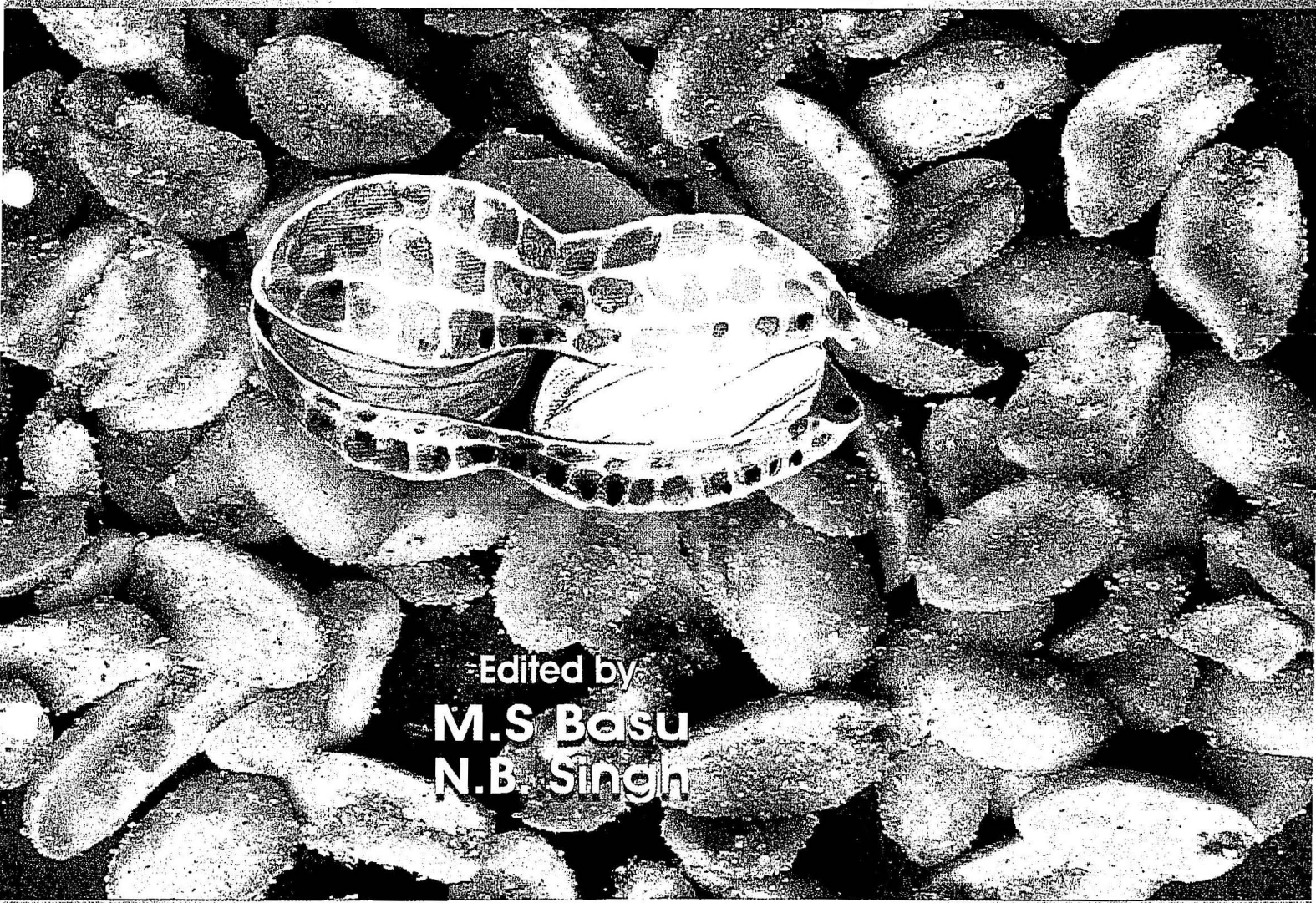


# Groundnut Research in India



Edited by  
**M.S. Basu**  
**N.B. Singh**

## National Research Centre for Groundnut

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# BREEDING METHODS OF CULTIVAR DEVELOPMENT IN GROUNDNUT

S.L. Dwivedi and S.N. Nigam

## I. INTRODUCTION

Cultivated groundnut (*Arachis hypogaea* L.) is a segmental amphidiploid ( $2n = 4x = 20$ ) with a basic chromosome number ( $x$ ) of 10. However, it behaves as a diploid. It is believed to have originated from a single hybridization event between *A. duranensis* and *A. ipaensis* not too distant in the past (Kochert et al. 1996). Archaeological evidence from excavations in Peru place the origin of *A. hypogaea* at least 3500 years ago (Singh and Simpson, 1994).

The cultivated groundnut is classified into two main sub-species: *hypogaea* (no flowering on the main stem and alternate branching) and *fastigiata* (flowering on the main stem and sequential branching). The subsp. *hypogaea* contains two botanical varieties—*hypogaea* (common name: Virginia) and *hirsuta*. The subsp. *fastigiata* contains four botanical varieties—*fastigiata* (common name: Valencia), *peruviana*, *aequatoriana*, and *vulgaris* (common name: Spanish). All the six botanical varieties have distinguishing morphological characteristics that separate them from one another (Krapovickas and Gregory, 1994).

Groundnut is a self-pollinated crop but natural hybrids due to out crossing (< 2% with few exceptions) have been detected occasionally depending upon season, genotype, and location (Kushman and Beattle, 1946; Hammons, 1964; Culp et al. 1968; Gibbons and Tattersfield, 1969). Be the principal pollen vectors of natural cross-pollination in groundnut. Out crossing in groundnut is a non-random event, with highest in Valencia followed by Virginia and Spanish groundnut (Lal et al. 2003).

## II. GENETIC VARIABILITY IN CULTIVATED AND WILD SPECIES

The genetic diversity in groundnut has been classified into four gene pools. The primary gene pool consists of land races of the cultivated groundnut and *A. monticola*; the secondary gene pool consists of diploid species cross compatible with *A. hypogaea*; the tertiary gene pool consists of section *Procumbensae*; and the quaternary gene pool consists of the rest of the species that are either cross-incompatible or weakly cross compatible with section *Arachis* and are grouped into six other sections (Singh and Simpson 1994). ICRISAT Center, Patancheru, India hosts the world collection of over 15000 accessions of the cultivated and 450 of wild *Arachis* species. These germplasm accessions differ in many morpho-physiological, reproductive, and quality traits, and in response to biotic and

abiotic stresses (Singh and Nigam 1997; Rajgopal et al. 1997; Upadhyaya et al. 2001, 2003). For enhancing the use of germplasm in groundnut breeding, a core collection consisting of 1704 accessions and a minicore collection consisting of 184 accessions have been developed (Upadhyaya et al. 2002, 2003). The accessions included in the core have potential to offer new sources of variation for use in breeding programs. Lately, molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) have been used to assess the genetic diversity among the select group of groundnut germplasm possessing beneficial traits. These studies revealed sufficient variability at the molecular level that could be tapped to identify markers associated with beneficial traits and possibly effect marker assisted genetic enhancement in groundnut (Dwivedi et al. 2001, 2002, 2003; Dwivedi and Gurtu, 2002; Dwivedi and Varma, 2002; Hopkins et al. 1999; Ferguson et al. 2003).

Wild *Arachis* species have shown great variability for morphological traits as well for resistance to pest and diseases, and several introgressed lines originating from interspecific crosses are now available for genetic enhancement in groundnut (Singh et al. 1991; Nigam et al. 1992; Simpson et al. 1993; Reddy et al. 1996; Simpson and Starr 2001; Stalker and Lynch 2002; Stalker et al. 2002a,b).

Natural out crossing provides additional source of genetic variation that could be exploited in breeding programs (Nigam et al. 1983). Groundnut cultivars, ICGV# 87119 (ICGS 1), 87123 (ICGS 11), 87128 (ICGS 44), and 87187 (ICGS 37) released in India and ICGV 87127 (ICGS 35) released in Korea originate from natural hybrid populations of Robut 33-1 (Nigam et al. 1990a, b; 1991; 1992; 1994).

## III. ARTIFICIAL HYBRIDIZATION IN GROUNDNUT

Nigam et al. (1990) published an information bulletin detailing various steps involved in artificial hybridization that researchers should follow for making successful crosses in groundnut. A success rate of 50% in the field and 70% in greenhouse has been obtained at ICRISAT. The skills of the operator and environmental conditions influence the success rate. Rainy season is preferred season for crossing because of high natural humidity. Hybridization can also be effected under greenhouse conditions where there is a better control of light, humidity, and temperature. Emasculation is normally done in the afternoon and pollination the following

morning when stigma is receptive to receive the pollen. Hybridization should be restricted to the early phase of flowering because of higher success rates in the production of mature pods from early-formed flowers.

#### IV. BREEDING METHODS EMPLOYED AND CULTIVAR DEVELOPED IN GROUNDNUT

##### (A) Breeding methods

Groundnut is a highly self-pollinated crop and breeding methods used are similar to those used in other self-pollinated crops and are described in greater details elsewhere (Allard, 1960; Knauff and Wynne, 1995; Knauff and Ozias-Akins, 1995; Chopra, 2000). They differ in the procedures used to handle segregating populations in early generations. The choice of the parents, the nature of gene action, the population size, the phenotypic screens, the selection method employed, and the intensity of selection play an important role in the success of any breeding program. Below, we cite a few examples that demonstrate the utility of different breeding methods employed in cultivar development in groundnut.

**Mass selection:** Mass selection in groundnut has been most effective in intersubspecific crosses and less effective in intrasubspecific crosses (Holley and Wynne, 1986). It is generally effective for selecting traits with high heritability such as branching habit, pod and seed size, seed coat color, and for earliness in early segregating generations. Substantial improvement in seed size was reported after one generation of mass selection from the bulk population of Zambian land races of Chalimbana in groundnut (Thomas et al. 1974). Even in the case of pod yield where heritability is generally not high, using mass selection for pod yield per plant in  $F_3$  -  $F_5$  generations, Patra et al. (1992) demonstrated significant improvement in pod yield, plant height, and harvest index in  $F_5$  lines in several crosses in groundnut. However, this method was not effective when combining high yield and leaf spot resistance possibly due to negative relationship between the two traits (Knauff et al. 1993).

**Pedigree selection:** In the pedigree method, accurate records are kept of the line of descent or pedigree of each plant. It is the most commonly employed breeding method in groundnut. With selection for market preferred, highly heritable traits in early segregating generations, the size of individual segregating population is quickly reduced. Selection for yield and other quantitative traits is practiced in later generations. However, this method is laborious and time and resource consuming. Alternatives to pedigree method are bulk pedigree method and modified pedigree method, which to a large extent overcome the limitation of the conventional pedigree method.

**Back cross:** This method of breeding has not been used extensively in groundnut due to paucity of simply inherited traits. However, this method has often been used at

ICRISAT and in groundnut breeding programs in the USA to transfer gene for resistance to foliar diseases from wild *Arachis* to cultivated groundnut. Back cross breeding method has also been used to transfer high oleic fatty acid trait into few large-seeded high yielding cultivars such as SunOleic 95R and SunOleic 97R in the USA (Gorbet and Knauff 1997, 2000). It is likely that this method of breeding will be more useful in transferring resistance genes from groundnut transgenics to other locally adapted varieties in future.

**Modified pedigree method:** The basic principle involved in this method is to advance segregating populations by selecting a single seed from each plant in  $F_2$  to later generations, and thus retain the entire spectrum of variability for selection to be effective in later generations (Brim, 1966). Rapid generation advance technique allows the growing of 2-3 generations per year thus saving time and lowering the cost in variety development. Branch et al. (1991) found that unselected bulks derived from single seed descent (SSD) performed equally well as selections obtained from a sequential selection scheme at several locations. Several breeding programs including at ICRISAT are now using modified pedigree method with SSD for population advancement in groundnut (Wynne 1976a; Wynne and Isleib, 1980; Wynne and Gregory, 1981; Hildebrand, 1985).

**Recurrent selection:** An alternative approach to overcome the breeding limitations of the pedigree method in self-pollinated crops is the use of recurrent selection. It permits the incorporation of diversity while providing opportunities for recombination. Guok et al. (1986) evaluated three cycles of selection for yield and determined the potential of future progress of selection in an interspecific cross, PI 10017 (*A. hypogaea*) x *A. cardenasii*, in groundnut. Two cycles of selection resulted in an increase in fruit yield of  $210 \pm 70$  kg  $ha^{-1}$  per cycle. Seed weight, shelling percentage, and extra large kernels increased significantly, while fruit length and other kernel characters decreased significantly over the two cycles of selection. However, a little variability was left among the lines after the second cycle of selection. This method provides many opportunities for recombination in early generation that reshuffle the entire spectrum of genetic variability to produce progenies with multiple beneficial traits. A comprehensive breeding procedure utilizing recurrent selection for groundnut has been outlined by Monteverde-Penso et al. 1987.

At ICRISAT, a modified version of recurrent selection is used where  $F_4$  or  $F_5$  bulks/progenies from different crosses are intercrossed to combine multiple traits.

**Early generation testing:** The value of early generation testing in self-pollinated crops has been disputed. Few studies revealed that early generation yield testing could be used to identify crosses from which high yielding segregants

could be selected (Harrington, 1940; Immer, 1941; Leffel and Hanson, 1961) whereas others questioned the predictive values of early generation ( $F_2$  and  $F_3$ ) tests to the performance of later generation selections (Weiss et al. 1947; Atkins and Murphy, 1949; Fowler and Heyne, 1955; Smith and Lambert, 1968). Early generation testing in groundnut involving different botanical varieties has been suggested as a useful breeding procedure for selecting component traits such as fruit length, sound mature kernels, fancy size pods but it has limited value in selecting for yield (Wynne, 1976b). Similarly, Ntare (1999) also observed little utility in selecting for yield and associated traits such as crop growth rate, reproductive duration and partitioning in early generations. However, Coffelt and Hammons (1974) from their study concluded that early generation yield trials may be an acceptable breeding procedure for selection of groundnut varieties.

**Complex crosses:** Instead of recurrent selection that requires making a large number of crosses, complex crosses have been proposed as a method of breeding to recover recombinants with multiple beneficial traits in groundnut. A few studies in groundnut revealed the superiority of three-way crosses over single crosses for improving physiological traits and yield components (Bandyopadhyaya et al. 1985; Arunachalam et al. 1985; Varman and Raveendran 1997). Early generation intermating produced superior progenies for yield and yield components in groundnut (Dutta et al. 1986). For complex crosses to be successful in breeding programs, a large segregating population from genuine hybrids must be generated to allow for selection of segregants with desired combination of reshuffled genes.

**Multiline varieties:** Multiline varieties in groundnut are composites used breeding method of cultivar development at the University of Florida, USA that released six groundnut cultivars with wide adaptation in the country. These cultivars are composite of 4 to 10 sister lines that are similar and indistinguishable from each other. Such cultivars have shown adaptation to wide geographical area, more stable yield across seasons, and broader protection against pests and diseases.

**Mutation breeding:** Both physical (Gamma radiation) and chemical mutagens (Ethyl methanesulfonate) have been used to create genetic variability in groundnut. Mutants with early maturity, resistance to rust and leaf spot, increased number of pods and seeds per plant, pod and seed weight per plant, shelling percentage, 100-seed weight, oil content, and O/L ratio are reported in groundnut (Patil et al. 1995; Naik and Nadaf, 1997; Mouli et al. 1989; Dwivedi et al. 1998; Gowda et al. 2002). The Bhabha Atomic Research Centre, Trombay, Mumbai, India did a pioneering work on induced mutants in groundnut, using both physical and chemical mutagens, and released mutants (TG 7 to TG 13)

with 27-46% more pod yield, 10-60% increased seed weight, and 2-4% higher oil content (Patil, 1973). Induced mutants have also been used in breeding programs as a source of noble variation. For example, TAG 24 variety was developed from a complex cross involving radiation-induced mutants. It is a dwarf, Spanish bunch cultivar released in 1991 in India that matures early, has high harvest index, and is tolerant of bud necrosis disease (Patil et al. 1995). A judicious use of mutation and recombination breeding can lead to significant gain in varietal improvement in groundnut.

### **(B) Genotype by environment interaction**

Large genotype (G) by environment (E) interaction has been reported in groundnut, thus necessitating the need for multiyear and multi-location testing before release of a cultivar (Shorter and Hammons 1985; Norden et al. 1986; Anderson et al. 1989; Coffelt et al. 1993). Photoperiod, temperature, and photoperiod by temperature interactions influence partitioning and thereby, adaptation of groundnut genotypes to new environments (Nigam et al. 1994, 1998). One way to minimize G x E interaction is to adopt a sequential selection method of evaluating breeding populations across diverse locations. It involves cycling early generation selections through different environments, as opposed to practicing selection at the same location each year. Branch et al (1991) compared the pedigree and sequential selections and single seed descent each of 3 years for yield and leaf spot resistance in groundnut. The sequential selections were comparable to or significantly better than the pedigree selections in leaf spot rating and yield performance. Individual sequential selections were also found with combined leaf spot resistance and high yield. This method, therefore, could be used as an alternative approach to selection of progenies with wider adaptation and yield potential. Its use allows selection to be practiced under much broader environmental regimes within early segregating populations, consequently enhancing the development of widely adapted genotypes. At ICRISAT, the groundnut breeding populations are generation advanced both in rainy and post-rainy seasons that differ in temperature and photoperiod regimes. Some times, they are also alternated between high-input and low-input growing conditions. This sequential selection under diverse growing conditions helps to build in wider adaptation in groundnut genotypes.

### **(C) Biotechnological approach to crop improvement in groundnut**

**1. Transgenics:** An efficient tissue culture and transformation system to introduce foreign DNA into groundnut has been developed (Sharma and Anjaiah, 2000). Transgenic plants carrying genes for resistance to tomato spotted wilt virus and lesser cornstalk borer in USA (Holbrook and Stalker, 2003), and transgenic groundnuts

with *IPCV<sub>p</sub>* or *IPCV<sub>replacase</sub>* gene for resistance to peanut clump virus, *GRAV<sub>p</sub>* gene for resistance to groundnut rosette assistor virus, rice chitinase gene for resistance to fungal diseases, and drought responsive elements (DRE) of *Arabidopsis* (rd29A:DREB1A) for improving drought tolerance in groundnut have been produced at ICRISAT that are in various stages of characterization under containment glasshouse and/or controlled field evaluations (ICRISAT, 2002). It is hoped that once favorable genes are introduced into cultivated groundnut, these transgenics will be routinely used to transfer these genes into other adapted cultivars by conventional breeding techniques.

**2. Marker-assisted selection:** Marker-assisted selection offers great promise for improving the efficiency of conventional plant breeding. An RFLP-based tetraploid genetic linkage map of groundnut derived from a BC<sub>1</sub> population of TxAG6 with Florunner has been developed that mapped 370 RFLP loci into 23 linkage groups with a total map distance of approximately 2210 cM (Burow et al. 2001). However, these RFLP loci are unlikely to detect alleles in *A. hypogaea* x *A. hypogaea* crosses. In the last few years, there has been substantial progress on development of SSR markers (Hopkins et al. 1999; Ferguson et al. 2003; ICRISAT, 2002) that have been found polymorphic among cultivated groundnut germplasm (see section II). Highly polymorphic germplasm have been used to develop recombinant inbred lines that will soon be available to researchers to identify genomic regions associated with beneficial traits and for constructing SSR-based genetic linkage map in groundnut.

#### **(D) Status of cultivar development in groundnut using different breeding methods in India**

The first groundnut variety released in India was Spanish Improved. It was released in 1905 and was selected from a Spanish groundnut population. Then, AK 12-24 and TMV 2, both selected from local populations, came in on the scene around 1940. From 1940 to 1960, there was an almost complete lull in varietal development activities in groundnut in the country. Groundnut breeding activities picked up from 1960 onwards and 129 varieties have been released till date in India (MS Basu and AL Rathnakumar, pers. commun.). The past breeding efforts focused on introduction and selection in introduced or local populations. Since 1980, the use of exotic germplasm and hybridization followed by selection and emphasis on stress resistance breeding increased in the national breeding programs. Inclusion of groundnut crop in ICRISAT's mandate in 1976 facilitated these changes in national breeding program strategies as more exotic germplasm became available, sources of resistance to various stress factors were identified and screening techniques were developed. On average, 46% of the new varietal proposals between 1985 and 1995 in the

country dealt with varieties, which had exotic germplasm in their parentage.

Of the 129 groundnut varieties released during the period from 1900 to 2003, 43 originated from introduced materials/land races followed by mass selection; 8 originated from mutation breeding, and 78 originated from the direct hybridization and selection. These figures clearly demonstrate that groundnut breeders are now relying increasingly on cross breeding and selection schemes. Earlier efforts were concentrated on yield *per se* with little attention to incorporating resistance to biotic and abiotic stresses. However, with increased availability of resistance sources and phenotypic screens, efforts are now directed towards developing cultivars with wide adaptation and multiple resistances to biotic and abiotic stresses.

There has been only limited use of genetic resources including wild *Arachis* species and their interspecific derivatives in breeding programs. It is necessary that diverse germplasm with beneficial traits are channeled into breeding programs to ensure broader genetic base of the breeding populations. DNA marker techniques have shown promise to discern variation at the molecular level that could be exploited to identify molecular tags linked with beneficial traits. These tags can be used in marker-assisted genetic enhancement in groundnut.

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