Standard Operating Procedures For Groundnut Breeding and Testing

Janila Pasupuelti, Surendra S Manohar, Dnyasehwar B Deshmukh,
Sunil Chaudhari, Papaiah V and Murali T Variath

ICRISAT INTERNATIONAL CROPS RESEARCH
INSTITUTE FOR THE SEMI-ARID TROPICS

Contents

1. Purpose and scope

The Standard Operating Procedures (SOP) is a reference or manual on breeding methodologies and testing procedures used at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to breed new groundnut varieties. It describes the tools that are currently being used for phenotyping and genotyping specific traits in groundnut. The SOP will be useful to groundnut researchers, scientific and technical staff and scholars and the NARS (National Agriculture Research System) partners.

2. Materials, equipment, and machinery for groundnut breeding operations

Various tools required for different operations of groundnut breeding are given below:

Hybridization: Needles, forceps, 90% ethanol, head lens (optional), colored threads to tag the emasculated and pollinated flowers, petri dish and cloth bags.

Field layout: Meter tape, pegs, field map, rope, lime (CaO) for marking boundaries in the field.

Equipment for recording observations: Digital tablet with bar code reader for data collection, camera, meter scale, electronic weighing balances (20 g to 10 kg and 1 kg to 100 kg), barcode labels, cloth and paper bags, and Near Infrared Reflectance Spectroscopy (NIRS) to estimate oil/protein/fatty acid content/moisture, seed counter, SPAD chlorophyll meter, germination towels and petri plates.

Machinery: Seed planter, barcode label printer, single plant threshers, dry plant threshers, pod and seed grader, electronic and manual pod sheller.

3. Steps in breeding and testing pipelines

Sections 3.1 to 3.4 describe the steps involved in breeding pipeline and section 3.5 describes the testing pipeline.

3.1. Selection of parents

The most important step in breeding is the selection of suitable parents for use in the crossing program. The parents are selected based on the breeding objectives or product concepts. At ICRISAT, the groundnut breeding program has three broad product concepts (PCs), namely:

- PC1: Short duration (85-100 days) and climate resilient varieties with resistance to foliar fungal diseases for oil, food and fodder purpose,

- PC2: Medium duration (100-120 days) varieties with resistance to foliar fungal and soil-borne diseases along with quality traits (high oil and oleic acid content) and for dual purposes, and

- PC3: Long duration (120-150 days) varieties with large seed size, high protein content, resistance to foliar fungal and soil-borne diseases for food and confectionary purposes.

The target trait(s), diversity based on pedigree and morphology, and available historical data are considered while selecting suitable parents. Historical data constitutes the performance of elite lines in the testing pipelines that includes both on-station and multi-location testing. The diversity and historical data are important for recycling elite parents in the breeding program. When selection is made visually, the choice of pollen and female parent is determined by morphological traits that enable confirming the hybridity of F_1 plants by visual observations such as flower color, growth and branching habit. For traits such as high oleic acid, rust and late leaf spot resistance, nematode resistance etc., where molecular markers are available, marker assisted breeding methods are used to assist the selection of hybrid progenies. The choice of male and female parent depends on the breeding objective. If the breeding objective is to transfer a trait from an interspecific derivative into a cultivated elite variety then the elite

parent is used as a female parent. For genetic and mapping studies involving crossing of two elite varieties differing the target trait, any one of the parents can be used as pistillate/pollen parent and the choice rests with the breeder. For some traits, the choice of parents is guided by the genetic nature of the trait.

3.2. Hybridization

It is very important to ensure that all the plants to be used in the hybridization program are 'true-to-type'. After selecting the parents for a cross, the plan of hybridization nursery is generated through Breeding Management System (BMS). The male and female parents are planted on a field or glass house as shown in Figure. 1. A row of 4m provides space for about 40 plants to carry out the crossing of 200-250 flowers per cross. The row-to-row spacing adopted is usually 60cm apart and plant-to-plant spacing is 10cm.
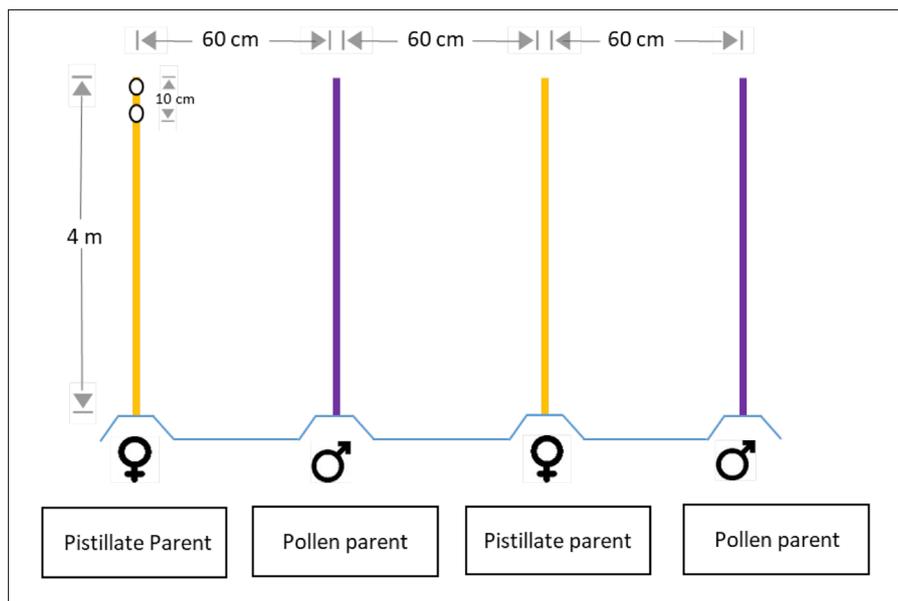

Figure 1 Field layout of hybridization block.

The wide row-to-row spacing gives adequate space for the researchers/students/technicians to sit and carry out emasculation and pollination work with ease. Staggered planting of parental lines may be necessary to ensure synchronization of their flowering periods and also to ensure sufficient F_0 seeds are available for sowing. At ICRISAT the success rate for crossing is 70 to 80% and this may vary depending on the skill of the person involved and environmental conditions, temperature and humidity in particular. The number of pollinations to be made is usually determined by the genetic nature of the target trait and the breeding program. For example, to develop a mapping population more F_0 seeds may be required to capture the diversity and ensure that sufficient population is available for carrying out mapping studies. In the case of traits which are controlled by one or two major genes, the population size can be reduced as the major objective is to introgress the trait from the donor to the cultivated elite lines. In general, for an F_2 size of 1000, 40-50 flowers are pollinated to obtain about 50 F_0 kernels. The number of flowers can be drastically cut down to 15-20 to obtain about 10-15 F_0 kernels when special hybridization houses are available to raise F_1 plants so as to harvest at least 30-40 fully mature pods on each F_1 plant.

Emasculation: Emasculation (removal of anthers from flower buds from those genotypes used as a female parent before their dehiscence to avoid self-pollination) is carried out between 1330-1630 hours at ICRISAT Center (18° N, 78° E, 545 MAMSL). Stages in emasculation of a bud are:

1. Selecting the right-sized bud for emasculation and removing other buds and flowers at node (node cleaning).
2. Holding the bud, removing the single sepal and folding down fused sepal using forceps.
3. Opening the standard petal, pulling down the wings and locking them with standard petal.
4. Pulling back the keel petal to expose the anthers and removing the anthers without damaging the stigma.
5. Emasculated flower showing only style and stigma.

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6. Folding back standard, wing, and keel petals to their normal positions after emasculation. This prevents cross-pollination and allows recovery of style from damages suffered during emasculation.
7. Tying a date-coded color thread on the stem just above the node of the emasculated flower for identification purpose.

Figure 2a Selecting the right-sized bud.

Figure 2b Removing other buds at the node (node cleaning).

Figure 2c The right way of holding the bud and removing the singe sepal.

Figure 2d Opening the standard petal.

Figure 2e Removing the anthers.

Figure 2f Emasculated flower showing only style and stigma.

Figure 2g Tying a date-coded color thread on the stem for the identification of the emasculated bud for pollination.

<u>Pollination</u>: To achieve a high success rate, pollination should be carried out early in the morning before sun rise and immediately after the bud opens. At ICRISAT, pollinations are done between 06.00 and 08.00 hrs. For pollination, a healthy flower from a pre-identified parent plant is removed by breaking the hypanthium. The calyx, standard, and wing petals are detached for ease of operation. The keel petal is gently pressed between the thumb and index finger to squeeze out the sticky pollen mass from the anthers. The sticky lump of pollen is deposited on the tip of the stigma of the emasculated flower. It is possible to pollinate up to 8-10 emasculated flowers with one pollen flower depending on the environmental conditions especially temperature at the time of pollination. The forceps and fingers of the operator should be wiped with alcohol when changing from one pollen parent to another to avoid contamination with unwanted pollen.

Figure 3a Emasculated flower ready for pollination.

Figure 3b A healthy flower from a male parent plant taken for pollination.

Figure 3c Calyx, standard petal and wings for flower taken from male parent are removed and pollen mass squeezed out, ready for pollination.

Figure 3d Depositing pollen on the stigma.

3.3. Hybridity confirmation of F_1 plants

The F_0 seeds are planted in the field or in a glasshouse to raise F_1 plants along with their female and male parents as per the field map depicted in Figure 4. The hybridity of F_1 plants is confirmed by comparing the vigour of F_1 plants to either of the parents and by checking for the presence of morphological traits from pollen parent. The morphological traits are observed during the various crop growth stage, at around 60-70 days after planting (DAP) and sometimes, if required pod features are also compared (Table 1). The selfed plants (female parent type), if any are removed. Hybridity confirmation can also be done using DNA markers when the pollen parent has specific traits for example high oleic acid content, resistance to LLS and rust diseases. The morphological confirmation is cost-effective and non-ambiguous.
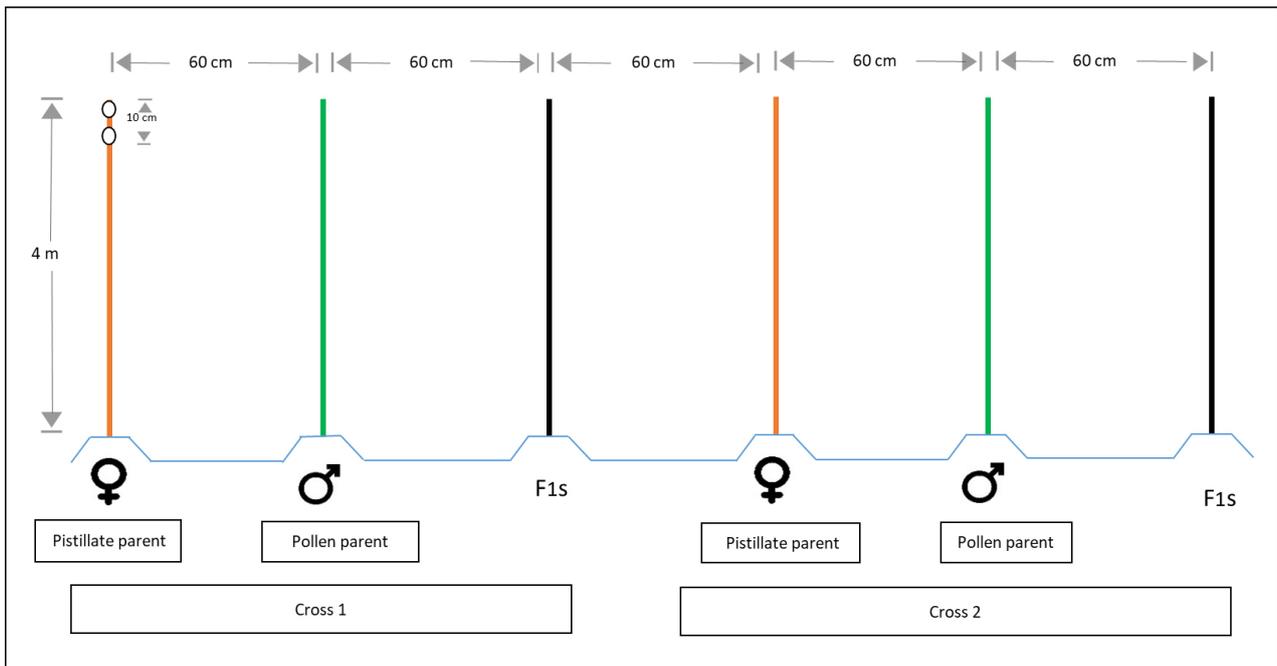
Figure 4 Field layout for F$_1$'s nursery.

Table 1 Morphological traits of groundnut to be used to check the hybridity of F1's.

Morphological traits	Female parent phenotype	Male parent phenotype	F$_1$ plant phenotype
Flowering pattern	Sequential flowering	Alternate flowering	Alternate flowering
Growth habit	Decumbent 3	Decumbent 2	Decumbent 2
	Decumbent 3	Decumbent 1	Decumbent 1
Leaf color	Green	Dark green	Dark green
	Green	Light green	Light green
Leaf size	Small-	Medium—	Medium-
Flower color	Orange	Garnet	Garnet
Stem pigmentation	Absent	Present	Present
Peg pigmentation	Absent	Present	Present
Leaf shape	Normal	Narrow	Narrow
Seed coat color	Tan/ light tan	Red	Red
Reticulation on pod surface	Present	Absent (Smooth pods)	Absent (Smooth pods)

* observed on the pods/seeds harvested from F$_1$ plant.

3.4. Handling from F$_2$ generation onwards

The F$_2$ seeds harvested from hybridity confirmed that F$_1$ plants are bulk harvested and advanced to next generations as per the methods described below. The modified pedigree methods, namely single seed descent and bulk-pedigree methods are routinely used. Most of the targeted traits such as, pod yield, shelling outturn, seed size, water deficit stress adaptation, oil and protein content and disease resistance are quantitatively inherited. Therefore, selections are made in advanced generations (F$_4$ onwards) when they attain a considerable level of homozygosity. Single-seed decent is a cost-effective and efficient method to breed groundnut varieties.

3.4.1. Single Seed Descent (SSD) method

This method is most suitable for handling large population size such as 800-1000 and is used either singly or in combination with other methods. A single seed obtained from a single pod from each F$_2$ plant is advanced to next generation till F$_{4/5}$, consequently the population size in F$_2$, F$_3$, F$_4$ and F$_5$ remains nearly the

same. Single plant selections are made in F_4 and F_5 generation and harvested as single plant bulks to raise F_5 or F_6 progenies, respectively. The uniform plants from F_5 or F_6 progenies are bulked or again single plants are selected in F_5 or F_6 generation. In F_6 or F_7 generation the bulks are advanced to seed increase nursery to generate sufficient seed for the testing pipeline (Figure 5). For some of the diverse crosses, there might be delay in selection as homozygosity can be reached at F8 generation. Selection is done at the time of harvesting. For single plants, besides the plant architecture, number of mature pods, pod size and shape, kernel size and shape are visually observed to facilitate selection. The estimate of harvest index based on the pods and haulm yield is an important criterion for selection. For early maturity, selection of number of mature pods at harvest (90 DAP) is important. Pod maturity can be determined either by the sound produced by the pods when they are pressed or by observing the inner pod wall which will turn blackish in mature pods. For bulk selection, along with the above criteria, uniformity of the selected plants is important. For selection purpose, plants from each progeny rows with large number of pods are set aside and from them the pods with good filling and desirable pod and kernel features are selected. The pods with prominent beaks and deep constriction should be avoided in selections. The SSD method is used to develop Recombinant Inbred Line (RIL) population.
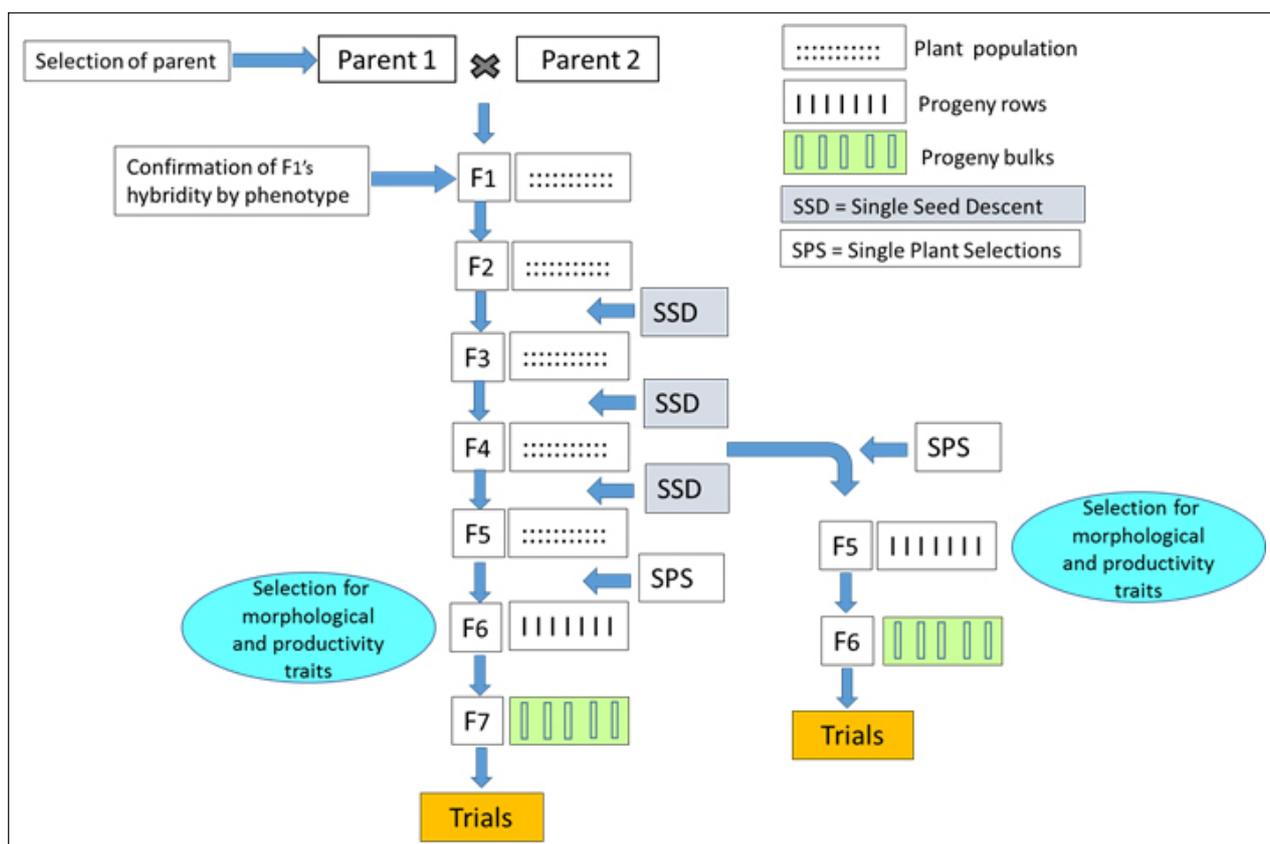

Figure 5 Single Seed Descent (SSD) method for Groundnut breeding.

3.4.2. Combination of SSD and Bulk-pedigree method

In this method of handling the crosses, along with single pods harvested from all individual plants in F_2 and F_3, about 2-20 plants with superior traits such as more number of mature pods per plant are selected, bulked and advanced to subsequent generations (Figure 6). The selections are made so that superior $F_{2/3}$ plants are not lost. In F_3 generation there will be an SSD bulk of the same size as F_2 and the selected bulk. The selected bulk constitutes the seed from all the selected plants. To optimize the space when there are more number of selected plants about 10 pods from each plant are harvested and bulked. Selections are again made in selected bulk to advance to next generation. Single plants from both SSD and selected bulks in F_4 and F_5 are chosen.
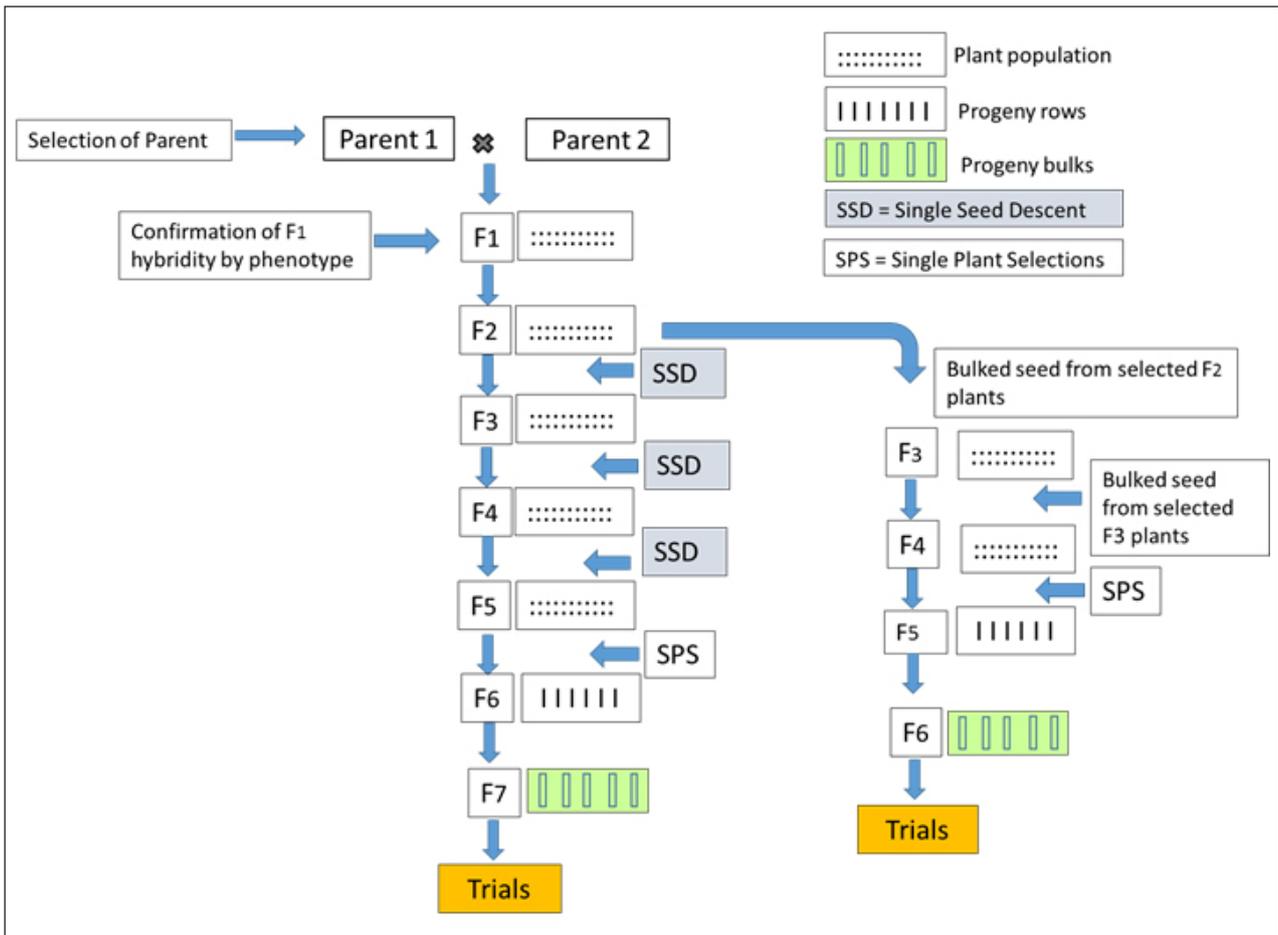
Figure 6 Combination of SSD and Bulk-pedigree methods for groundnut breeding.

3.4.3. Combination of SSD and Pedigree methods

In this method of handling the crosses along with single pods harvested from all individual plants in F_2 and F_3, about 2-20 $F_{2/3}$ plants based on plant type and morphological feature of pods and/or kernels like seed coat color and advanced to subsequent generations as single plant progenies (Figure 7). Therefore, in F_3 generation there will be an SSD bulk along with the selected progenies and the selection continues for F_4 and F_5 generations. After this bulk selections are made based on the uniformity of the population. This method is not frequently used.

3.4.4. Marker-Assisted Selection

In Marker Assisted Selection (MAS) method, selections are made in F_1 and F_2 generation using molecular markers linked to target trait and advanced as single plant progenies in F_2 and F_3 generations. In F_1 generation, confirmation is made for hybridity/heterozygosity and the validated hybrid plants are selfed to obtain F_2s which are again confirmed for homozygosity of the linked markers. In MAS the initial selection is made for the target trait and in later generations ($F_{5/6}$) selection is made for plants that closely resemble recipient parent features. Single or bulk selections depend on the uniformity in the population (Figure 8). The selected single plants/bulks go through seed increase before moving to testing in yield evaluation trials. The MAS is similar to the pedigree breeding method. Alternately, the F_2 population can be advanced to $F_{4/5}$ following SSD and F_4 or F_5 plants can be genotyped for selection and advanced as selected F_5 or F_6 progenies. Such a scheme will be useful when rapid generation advancement methods are available.
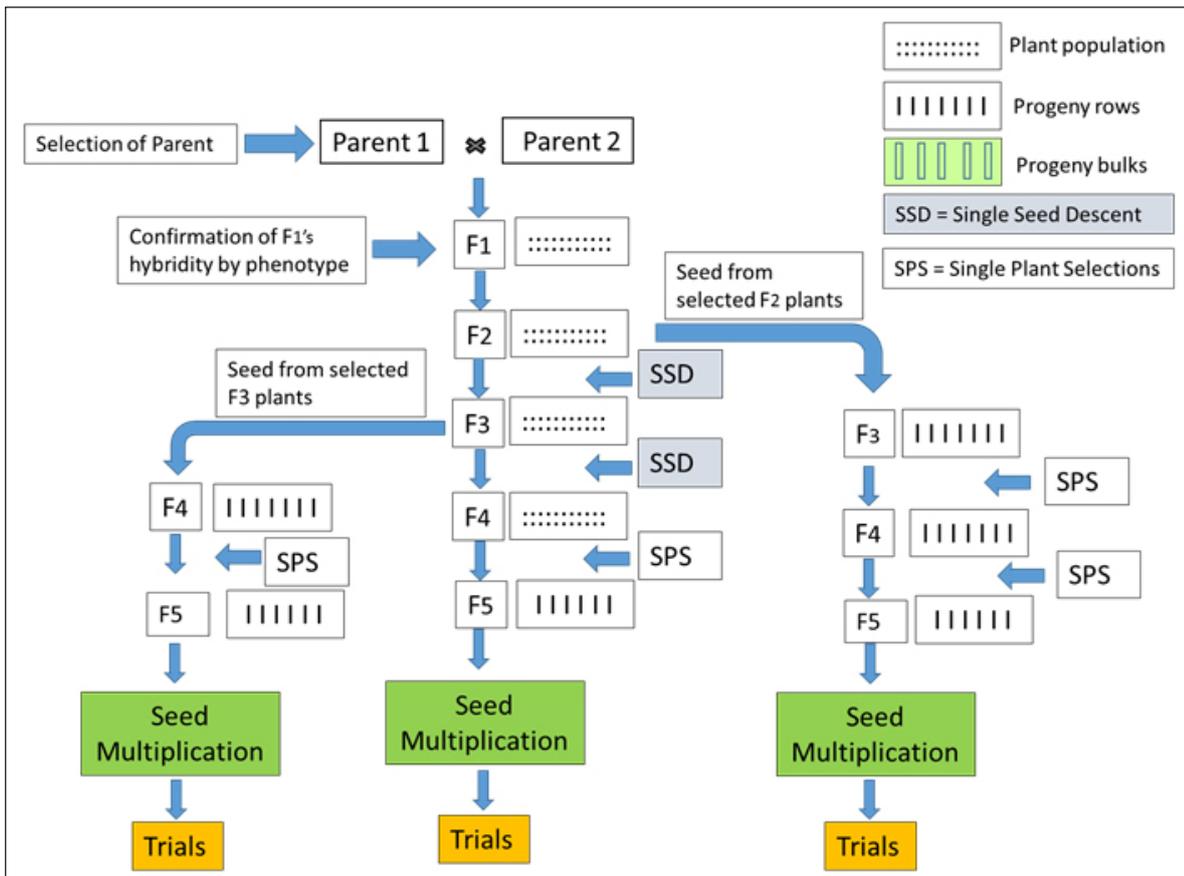
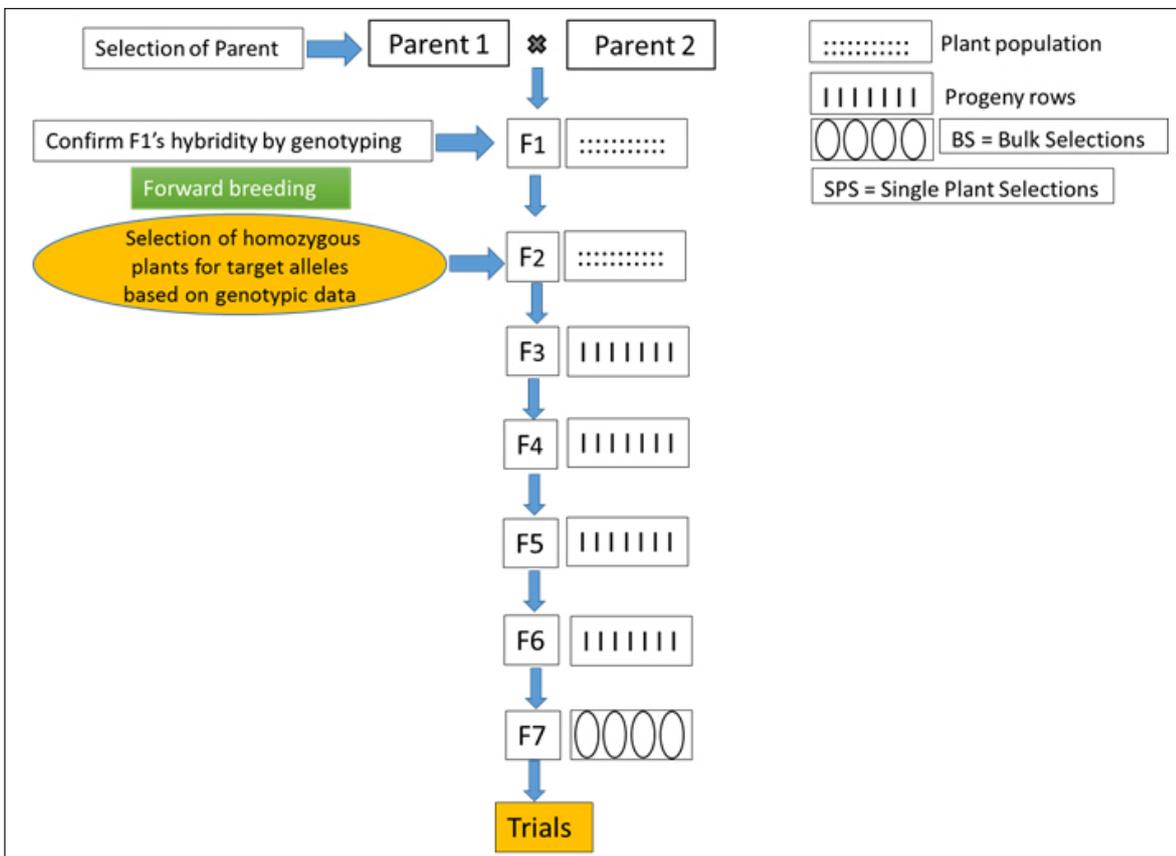*Figure 7 Combination of SSD and Pedigree methods.*

Figure 8 Marker-Assisted Selection (MAS) in groundnut.

3.4.5. Marker-Assisted Backcrossing

Market Assisted Backcrossing (MABC) aims to improve a mega-variety or elite line for a specific trait using molecular markers. In MABC the hybridity of F_1's is confirmed with markers and validated F_1s are crossed to recurrent parents 2-3 times to obtain BC_1F_1, BC_2F_1 and BC_3F_1 generations. Hybridity of back crossed F_1 generations is confirmed using markers and the validated hybrid plants are selfed to obtain BC_1F_2, BC_2F_2 and BC_3F_2 generations. Marker homozygotes are selected in BC_1F_2, BC_2F_2 and BC_3F_2 generations (Figure 9). The selected plant progenies from $BC_{2/3}F_2$ generation are advanced to $BC_{2/3}F_3$ and $BC_{2/3}F_4$ generations by selection for best plants based on morphology and resemblance to the recurrent parent. The selected plants from $BC_{2/3}F_4$ or $BC_{2/3}F_5$ progenies are bulked and put to seed increase nursery if they are uniform. Phenotyping is carried out in later generations for target traits.
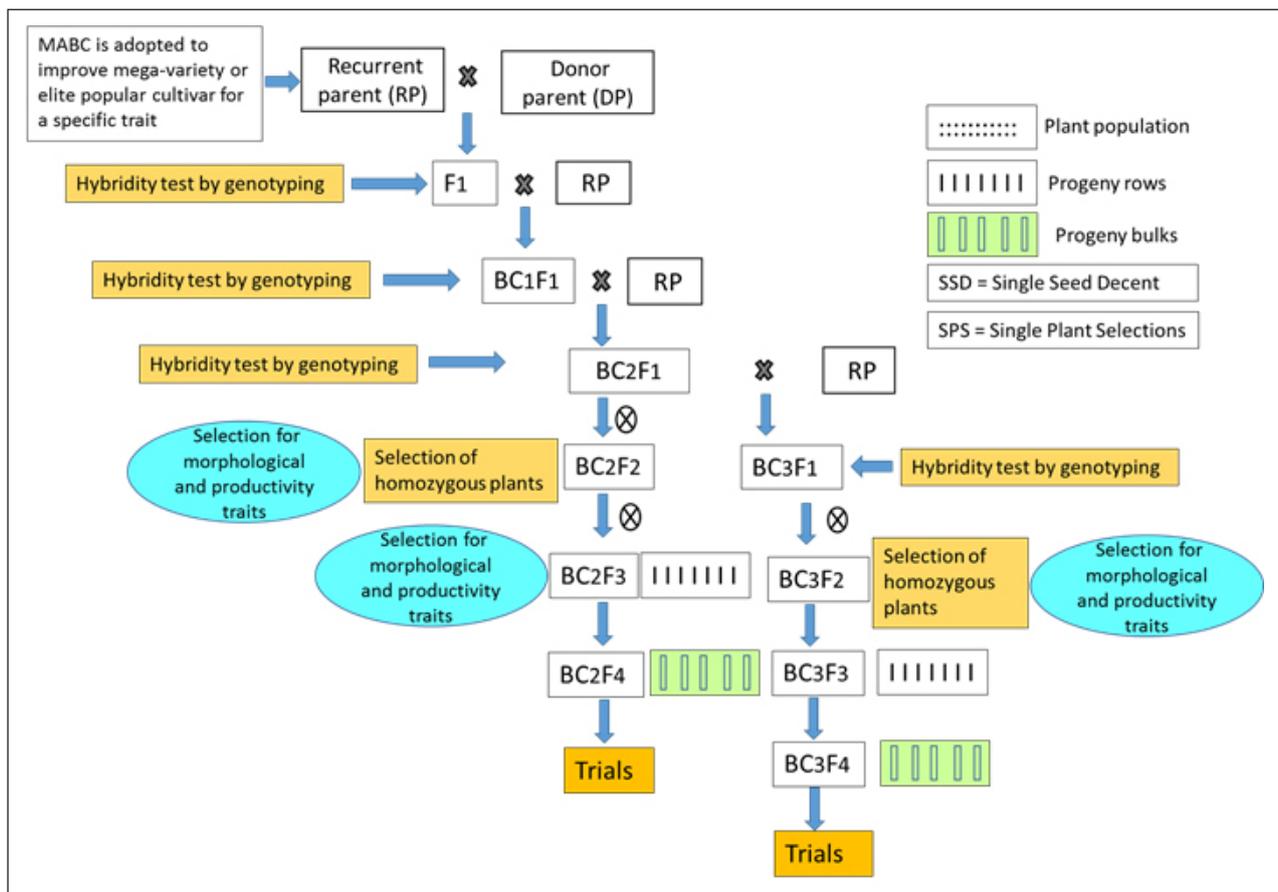
Figure 9 Marker-Assisted Backcross (MABC) breeding scheme for groundnut.

3.4.6. Phenotyping

Phenotyping is the process of classifying plants/progenies/lines/varieties based on their observable or recordable features. The phenotyping tools for groundnut are described below.

3.4.6.1. Morphological traits

Growth habit is classified into six classes as per groundnut descriptors (Figure 10a) and the branching pattern can be alternate, sequential and irregular (Figure 10b) based on the presence or absence of flowers on main axis and pattern of flowers on lateral branches.

Figure 10a Growth habit of groundnut

Figure 10b Branching pattern in groundnut.

3.4.6.2. Pod and kernel weight, shelling outturn, 100 seed weight

At the time of harvest the moisture content of the pods will be about 25-30%. Therefore, the pods are sun-dried until 8-10% moisture is left. Well dried pods are cleaned of soil and other particles using pod grader before recording pod yield per plot. Randomly selected pods (ca. 200 g) from each sample are weighed (pod weight), shelle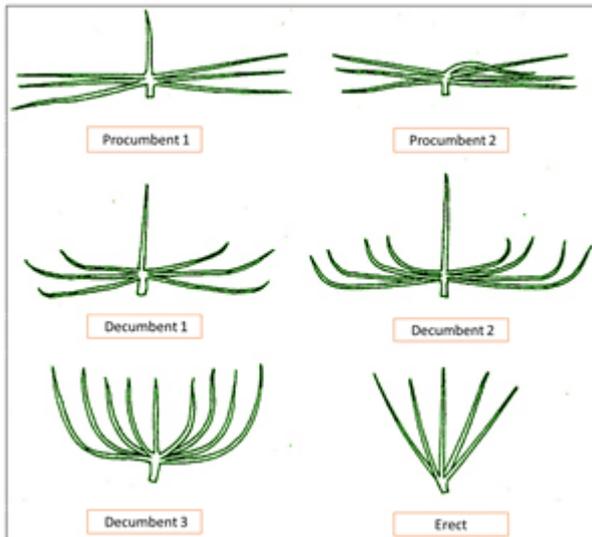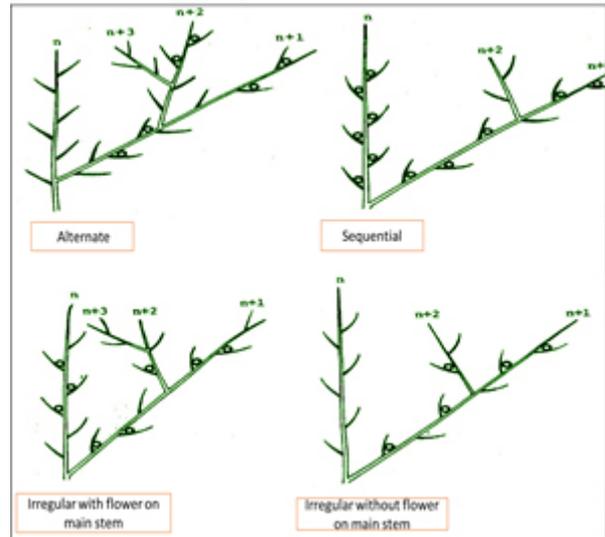d and the kernels are weighed (kernel weight) to derive shelling outturn as kernel weight/pod weight and expressed as percentage. The weight of 100 mature and sound kernels is weighted in an electronic balance to obtain 100-seed weight.

3.4.6.3. Estimation of oil, protein, fatty acid and moisture content using Near Infrared Reflectance Spectroscope (NIRS)

The Near Infrared Reflectance Spectroscope (NIRS) is a non-destructive, rapid and cost-effect method to estimate oil, protein, fatty acid and moisture content of seeds. To use NIRS, the calibration equations need to be developed based on chemical methods. At ICRISAT, calibrations were made using rectangular cup for the said quality parameters. The kernels are filled in a rectangular cup and scanned to get the measurements for quality parameters. Calibrations and measurements can also be made using a single seed or meal. A minimum sample size of 100 g of mature and sound kernels are required to fill the cup and obtain unbiased estimates for the quality traits using NIRS.

3.4.6.4. Screening for early maturity

Cumulative Thermal Time (CTT) which measures the heat units accumulated over a period of time, is used to measure maturity rather than DAP. At ICRISAT, Patancheru for 90 DAP, the number of heat unit is 1470. To make decisions on advancement of fixed lines to next cycle of testing, same set of entries are harvested at two dates. First at 80 DAP (1240 CTT) and second at 90 DAP (1470 CTT). The entries that are high yielding and do not record significant yield increment at 90 DAP as compared to 80 DAP are advanced. In segregating generations, the plants/progenies with pod maturity in terms of the proportion of fully mature pods and extent of pod filling at harvest time (90 DAP), high shelling outturn (70% or greater) and 100-seed weight (35-40 g) are used as selection criteria. The second round of selection is done wherein after drying and shelling, the proportion of shriveled kernels is used as a criteria to reject a selected plant/progeny/fixed line. A three-tier yield evaluation such as, preliminary, advance and elite trials are conducted. The preliminary and advance trials are harvested at 1470 CTT, while the elite trial at 1240 CTT (equivalent to 80 DAS) and 1470 CTT (equivalent to 90 days) at Patancheru, India. The formula for calculation of CTT is given below.

$$CTT\ (°Cd) = \sum_{P}^{H} \frac{(T\max + T\min)}{2} - T\,base$$

Where,

T Max= Daily maximum temperature

T Min= Daily Minimum temperature

T Base= Mean base temperature for peanut (T Base= 10° C)

P= Planting date

H = Harvesting date

3.4.6.5. An empirical approach for water deficit stress screening

In segregating generations, number of mature pods/plant, extent of pod filling, high harvest index (HI), greater water use efficiency (WUE) and visual shelling outturn under water-deficit stress are used as selection criteria for selecting water stress tolerant genotypes. In early generation testing and yield trials, pod yield under well-watered and imposed water deficit stress is measured and the entries with least penalty in pod yield and shelling outturn under stress and well-watered conditions are advanced. For this, the trial is conducted in post-rainy season under drip-irrigation. In stress nursery, the water is withheld after 60 DAP (1000 CTT) and irrigation is given when wilting symptoms appear or sometime alternate irrigations are withheld after 60 DAP. This imposes stress that represents mid- and end-of-season water deficit stress ecologies. The SPAD meter readings and wilting scores on 1 to 5 scale (where, 1 = no wilting symptoms, 2 = few leaves wilted in few plants from the plot, 3 = majority of plants in a plot have wilted leaves but none have reached permanent wilting, 4 = a few plants show permanent wilting, and 5 = most plants show symptoms of permanent wilting) are recorded at 80 DAP. The SPAD meter reading is recorded in both well-watered and stress nurseries while the wilting score is recorded in stress nursery. The SPAD chlorophyll meter reading (SCMR) and wilting scores are used in combination with pod yield to make a decision on selection.

3.4.6.6. Screening for foliar fungal diseases and stem rot

Screening for foliar fungal diseases (rust and late leaf spot) is carried out in the rainy season when the environment is favorable for disease infection and spread. Screening for both the diseases is done in the same nursery. Artificial inoculation method under infector row system is used. For trials, an infector (high susceptible variety) is planted after every four test plots (Figure 11a) and for generations after every nine rows (Figure 11b). The entire plot is surrounded by infector rows. At 40 DAP, infected plants from greenhouse are transplanted along the infector rows and at 50 DAP spore and conidia suspension of rust and LLS respectively is sprayed on the nursery. This is followed by perfo-irrigating the crop to enable the development of microclimate favoring infection and spread. Scoring for disease is done on 1 to 9 scale, 1 being resistant and 9 being susceptible at 75, 90 and 105 DAP. The scores of the segregating generations or advanced breeding lines along with pod yield are compared with resistant and susceptible checks of same maturity group to make decisions on selection. For early maturing varieties (100-115) the score at 90 DAP or sometimes at 80 DAP is useful, whereas for medium duration varieties (120-125 days), the score at 105 DAP is informative for decision making.

Stem rot sick nursery was developed and maintained for the screening of test entries. Under controlled condition in a glasshouse, the screening for stem rot was standardized and used. Plant mortality score is recorded at 45, 75, 90 and 105 DAP. A mortality rate of <20% is considered as resistant and compared with resistant and susceptible checks.

3.4.6.7. Haulm yield and quality

The dry plant weight after separating pods is measured to obtain information on haulm yield, whereas for estimating haulm quality parameters, samples are sent to the International Livestock Research Institute (ILRI) on ICRISAT campus. Haulm quality parameters such as nitrogen content and *in-vitro* organic matter digestibility (IVOMD) would be estimated using the Near Infrared Reflectance Spectroscopy (NIRS).
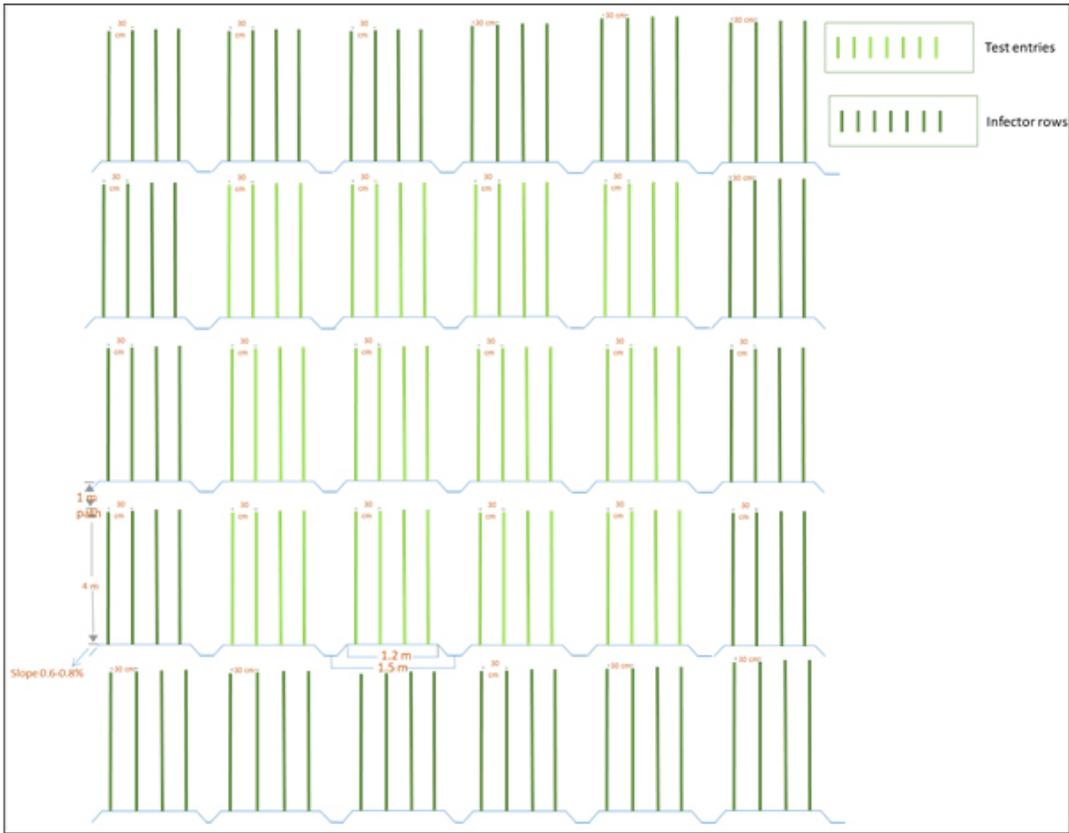
Figure 11a Schematic representation of foliar fungal disease nursery trials grown on raised beds.

Figure 11b Schematic representation of foliar fungal disease nursery for generations grown on ridges.

3.4.6.8. Fresh seed dormancy

In groundnut, fresh seed dormancy of two to three weeks is desirable as it prevents in-situ germination under unfavorable conditions and also enables harvesting the crop with extended period. To assess fresh seed dormancy, freshly harvested pods are dried for 2-3 days and mature seeds from test genotypes are tested. The seeds are kept on germination paper in petri plates and regularly watered. Usually three replications each with 50 seeds are made. The data on number of seeds germinated are recorded every day for up to three weeks. The seeds are surface sterilized before keeping them for germination to prevent possible fungal infections. When seeds don't germinate they tend to decay after a week. Therefore, after first and second week, new set of seeds are kept for germination from the same seed lot. Percent germination is calculated and the genotypes which show dormancy for a period of two weeks or more are selected.

Figure 12 Germination test to assess fresh seed dormancy. On the left is a non-dormant line (ICGV 03051) and on the right is a dormant line (ICGV 15396).

3.4.7. Genotyping tools

Markers are available for high oleic trait and resistance to rust and late leaf spot (LLS) in groundnut. The QTL region on linkage group (LG) B03 explains up to 82.62% of the phenotypic variance (PV) for rust resistance and a major QTL on LG B10 explains up to 62.34% PV for LLS resistance. One region present on linkage group B03 represents three QTLs each for LLS explaining up to 67.98% PV and one for rust explaining up to 82.96% PV. Among the two alleles *ahFAD2A* and *ahFAD2B* (FAD-Fatty acid desaturase) responsible for converting oleic to linoleic acid, *ahFAD2B* is believed to regulate the accumulation of more than 75% oleic acid content in kernels. Thus this allele is targeted in the breeding program. With the identification of SNPs (Single Nucleotide Polymorphism markers) for the three target traits, high throughput plant genotyping (HTPG) platform is utilized to make selection decisions in early generations. Leaf discs are collected and sent to Intertek Private Ltd. for SNP genotyping which involves a set of 10 diagnostic SNP's in a panel for the above three traits costing USD2 per sample including DNA isolation.

3.5 Testing pipeline

The groundnut testing pipeline often involves testing of fixed lines (F_6 and later generations) coming through cycles of selections. Hence, only the best lines are tested in the trials for superior yield performance in comparison to the best available check/control. For some traits such as foliar disease resistance, water stress etc., early generation testing of segregating populations in target sites is recommended to avoid rejection of plants that may show superior performance at the target location when compared to the non-target location. For foliar disease resistance testing of segregating progenies in hotspot locations such as Dharwad and Aliyarnagar will enable better selection of resistant plants when compared to Gujarat where the natural disease incidence is low due to unfavorable environment for the fungus. The phenotyping tools described for breeding pipeline also holds good for testing pipelines as well.

3.5.1 Early generation testing at target-sites

The target sites for specific traits were identified for testing of segregating or early generation material (non-replicated). They include Dharwad-Karnataka and Aliyarnagar-Tamil Nadu for foliar fungal diseases, Vijayapura-Karnataka and Anantapur-Andhra Pradesh for water deficit stress environment, Jaipur and Bikaner in Rajasthan for confectionary traits, Ludhiana-Punjab for collar rot, and Junagadh-Gujarat for stem rot. Crossing is done at ICRISAT and the segregating populations are sent to these locations for making selections in the target sites.

3.5.2. On-station testing

A three-tier evaluation system is traditionally followed at ICRISAT for on-station testing which involves preliminary, advanced and elite trials. Each trial is conducted for two seasons, rainy and post-rainy. These are replicated trials involving checks and the best bet entries from the preliminary trials are promoted to the advanced trial. Similarly, the best-performing entries from advanced trial are forwarded to the elite trials. The best-best performers are constituted for International Nurseries to share the lines with collaborative partners across the globe. To optimize resources, 'observation trial', a non-replicated trial is done before the preliminary trial to eliminate poor performers before they move to the replicated trials. In some special cases where the target trait is new and in high market demand, entries are advanced to multi-location testing following preliminary/advanced trials at ICRISAT. The most common format of testing has been the observation trial followed by preliminary trials for two seasons moving on to multi-location testing. The lines are advanced based on statistical significance over the best check/control. More than one check is included in the trials and often ruling varieties are included as checks. The list of traits with recorded observations and description are given in Table 3.

3.5.3. Multi-location testing

Multi-location testing is done to enable the selection and release of superior performing varieties. The lines showing best performance over the locations are selected and advanced to International Nurseries at ICRISAT. Lines are shared with partners across the globe as part of the variety release trials of respective countries. In India, a well-established All India Coordinated Research Project on Groundnut (AICRP-G) enables national testing to release varieties for cultivation.

4. Data recording, management, analysis and interpretation

Digital tools such as tablets with barcode reader and software applications like 'Field Score' and 'Field Book' are being used for data recording. The data recorded on different traits from various trials are uploaded into the Breeding Management System (BMS). The BMS combines interconnected software applications to manage breeding nurseries and data from trials across all phases of the crop improvement cycle. The breeder can easily manage trials, nurseries and seed inventory, run statistical analyses and support breeding decisions through the gradual integration of genetic markers while keeping a safe, standardized and centralized record of data from one generation to the next on the same platform. For pedigree information and nurseries, Pedigree Management System (PMS) has been used in the past and the data on pedigree information are available from 1986 to present. A data management system i.e.,

Table 3. List of traits in groundnut.

Name of the Trait	Description of the Trait
Days of 75% emergence	Visual score of 75% emergence
Days of 75% flowering	Number of days from planting to initiate flowers in 75% of plants in a plot
Final plant stand	Number of plants in a plot at the time of harvest
Days to harvest	Number of days from planting to maturity
Pod yield (g/plot)	Weight of dry pods from a unit plot
Shelling percent	Proportion of kernel weight to weight of total pod shelled expressed as percent
100 kernel weight	Measurement in grams of 100 well-developed whole air-dried kernels
Kernel appearance and uniformity	Visual scoring of the appearance of seeds and their uniformity
Kernels per pod	Number of kernels present in majority of pods
Pod Number	Is a count of the number of pods per plant grown in the plot or field
Kernel yield (g/plot)	Weight of kernels harvested from a plot/plant
Fresh seed dormancy	Duration of fresh seed dormancy is evaluated through imposing conditions during germination for freshly harvested seeds in petri-dishes
Biomass	The dry weight of the whole plant or plant organs
Growth habit$	A qualitative description of the growth habit of the plant, whether erect/semi-erect/prostrate/spreading/semi-spreading
Branching habit	A qualitative description based on flower arrangement on main branch as present or absent and on primary branches as sequential or alternate
Stem pigmentation$	A qualitative trait indicating the extent of anthocyanin pigmentation recorded as present or absent
Peg pigmentation$	A qualitative trait indicating the extent of anthocyanin pigmentation in the peg
Leaf color$	A qualitative expression of leaf color recorded as light green, green and dark green
Leaf shape$	Visual appearance of leaf architecture recorded as Cuneate/Obcuneate/Elliptic/Oblong-Elliptic/Nerrow-eliptic/Wide-elliptic/Suborbicular/Orbicular/Ovate/Obovate/Oblong/Oblong-lanceolate/Lanceolate/Limear-laneolate
Leaf size$	A quantitative expression for the mass of a leaf
Leaf area	A quantitative measurement of whole plant leaf area
Flower color (standard petal)$	A qualitative trait indicating the flower color to be recorded as yellow/orange/garnet/white
Marking on standard petal $	Color appearance of wing petal recorded as yellow/orange/garnet/white
Flower color (crescent)$	A qualitative trait indicating flower crescent color to be recorded as yellow/orange/garnet/white
Pod constriction$	Visual Shape of pod recorded as absent/slight/moderate/deep/very deep
Pod beak$	Tip of the pod recorded as absent/slight/moderate/prominent/very prominent
Pod reticulation$	The texture of the pod recorded as smooth/moderate/prominent
Pod ridge$	The texture of each seed in pod recorded as absent/moderate/ prominent
Pod shell thickness$ (mm)	A quantitative measurement of pod thickness measured through Vernier calipers
Pod size (cm)	Length of pod recorded through measuring scale or Vernier calipers

Continued

Table 3. *Continued*

Name of the Trait	Description of the Trait
Seed color[$]	A qualitative trait indicating seed coat color that could be Purple/Red/Dard red/Tan/Light Tan/Pale tan, and different shades and combinations of these colors
Seed size[$]	Seed size is recorded as Small/Medium/Medium large/Large
Seed shape	A qualitative trait indicating seed shape to be either Round/Flat end/Elongated
Cultivar group	Identified based on growth habit and branching pattern, genotypes belonging to the same group are evaluated separate trials
Hairness on main stem[$]	Morphological character measuring the density of hairs on main stem
Hairness on mature Leaflet[$]	Morphological character measuring the density of hairs on the leaf
Harvest index	Proportion of grain yield over total biomass yield expressed as percent
SPAD chlorophyll meter reading at the start of stress	A qualitative measurement of leaf chlorophyll content recorded through SPAD chlorophyll meter after imposing water deficit stress (at around 80 DAP)
Mature and sound seed percent	Proportion of mature seed to total seed weight as percent
Seed Ends[$]	A qualitative trait indicating seed end shape to be either beak head or bold
Seedling root to shoot ratio	Average value of root/shoot dry weight
Specific leaf area	Leaf area/leaf weight
Moisture content (%)	% moisture in the kernels measured using NIRS method or % moisture measured in pods using a moisture meter
Oil content (%)	% oil in the kernels measured using NIRS or Soxhlet method
Protein content (%)	% protein in the kernels measures using NIRS method or measured in pods using wet lab methods
Oleic acid (%)	Fatty acid measured using Gas Chromatography or NIRS
Linoleic acid (%)	Fatty acid measured using Gas Chromatography or NIRS
Palmitic acid (%)	Fatty acid measured using Gas Chromatography or NIRS
Stearic acid (%)	Fatty acid measured using Gas Chromatography or NIRS
Oleic/linoleic acid ratio	Proportion of oleic/linoleic acid content in oil
Insect pest score [#]	Visual score of insect pest damage
Disease score [#]	Visual severity rating for plants infected with leaf spots and/or rust
Disease score RO: Rosette[#]	Visual estimate of diseased plants
Disease score BND[#]	Visual severity scores for plants infected with bud necrosis disease

[#] as per the scores are given in Janila and Nigam (2013).
[$] refer to IBPGR and ICRISAT, Descriptors of Groundnut (1992)

AGROBASE was also used during 2011 to 2014 to manage data from preliminary, advance and elite trials. The data from 2001 to 2010 were uploaded to it. From 2015 onwards an Integrated Breeding Platform called Breeding Management System (BMS) is being used for pedigree management, maintaining and conducting crossing nurseries, generating layouts and randomization of different trials as per the experimental design and management and analysis of genotypic and phenotypic data. Data recorded from different trials are subject to statictical analysis such as analysis of variances and covariances, genotypic and phenotypic coefficient of variation, heritability and genetic advance as percent of mean to make selection decisions. The data recorded from multi-environments are subject to combined analysis of variance to know about the genotype, environment and genotype × environment interaction variances for different traits along with the above mentioned statistical parameters. Stability analysis is also done to identify genotypes adoptable to wide range of environments. Genstat is often used for different statistical analysis.

5. Naming conventions for nurseries and trials

A new naming convention has been prepared at ICRISAT for all the six mandate crops and is being followed from 2017 rainy season onwards. Streamlining the naming convention is required as we moved to BMS, a more robust data-management system across our centres. The naming convention and examples of naming crosses, advance breeding lines and RILS are given below.

5.1. Streamline of trial and nursery naming convention

Trials:	TG R 17 PAT PYT SD 01	Nursery:	NG R 17 PAT F1 SD 01
Character	**Description**	**Suggested Values**	
1	Trial or Nursery	T, N	
2	First Letter of Crop	C, F, G, M, P, S	
3	Season	R, P etc.	
4-5	Year	00-99	
6-8	Location code as in BMS	As in BMS (eg., PAT for Patancheru)	
9-13	Stage of nursery or trial	Nursery: F1, F2, BC1F1, RIL, Trials: PYT, IVT, AVT	
14-16	Product/type	(HT) Heat Tolerance, (NUE) Nitrogen Use Efficiency, (FDR) Foliar Disease Resistance etc.	
17-18	Trial unique number	01-99	

5.2. Naming convention of Crosses

IC	G	X		17	1	001
ICRISAT	Crop G=Groundnut	Cross	One space	Year (17 for 2017)	Location 1 = Patancheru 2 = Kenya 3 = Ethiopia 4 = Malawi 5 = Zimbabwe 6 = Mali 7 = Niger 8 = Nigeria	Serial number of cross from 001 to 999

5.3. Naming convention of advance breeding line

IC	G	V		17	1	001
ICRISAT	Crop G = Groundnut	Breeding line	One space	Year (17 for 2017)	Location 1 = Patancheru 2 = Kenya 3 = Ethiopia 4 = Malawi 5 = Zimbabwe 6 = Mali 7 = Niger 8 = Nigeria	Serial number of lines from 001 to 999 ((001 to 500 for Patancheru, 501 to 800 for ESA and 801 to 999 for WCA)

5.4. Naming convention of Recombinant Inbred Lines

IC	G	R		17	1001
ICRISAT	Crop G = Groundnut	R=Recombinant Inbred Line (RIL)	One space	Year (17 for 2017)	Serial number of line from 1001 to continue

5.5. Pedigree record maintenance

At ICRISAT, the pedigree record of each cross is maintained along with details of the breeding procedure followed and selections done in every generation. An example of full pedigree record and its interpretation are given below (Figure 12a and 12b).
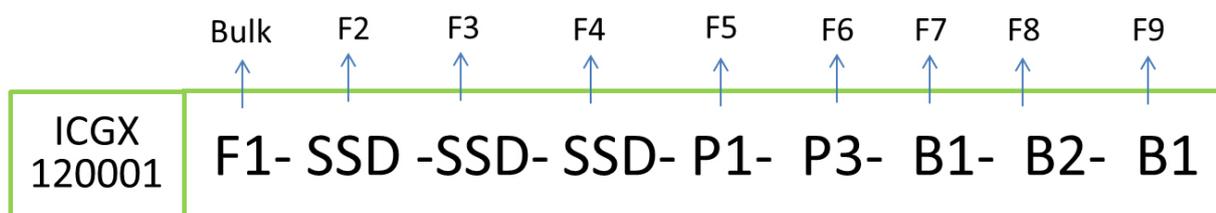

Figure 12a Pedigree record of a cross bred using Single Seed Descent (SSD) method for groundnut breeding (P1 & P2=Plant number; B1 & B2= Bulk one and two).
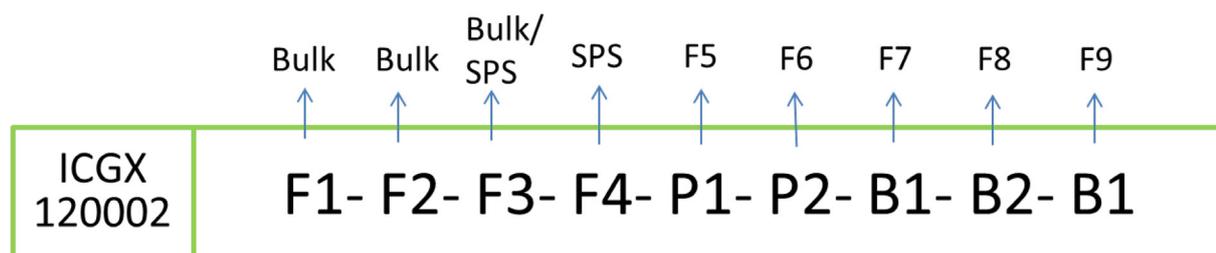

Figure 12b Pedigree record of a groundnut cross bred using combination of Bulk-pedigree methods (SPS= Single plant selection; P1 & P2=Plant number; B1 & B2= Bulk one and two).

6. Suggested reading

IBPGR and **ICRISAT.** 1992. *Descriptors of Groundnut. International Board of Plant Genetic Resources*, Rome, Italy. International Crops Research Institute for Semi-Arid Tropics, Hyderabad, India. https://www.bioversityinternational.org/fileadmin/userupload/onlinelibrary/publications/pdfs/431.pdf

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For more details please contact Dr P Janila, Principal Scientist-Groundnut Breeding, ICRISAT. p.janila@cgiar.org

This manual has been prepared by Janila Pasupuelti, Surendra S Manohar, Dnyasehwar B Deshmukh, Sunil Chaudhari, Papaiah V, and Murali T Variath, ICRISAT. 2017.

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