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Development and exploitation of novel genetic markers in the improvement of chickpea and pigeonpea

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Background

The chickpea and pigeonpea breeding projects in ICRISAT are aimed at alleviating the constraints to the production of the crops in many regions of Asia and Africa. Production constraints for chickpea include: *Fusarium* wilt, root rot caused by *Rhizoctonia* spp., *Botrytis* mould, *Ascochyta* blight, viruses, *Helicoverpa* insect, low night temperatures, and drought. Constraints for pigeonpea include: *Phytophthora* blight, viruses, nematodes, *Helicoverpa* insect, and drought. Depending on the geographical region in which the crops are grown, there are benefits to be gained from planting varieties that have particular crop durations. Extra short, short, medium and long duration forms of pigeonpea and chickpea are being developed to meet these needs.

These important criteria are utilised by breeders in selecting improved varieties. Selecting for the optimal expression of any one of these characters and combining as many as possible of the desirable attributes can pose enormous challenges for the breeder. The development of DNA markers has provided an opportunity for assisting breeders in the procedures of selection. The identification of a DNA marker closely linked to the agronomic character sought allows selection for the DNA marker in segregating populations, rather than for the agronomic character, and selections can be made at an early stage of the plant's development rather than waiting for the plant to grow to maturity. Of greater importance is the ability to select those attributes which are difficult to detect for any one of a number of reasons. The latter may arise due to the character's susceptibility to environmental factors, to its being influenced by other segregating genes, to the complexity of the assay used to detect it, to inadequate inoculum in the case of a pathogen, or to the temporary absence of a vector in the case of a virus disease.

Project purpose

- Develop novel genetic markers which can be used in breeding programmes
- Define DNA markers tightly linked to specific agronomic traits in chickpea and pigeonpea
- Develop a genetic map for both crops which allow other traits (both qualitative and quantitative) to be located and selected in further breeding programmes

Outputs

For both chickpea and pigeonpea the pattern of diversity within a small sample of lines was assessed by RFLP analysis and compared to the diversity in *Pisum* (Figure 1). Informative markers were more difficult to find in both chickpea and pigeonpea than is the case for pea. This puts a high premium on surveys of genetic diversity and implies that judicious choice of maximally informative crosses is important for the successful application of molecular marker techniques to breeding objectives in these species.

Both molecular marker techniques – restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) analysis – have been shown to be of value as markers in the two target species.

RFLP probes from a cDNA bank from *Pisum* provided polymorphic markers for bothspecies (Figure 2). A member of the *Ty*1-*copia* class of retroelements proved to be polymorphic in chickpea. It seems likely that a similar type of element could be found in

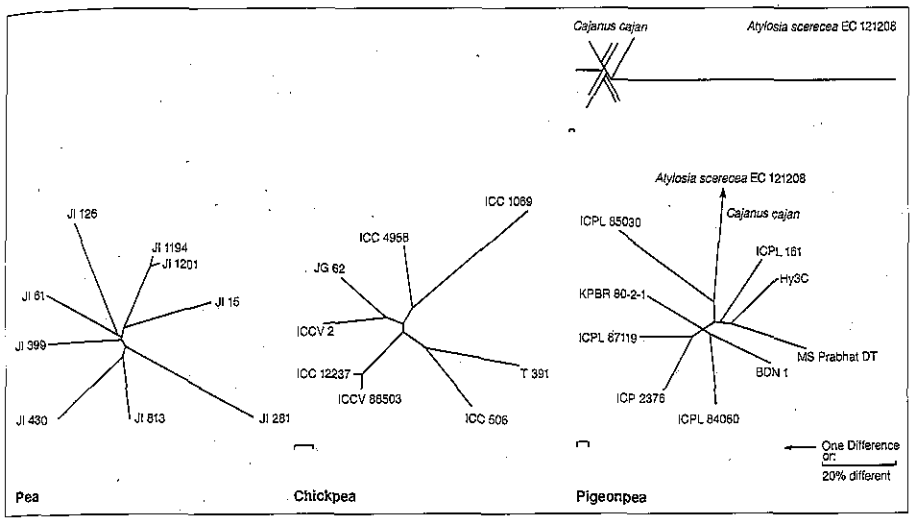

Figure 1 RFLP analysis of genetic diversity among accessions of three legume species

Cajanus, but that the particular element used in this project was either absent from this species, or diverged to such an extent that its presence could not be detected. The (GAXA)n family of repeats has been studied in *Pisum*, and preliminary results suggest that this class of sequence could be highly informative in both *Cicer* and *Cajanus*. Studies in *Pisum* from this project, however, suggest that this marker type is likely to cause problems in both diversity studies and linkage analysis. Despite the extreme variability of the hybridisation patterns obtained with this probe, it is unlikely to be useful in genetic analysis. Experiments in the F_2 of ICCV2 x JG62 confirm that the complex hybridisation patterns cannot be scored reliably, and that the banding patterns do not appear to segregate in a simple Mendelian fashion.

AFLP markers have been found to be informative in both species, and have been used in linkage analysis of F_2 populations of both species. This worked much better for chickpea than pigeonpea. The reason for this is not clear; although the pigeonpea F_2 population did not, in general, show the segregation patterns expected from the parental screens, the individual F_2 plants showed essentially the same banding pattern, implying that the AFLP method is sufficiently reproducible to undertake segregation analysis in a pigeonpea RI population.

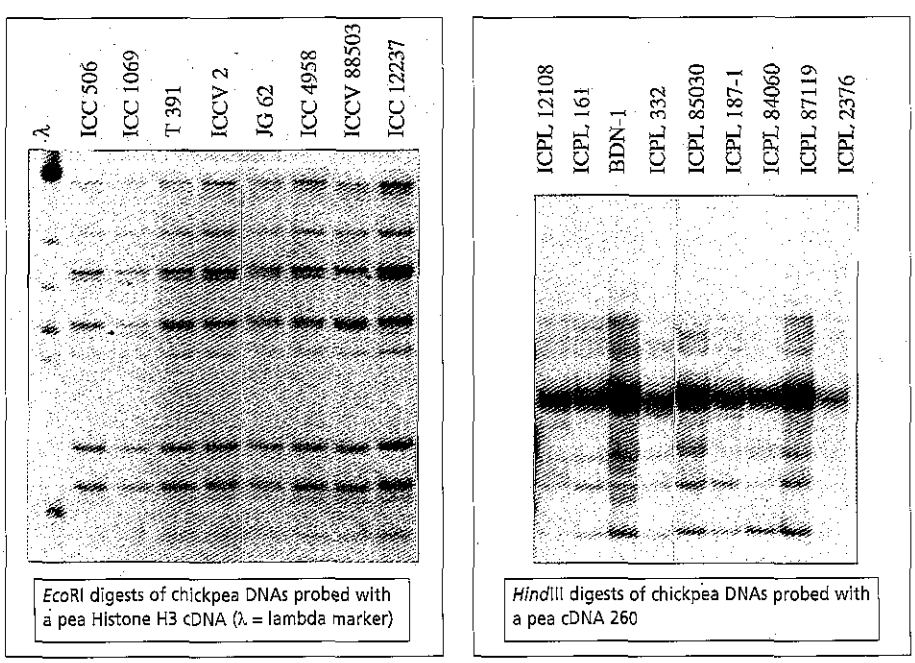

Figure 2 Southern blots of pigeonpea and chickpea DNA digests reveal informative markers when pea cDNAs are used as hybridisation probes. This marker system does not reveal markers as efficiently as AFLP, but can provide co-dominant anchor loci in map construction and for comparative genetic analysis

The F_3 seed sent to ICRISAT were scored for flower colour, allowing the identification of F_3 individuals which were heterozygotes at this locus, and also confirming the monofactorial inheritance of the trait. The segregation data for this trait were updated accordingly, but no linkage to a marker locus has been detected. The final version of the linkage map is shown in Figure 3. The number of loci needed for generation of a complete low density linkage map is about 130 markers.

Some of the presumed polymorphic markers from this project may correspond to DNA methylation differences rather than structural alterations (e.g., point mutation or insertion/deletion events). This confirms the utility of AFLP markers which have been scored reliably and successfully in the F_2 population, and suggests that such a rich marker method is nearly essential for chickpea genetics. The application of AFLPs to the analysis of chickpea should probably avoid the use of methyl-sensitive enzymes (such as *Pst*I) which were used in this project. The enzyme *Hind*III is not sensitive to C methylation and could be used as a replacement for *Eco*RI that is usually supplied with commercial kits.

Impact and uptake

The project has demonstrated that AFLP analysis is the method of choice for marker analysis in chickpea and pigeonpea. In part this is because the diversity within these species is relatively low, which means that searching for RFLP markers is time-consuming. From the AFLP analyses in pea, chickpea and pigeonpea there are good estimates of the fraction of restriction fragments which distinguish the parents of segregating populations studied. The pea lines differ, on average, for about 14% of AFLP bands, the chickpea lines ICCV2 and JG62 differ for about 2% of AFLP bands,

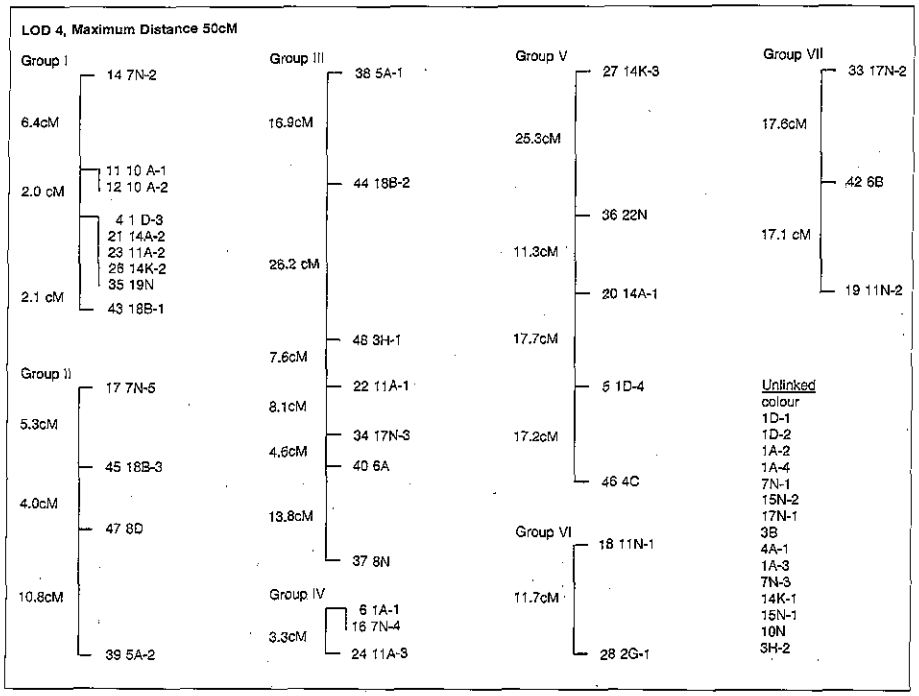

Figure 3 Linkage map from ICCV2 x JG62 F_2 in chickpea

and the pigeonpea lines ICPL161 and MS Prabhat DT differ for about 3% of bands. This means that highly informative crosses will be more difficult to find than is the case for pea.

The choice of parents for mapping populations is critical, especially given the low frequency of polymorphism observed. Consequently, a major recommendation from the project is:

- A diversity analysis of chickpea and the pigeonpea germplasm available at ICRISAT should be undertaken using the AFLP method, and should include several lines directly used in ICRISAT breeding programmes. The analysis should use a small number of primer combinations, and could sample the available germplasm on the basis of existing information. This analysis will define suitable parents for the development of mapping populations at ICRISAT.
- Recombinant inbred populations segregating for traits of interest are needed for the identification of molecular markers for marker-assisted breeding. Several of the traits of interest to ICRISAT breeders are extremely difficult to measure, and probably subject to variation between growing seasons. *Helicoverpa* resistance is a case in point, and the development of RI populations generated from crosses between differential parents are being established.

A substantive transfer of AFLP technology to ICRISAT, through JIC staff collaboration in a course in AFLP analysis conducted by ICRISAT in 1996, has been achieved. This method can now be applied to any of the segregating populations available at ICRISAT, and not solely those of chickpea and pigeonpea. The F_3 seed of the mapping population was transferred to ICRISAT, and is well on the way to generating a recombinant inbred population suitablefor linkage analysis. This population will also be progeny tested for *Fusarium* wilt resistance in order to identify a marker linked to the determinant of *Fusarium* wilt resistance segregating in this population.

The scope, materials and expertise exist to achieve the completion of the molecular map and the identification of genetic markers for key agronomic traits of chickpea. These are important and strategic objectives which will strengthen the capacity of national programmes to undertake marker-aided selection. ICRISAT is currently establishing the capability to continue mapping and marker studies at Patancheru, and is eager to involve the expertise of JIC in completing this work to the point of developing technologies for use by National Agricultural Research Systems (NARS).