</u>: Number of days from sowing (first irrigation) to the stage when 90% of pods have matured and turned yellow in an accession.

<u>Pods per plant</u>: Average number of fully formed pods per plant from 5 representative plants at maturity.

<u>Seeds per pod</u>: Number of seeds per pod estimated by dividing the total number of seeds by the total number of pods harvested from 5 representative plants.

Seed color: Color of mature seeds stored not longer than 5 months.

BL	Black	YB	Yellow brown
В	Brown	OY	Orange yellow
LB	Light brown	0	Orange
DB	Dark brown	YE	Yellow beige
RB	Reddish brown	1	Ivory
GB	Greyish brown	G	Green
SB	Salmon brown	LG	Light green
OB	Orange brown	BR	Brown reddish
GR	Grey	Μ	Variegated
BB	Brown beige	BM	Black brown mosaic
Υ	Yellow	LO	Light orange
LY	Light yellow		

Dots on seed coat: Presence or absence of minute black dots on the seed coat.

- A Absent
- P Present

Seed shape: Shape of mature seeds (Fig. 10A.3.2).

- ANG Angular, ram's head
- OWL Irregular round, owl's head
- PEA Pea-shaped, smooth round



Figure 10A.3.2. Seed shape in chickpea.

Seed surface: Seed surface observed from dry mature seed (Fig. 10A.3.3).

- R Rough wrinkled with uneven surface.
- T Tuberculated sticky because of tiny projections.
- S Smooth.

Seed weight (g): Weight of 100 seeds at 10% moisture content.

<u>Seed yield</u> (kg ha<sup>-1</sup>): Seed yield from all the plants of the plot. Plant stand is also counted. If the plant stand is at least 60% of the optimum number, then plot yield is converted to seed yield in kg ha<sup>-1</sup>.



Figure 10A.3.3. Testa texture in chickpea.

<u>Protein content</u> (%): The percentage of crude protein in the freshly harvested seeds, estimated using dye-binding method or automatic protein analyzer.

#### Diseases

<u>Wilt</u>: Scoring for fusarium wilt (causal organism: *Fusarium oxysporum*) resistance. Accessions sown in wilt-sick plots, plant mortality counted at the end of the season and converted into percentage.

- R Resistant: <10% mortality
- M Moderately resistant: 10-20% mortality
- S Susceptible: >20% mortality

<u>Ascochyta blight</u>: Scoring for ascochyta blight (causal organism: *Ascochyta rabiei*) resistance. Ten day-old seedlings are inoculated in a plant propagator and disease severity scored after 15-day incubation on a 1–9 scale.

- 1 No damage
- 9 Severe damage

<u>Colletotrichum blight</u>: Scored for colletotrichum blight, caused by *Colletotrichum dematium*. Screening done by artificial inoculation with the pathogen twice and scored on a 1–9 scale.

- 1 No damage
- 9 Severe damage

<u>Botrytis grey mold</u>: Screening done using isolation plant propagator. Ten-day old seedlings inoculated and disease severity scored 15 days after inoculation on a 1–9 scale.

- 1 No damage
- 9 Severe damage

## **10A.4.** Descriptors for characterization of pigeonpea

### **Vegetative phase**

Growth habit: Pattern of growth and plant habit.

- C Compact having relatively few branches, borne at narrow angles to the stem
- S Spreading having relatively many branches, resulting in a broad canopy
- SS Semi-spreading intermediate between the above two types

Plant height (cm): Average height of three randomly chosen plants measured at maturity.

<u>Primary branches number</u>: Average number of branches borne on the main stem, recorded from three plants at the time of harvest.

<u>Secondary branches number</u>: Average number of branches borne on the primary branches, recorded from three plants at the time of harvest.

Plant pigmentation: Color of the stem at the time of 50% flowering.

- D Dark purple
- G Green
- P Purple
- R Sun red

### **Reproductive phase**

<u>Days to 50% flowering</u>: Days from effective sowing date to when 50% of the plants in the plot have at least one open flower.

Flowering Pattern: The pattern of flowering habit (Fig.10A.4.1).

DT (Determinate):	Apical buds of the main shoots develop into inflorescence, the sequence of inflorescence production is basipetal.
NDT (Indeterminate):	Inflorescences develop as axillary racemes from all over the branches, flowering proceeds acropetally from base to apex both within the racemes and on the branches.
SDT (Semi-determinate):	Flowering starts at nodes behind the apex and proceeds both acropetally and basipetally.

Flower color: The main color of the standard petal recorded from the plot.

- I Ivory
- L Light yellow
- OY Orange yellow
- Y Yellow



Figure 10A.4.1. Flowering pattern in pigeonpea – Indeterminate (A), semi-determinate (B) and determinate (C).

Streak color: Color of streaks on the dorsal side of the standard petal.

- NO None
- Pu Purple
- R Red

Streak pattern: Pattern of streaks on the dorsal side of the standard petal (Fig. 10A.4.2).

- FS Few streaks
- MS Medium streaks
- DS Dense streaks
- P Plain, uniform coverage
- NO None



Figure 10A.4.2. Pattern of streaks on standard petal in pigeonpea.

<u>Raceme number</u>: Average number of racemes per plant, recorded from three plants at the time of 50% flowering.

<u>Days to 75% maturity</u>: Number of days taken from effective sowing date to when 75% of the plants in the plot reach maturity.

Pod color: Main color of the pod (Fig.10A.4.3)

- DP Dark purple
- G Green
- M Mixed green and purple
- P Purple



Figure 10A.4.3. Diversity for pod color in pigeonpea germplasm at ICRISAT genebank.

<u>Seeds per pod</u>: Number of seeds per pod, determined from 10 pods randomly picked from three plants at harvest maturity.

Seed color pattern: Color pattern of seed coat recorded after drying (Fig. 10A.4.4).

- P Plain
- M Mottled
- S Speckled
- MS Mottled and speckled
- R Ringed



Figure 10A.4.4. Seed color pattern in pigeonpea.

Primary seed color: Main color of the seed coat recorded after drying (Fig.10A.4.5).

W	White	DP	Dark purple
BL	Black	LB	Light brown
С	Cream	LC	Light cream
0	Orange	LG	Light grey
G	Grey	RB	Reddish brown
Р	Purple		



Figure 10A.4.5. Diversity for seed color in pigeonpea germplasm.

<u>Secondary seed color</u>: Eventual other color on the seed coat, coded as in primary seed color.

<u>Seed eye color</u>: Color around hilum, recorded after drying, coded as in primary seed color.

Seed eye color width: Width of color around hilum, recorded after drying.

- N Narrow
- M Medium
- W Wide

Seed shape: Shape of the seed recorded after drying (Fig. 10A.4.6).

- O Oval
- P Pea (globular)
- S Square
- E Elongate



Figure 10A.4.6. Seed shape in pigeonpea.

Seed hilum: Presence or absence of strophiole.

- A Absent
- P Present

<u>Seed weight</u> (g): Weight of 100 seeds, from a random sample taken from the whole plot, recorded after the seed is sun dried.

<u>Shelling percentage</u>: Seed:pod ratio expressed as percentage based on weight from three randomly selected plants after harvesting and drying.

Protein content (%): Crude protein percentage of seed on dry weight basis.

Seed yield per plant (g): Average seed yield from three randomly selected plants.

<u>Harvest index</u> (%): Ratio of total seed yield to the total biological yield expressed as percentage from three plants.

### **10A.5.** Descriptors for characterization of groundnut

#### **Vegetative Phase**

<u>Days to emergence</u>: Number of days to 75% seedling emergence from the day of sowing or first irrigation.

<u>Growth habit</u>: Recorded at podding stage for plants at 10–15 cm interplant spacing (Fig. 10A.5.1).

- 1 Procumbent-1
- 2 Procumbent-2
- 3 Decumbent-1
- 4 Decumbent-2
- 5 Decumbent-3
- 6 Erect
- 7 Others

<u>Plant height</u> (cm): Height of main axis, measured from cotyledonary axil up to terminal bud, mean of 5 plants recorded 60–85 days after emergence.



Figure 10.A.5.1. Growth habit in groundnut.

<u>Plant pigmentation</u>: Presence of anthocyanin pigmentation in mature plants.

- 0 Absent
- + Present

<u>Stem hairiness</u>: Hairiness, observed on main axis.

- 1 Glabrous
- 3 Sub-glabrous, hairs in one or two rows along main stem
- 5 Moderately hairy, three or four rows along the main axis
- 7 Very hairy, most of the stem surface covered with hairs
- 9 Woolly, most of the stem surface covered with long hairs

Branching pattern: Pattern of cotyledonary branching (Fig. 10A.5.2).

- 1 Alternate
- 2 Sequential
- 3 Irregular with flowers on main stem
- 4 Irregular without flowers on main stem
- 5 Others



Figure 10A.5.2. Branching pattern in groundnut.

Primary branches number: Number of primary branches.

Leaflet color: Color of fully expanded leaf.

- 1 Yellow or yellow-green
- 2 Light green
- 3 Green
- 4 Dark green
- 5 Bluish green
- 6 Other

<u>Leaflet length</u> (mm): Length of apical leaflet of the fully expanded third leaf on the main stem. Mean of 5 leaflets recorded from different plants.

<u>Leaflet width</u> (mm): Width of fully expanded apical leaflet of the third leaf on the main stem, measured at its widest portion. Mean of 5 leaflets recorded from different plants.

<u>Leaflet shape</u>: Shape of fully expanded apical leaflet of the third leaf on the main stem (Fig. 10A.5.3).

Cuneate	9	Ovate
Obcuneate	10	Obovate
Elliptic	11	Oblong
Oblong-elliptic	12	Oblong-lanceolate
Narrow-elliptic	13	Lanceolate
Wide-elliptic	14	Linear-lanceolate
Suborbicular	15	Others
	Cuneate Obcuneate Elliptic Oblong-elliptic Narrow-elliptic Wide-elliptic Suborbicular	Cuneate9Obcuneate10Elliptic11Oblong-elliptic12Narrow-elliptic13Wide-elliptic14Suborbicular15

8 Orbicular

<u>Leaflet hairiness</u>: Hairiness on both surfaces, recorded from leaflets at the third node of the main stem.

- 1 Almost glabrous on both surfaces
- 2 Almost glabrous above, hairs below
- 3 Almost glabrous above, hairs and/or bristles below
- 4 Almost glabrous below, hairs above
- 5 Almost glabrous below, hairs and bristles above
- 6 Hairs on both surfaces, without bristles
- 7 Hairs on both surfaces, with bristles at least on one surface
- 8 Woolly without bristles
- 9 Woolly with bristles on one surface
- 10 Others

#### **Reproductive Phase**

<u>Days to 50% flowering</u>: Number of days from emergence to the day on which 50% plants of an accession have flowered.

<u>Flower color</u>: Color of front face of the standard petal excluding the crescent portion of fresh and fully opened flowers.

- 1 White
- 2 Lemon
- 3 Yellow
- 4 Orange-yellow
- 5 Orange



Figure 10A.5.3. Leaflet shape in groundnut.

- 6 Dark orange
- 7 Garnet/brick red
- 8 Others

Streak color: Color of the markings (crescent) on the front face of the standard petal.

- 1 White
- 2 Lemon
- 3 Yellow
- 4 Orange-yellow
- 5 Orange
- 6 Dark orange
- 7 Garnet or brick red
- 8 Others

Peg color: Pigmentation on peg.

- 0 Absent
- + Present

Days to maturity: Number of days from emergence to maturity.

- 1 <90
- 2 91-100
- 3 101-110
- 4 111-120
- 5 121-130
- 6 131-140
- 7 141-150
- 8 151-160
- 9 >160

Pod beak: Tip of the indehiscent fruit (Fig. 10A.5.4).

- 0 Absent
- 3 Slight
- 5 Moderate
- 7 Prominent
- 9 Very prominent

Pod constriction: Degree of pod constriction (Fig. 10A.5.5).

- 0 None
- 3 Slight
- 5 Moderate



Figure 10A.5.4. Pod beak in groundnut.



Figure 10A.5.5. Pod constriction in groundnut.

- 7 Deep
- 9 Very deep

<u>Pod reticulation</u>: Reticulation (venation, ribbing, ridging) on the shell of the pod.

- 0 None
- 3 Slight
- 5 Moderate
- 7 Prominent
- 9 Very prominent

Pod length (mm): Mean length of the pod, recorded from 10 mature pods (Fig. 10A.5.6).



Figure 10A.5.6. Diversity for pod and seed traits in groundnut.

Pod width (mm): Mean width of pod at widest point, recorded from 10 mature pods.

<u>Seeds per pod</u>: Number of seeds per pod. First number indicating most frequent number of seeds per pod, second indicating second most frequent number and so on.

- 1 2-1
- 2 2-3-1/2-1-3
- 3 3-2-1/3-1-2
- 4 2-3-4-1/2-4-3-1/2-3-1-4/2-4-1-3/2-1-3-4/2/1/4/3
- 5 3-2-4-1/3-2-1-4
- 6 3-4-2-1/3-4-1-2
- 7 4-3-2-1/4-2-3-1

- 8 4-3-1-2/4-2-1-3
- 9 3 or 4 seeded with occasional 5 seeded pods

<u>Seed color pattern</u>: Pattern of seed color, recorded within a month of harvest after complete drying (Fig. 10A.5.6)

- 1 One color
- 2 Variegated

<u>Primary seed color</u>: Major color of seeds recorded within one month of harvest after complete drying of mature, wrinkle free seeds (Fig.10A.5.6)

1	White	11	Salmon
2	Off-white	12	Light red
3	Yellow	13	Red
4	Very pale tan	14	Dark red
5	Pale tan	15	Purplish red/reddish purple
6	Light tan	16	Light purple
7	Tan	17	Purple
8	Dark tan	18	Dark purple
9	Greyed orange	19	Very dark purple
10	Rose	20	Other

Secondary seed color: Minor color of variegated seeds (Fig. 10A.5.6)

- 1 Blotched
- 2 Flecks of color
- 3 Striped
- 4 Tipped at the embryo end
- 5 Obscure or hazy
- 6 Others

<u>Seed length</u> (mm): Length of seed, recorded from an average of 10 mature seeds (Fig. 10A.5.6).

Seed width (mm): Width of seeds measured at mid point.

Shelling percentage: Shelling percentage recorded with seeds at about 8% moisture as

(Mass of mature seeds  $\times$  100)/(Mass of mature pods).

<u>Fresh seed dormancy</u> (%): Germination immediately after harvest and number of days to achieve 70% germination, eg, 65/12 indicates that 65% seed can germinate immediately after harvest, and seeds require 12 days to reach 70% germination.

# **10B. Molecular characterization**

The objective of molecular characterization of germplasm collections is to discern the diversity and population structure at DNA level and identify genetically diverse parents for mapping and use in breeding programs. As it is costly to characterize the entire collection, only selected sets such as core and mini core collections and trait-specific germplasm accessions are characterized to identify genetically diverse parents for use in crop improvement.

- Develop a composite collection of germplasm accessions from the entire collection for diversity, in such a way that it includes core or mini core collection and also trait specific germplasm.
- Genotype the accessions using available Simple Sequence Repeats (SSR) markers or Microsatellites.
- Analyze the genotypic data using Powermarker V3.0 and DARwin 5.0.
- Choose a reference set of 200-400 most diverse accessions using 'Max length sub tree' option of DARwin 5.0, which creates the subset of units minimizing the redundancy between units and limiting the loss of diversity (Fig. 10B.1-2).
- Assess the reference set for allelic richness and diversity.
- Reference set should capture maximum diversity of the composite collection.
- For example, as part of the Generation Challenge Program (GCP), scientists at ICRISAT in collaboration with partners such as ICARDA, Syria; CIRAD, France; EMBRAPA, Brazil; and CAAS, China have developed the composite collections of sorghum, pearl millet, chickpea, pigeonpea, groundnut, finger millet and foxtail millet (500-3,000 accessions) for molecular characterization.
- Reference sets of 200-400 accessions developed at ICRISAT had captured 78% alleles of composite collection in chickpea and sorghum, 95% in groundnut and pearl millet, 83% in pigeonpea, 89% in finger millet and 87% in foxtail millet.



Figure 10B.1. Tree of groundnut composite collection and reference set based on SSR markers.



Figure 10B.2. Tree of pigeonpea composite collection and reference set based on SSR markers.

# **10C. Multilocation evaluation**

There is a lack of reliable information on the performance of a large number of accessions, particularly, for traits of economic importance, which display large genotype×environment interactions and require multilocation evaluation. Multilocation evaluation of germplasm sets such as mini core collections, which can be handled easily and economically, for important agronomic traits in different countries, preferably at or near its place of origin, will provide the most reliable data (Fig. 10C.1).



Figure 10C.1. Evaluation of chickpea mini core collection at Patancheru location, India.

### Procedure for multilocation evaluation

- Plan well in advance, atleast a year ahead.
- Select sets of germplasm accessions.
- Select locations suitable for the selected accessions.
- Identify appropriate design for evaluation.
- Correspond with NARS, universities, NGOs, etc, in selected countries for collaborative evaluation.
- Arrange for the export of seed material well in advance by obtaining SMTA, IP and other necessary documents.
- Send the seed, list of material, characters to be recorded along with the procedures and planting plans, to all locations.
- Visit each location preferably at the time of planting to entrust the job and train the local staff to record the observations.
- If possible, visit the locations at the peak period of recording observations, preferably at the time of harvesting.
- Compile data from all locations and update the databases.

- Analyze the data using appropriate procedures and softwares. At ICRISAT, we use Residual Maximum Likelihood (REML) procedure for analysis of multilocational data.
- Identify promising accessions as sources for utilization.
- Publish the results in the form of catalogs, journal articles, etc.
- Assess the impact of multilocation evaluation on utilization of germplasm by crop improvement scientists.

# **10D. Diversity assessment**

Diversity in the germplasm collections could be mainly due to natural and human selection. Ecology, climatic, geographic location (latitude and longitude), and elevation of the collection site are the important factors that determine the patterns of diversity in the collections. Therefore, the collection team should remember to collect the above information at the time of collecting germplasm.

#### Assessment of diversity in the germplasm collections is essential to:

- Identify trait specific germplasm and its areas of adaptation
- Identify suitable locations for characterization and regeneration
- Classify the germplasm
- Select appropriate germplasm for distribution to scientists in different regions
- Develop representative core and mini core collection.

#### Diversity can be:

- Phenotypic diversity based on qualitative and quantitative traits
- Molecular diversity based on molecular markers such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP) and Simple Sequence Repeats (SSR).

Phenotypic diversity can be assessed by estimating the following statistical parameters for characters under study:

- Frequency distribution
- Range
- Mean
- Variance
- Diversity indices

Molecular diversity can be assessed by using different molecular markers such as AFLP, RFLP, SSR and SNP. Simple matching allele frequency-based distance matrix in DARwin 5.0 can be used to dissect the genetic structure and diversity, while Power Marker V 3.0 can be used to estimate basic statistics such as PIC, allelic richness, gene diversity, heterozygosity and occurrences of unique, rare, common and most frequent alleles in the population.