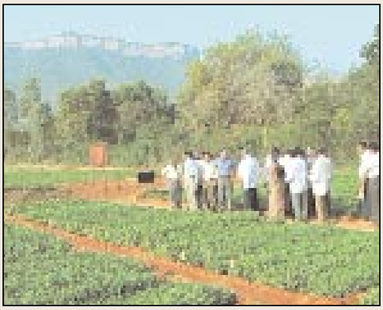
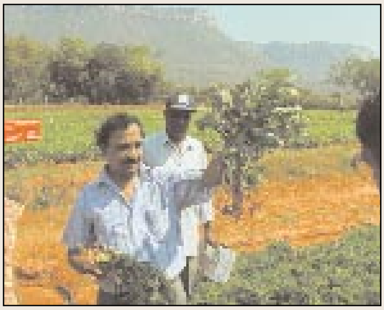


Inspecting aflatoxin screening plots at the Regional Agricultural Station, S.V. Agricultural College Campus, Tirupati, Andhra Pradesh, India.



Promising drought-resistant genotypes.

Hybridisation and Description of the Trait-based and Empirical Selection Programs

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Introduction

PARENTS SELECTED on the basis of the three main physiological traits (TE, W and HI) were used in a crossing program that was implemented at four locations in India and one location in Australia. Details of the crossing program are given below.

Crosses

There were four crosses at each centre. There were originally intended to be three common crosses and one cross involving the best locally-adapted line by a parent possessing the drought trait most deficient in the adapted line. For example, in the QDPI program, Streeton with good HI and T was crossed with a high TE parent, ICGV 86031.

At a workshop at ICRISAT in June 1997, Indian and Australian collaborators jointly decided the best crosses to be made. They considered factors such as maturity and level of expression of specific traits, as described by Rachaputi and Wright (2003). The aim was to ensure that parents which were deficient in one trait were crossed with another having high expression in that trait. Germplasm availability in both India and Australia was also taken into account. The crosses ultimately decided are shown in Table 1.

During the PN 9216 extension project (July 1997 to June 1998), potential parents were introduced into

Table 1. Crosses made at the five different breeding locations.

Location	Female Parent	Male Parent
All centres	ICGV 86031	TAG 24
All Indian centres	ICGS 76	CSMG 84-1
All Indian centres	ICGS 44	CSMG 84-1
ICRISAT	ICGS 44	ICGS 76
Jalgaon	JL 220	TAG 24
Tirupati	K 134	TAG 24
NRCG	GG 2	ICGV 86031
Kingaroy	Streeton	ICGV 86031
Kingaroy	Streeton	CSMG 84-1
Kingaroy	TAG 24	CSMG 84-1

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Breeding of Drought-resistant Peanuts – Edited by A.W. Cruickshank, N.C. Rachaputi, G.C. Wright and S.N. Nigam
ACIAR Proceedings No. 112 (printed version published in 2003)

Australia via the Australian Quarantine and Inspection Service (AQIS). Unfortunately ICGS 44 and ICGS 76 were not available for crossing in time. Comparable crosses were made with the best available material.

Minimising the impact of maturity

The June 1997 workshop discussed at length the issue of crop maturity and its potential confounding effect on the drought breeding selection experiments. Crop phenology can have a strong impact on pod yield performance under drought, via drought escape factors. Therefore during the evaluation phase selected lines must maintain a comparable maturity duration so that genotypic comparisons are not confounded by maturity differences, such as drought escape or pod loss.

It was ultimately decided that parents of relatively similar maturity (c. 110–120 days in India) be used in the hybridisation phase. This approach resulted in segregating populations of relatively uniform maturity on which selection was subsequently practiced. This ensured that any measured genetic gain in pod yield performance was achieved through selection for our drought 'resistance' traits.

To facilitate this process, a specific crop duration (in terms of a thermal time target such as 1500 Growing Degree Days (GDD)) was used as a selection criterion. This specific target varied slightly between locations, and was based on long-term climate analysis to determine optimum maturity for a region or location, using the analysis reported by Wright (1997).

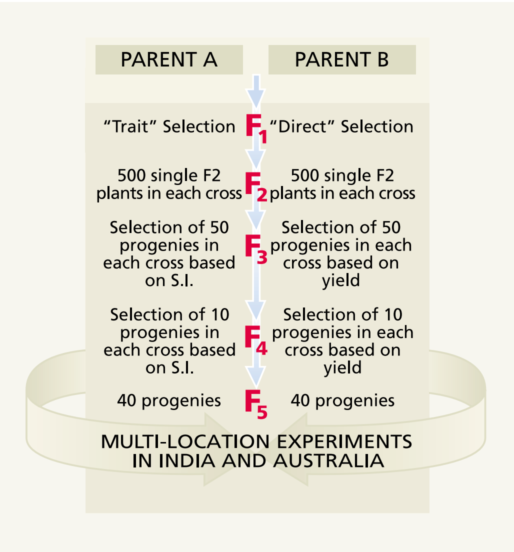

Figure 1. The protocol followed for hybridisation, selection and multi-location evaluation processes for 4 crosses at each breeding location in India and Australia.

It was anticipated that in the evaluation phase of the project, selected lines would be of similar maturity, but that some lines may have significantly different maturity. The latter could therefore be harvested at their 'optimal' maturity, and subsequently classified into separate maturity classes to enable a non-confounded analysis. In practice, the greatest maturity differences occurred among crosses. As crosses were kept separate through the selection phase, harvests of crosses could be staggered. This allowed harvest at near-optimal maturity.

Selection Protocols

Trait (indirect) program

This program combined high TE, HI and T traits using a Selection Index approach.

The trait-based approach necessarily involved intensive measurements on large numbers of progeny bulks from the F_3 onwards. These numbers were less than in a normal breeding program, but still comprised large numbers for intensive physiological measurement. Considering the existence of the apparent negative association between HI and TE, it is considered that these numbers of plants are justified in order to increase the chances of breaking the apparent genetic correlation.

The trait-based selections were made using a selection index (SI) approach described by Nigam and Chandra (2003). The form of SI was consistent over all crosses and locations. In the first round of selection there was one environment per location. In the second round there was both a 'stressed' and a 'non-stressed' environment at each location. In some cases the stressed environment was simply rainfed, in other cases it was a 'managed stress' created by selectively withholding irrigation.

The timetable of activities is represented in a flowchart (Figure 1) and outlined below.

- The F_1 plants from the initial crosses (c50 plants/cross) were grown out under non-stressed conditions as spaced plants to maximise seed multiplication.
- The F_2 seed from these crosses was grown out as spaced plants to maximise seed multiplication for the F_3 populations (assumed to be c1000 seeds/cross, based on c25 seeds/plant).This population was then divided equally between 'trait' and 'empirical' selection approaches (c500 F_2 plants/cross).
- $F_{2:3}$ progeny bulks (derived from the spaced F_2 plants, c50 seeds/row @ 20 cm spacing) were planted out and grown under water-non-limiting conditions.

- All $F_{2:3}$ progeny bulks were assessed for pod yield, TDM, TE (via SLA and SPAD), HI and T (using the reverse engineering approach of Wright *et al*. (1996), by sampling 0.5 m^2 quadrats at maturity. SPAD (and in some cases SLA) were measured 2–3 times during the crop growth cycle. As soon as possible after this data had been collated and analysed, a selection index (SI) value was calculated for each progeny, and the top 10% of progeny bulks (or the top 50 if n<500) carried forward to the $F_{2:4}$ generation. Some 400 progenies (including both trait-based and empirical selections), incorporating representative members from each cross, were carried forward at each centre.
- The carried forward $F_{2:4}$ progeny bulks were then planted out under both stressed and non-stressed conditions, and the same measurements made as for the F_3 generation. The ability to select progenies under both stressed and non-stressed conditions enabled an assessment of the relative merit of selection environment during the final evaluation studies. This further cycle of selection was implemented in the F_4 generation, and the top 10% (top 20% at Kingaroy) of the progenies were advanced.
- The selected $F_{2:4}$ families were used to generate five $F_{2:5}$ families at each breeding site for each selection method. In India, these $F_{2:5}$ families from both selection methods were advanced to $F_{2:6}$ and their seed increased. The replicated field trials, conducted in 2000-01, consisted of 192 $F_{2:6}$ families, three each from no-moisture-stress and managed-moisture-stress for trait selection method, and six from the empirical selection method for each cross/breeding site combination. In Australia, the $F_{2:5}$ seed was adequate to plant the multi-site evaluation.

Empirical (direct) program

In order to maintain consistency between empirical and trait-based selection protocols, the empirical selection procedure practised pod-yield selection at the same time as the trait-based measurements/selections (i.e. in $F_{2:3}$ and $F_{2:4}$ generations). In essence, the procedure was similar to the plan for trait-based selections, except that selections were made in an appropriate target environment as chosen by the relevant breeding program (for example, under rain-fed or irrigated conditions at the main experimental site, like normal practice for the local breeding program). By the end of the selection cycles, the empirical selection approach carried out at the four centres in India, and Kingaroy centre in Australia, supplied a subset of $F_{2:5}$ progenies for inclusion in the multi-location testing. As for the tait-based approach, selection for yield was strictly within maturity classes to avoid confounding the effects of crop phenology, drought escape and yield-determining traits.

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