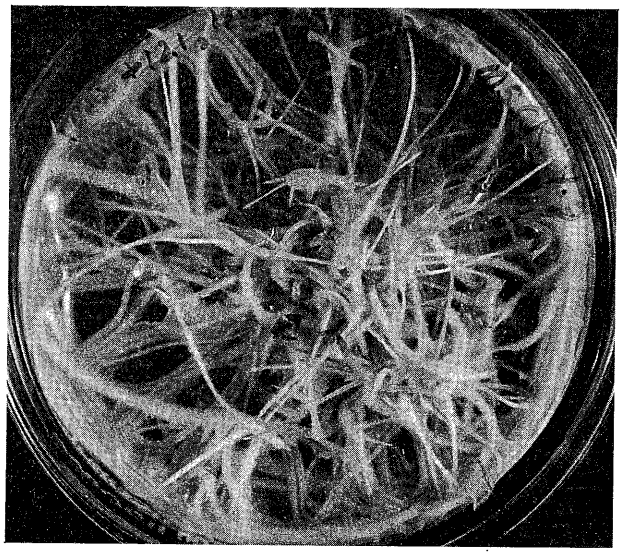

Figure 1. Profuse rooting as induced by *A. rhizogenes* with plasmid vector pBI 121.

for their viability, root morphology, mean root length of 10 longest roots, and tumor size.

All the transformed cultures survived on the selection media, while the normal stem pieces necrosed. This confirms the integration of Kanamycin or Hygromycin resistance genes. The pBI 121-infected chickpea explants produced roots which grew very fast and completely covered the surface of the medium within 3 weeks (Fig. 1). The roots were profuse, pure white, and did not produce tumors. The mean root length was 55 ± 10 cm, about 30 times more than that of roots induced by pBI 333 (2 ± 1 cm). As is the general case, the hairy roots that developed after infecting chickpea with pBI 333 were plagiotropic. However, in the case of pBI 121-infected chickpea, the roots spread only horizontally over the surface of the agar medium. The growth of pBI 333 roots was very slow and they produced tumors at many places on their surface (Fig. 2). Both the tumors and roots were light green in colour.

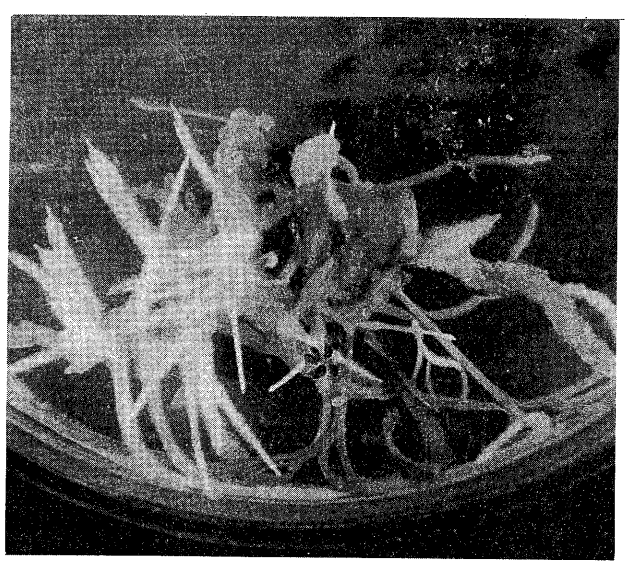

Figure 2. Rooting and tumorigenesis induced by *A. rhizogenes* with plasmid vector pBI 333.

GUS Assay

Hairy roots obtained from pBI 121 and pBI 333 infection were assayed for GUS gene activity by the histochemical method (Jefferson et al. 1987). They were incubated overnight with x-gluc at 37°C. All the roots turned blue, thereby confirming the presence of the GUS gene in the plant genome.

For obtaining agrobacteria-free cultures, four subcultures of hairy roots were on MS + antibiotic (Kanamycin or Hygromycin) + claforan. The hairy roots were then transferred for callus induction to B5 medium supplemented with 1.5 mg L^{-1} BAP and 1 mg L^{-1} NAA. Good callusing was observed after 30 days of incubation at 25 ± 1°C under fluorescent light/dark cycle of 16/8 h. Efforts are being made to regenerate plants from the transformed calli.

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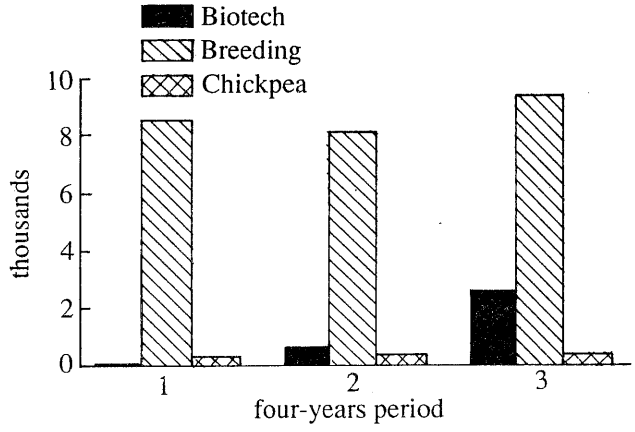
Biotechnology and Chickpea Breeding

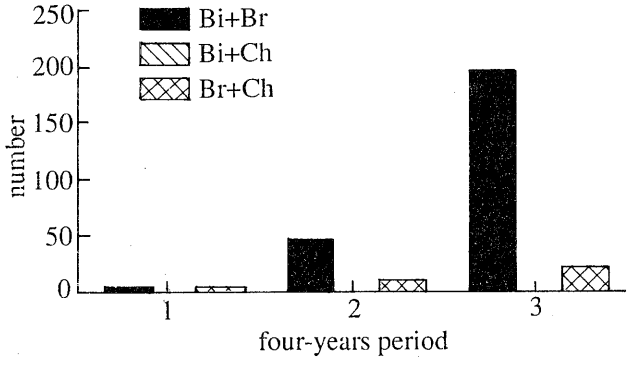
Henk A. van Rheenen (ICRISAT Center)

Searching through the AGRICOLA database for the periods (1) 1979-82; (2) 1983-86; and (3) 1987-91 on the



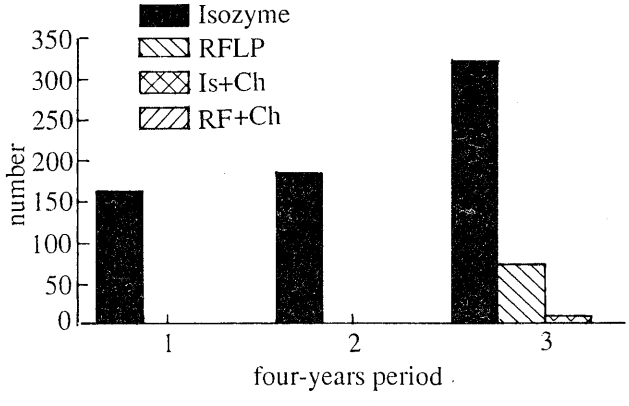
Period 1=79-82; 2=83-86; 3=87-91

1a.Biotech, Breeding, and Chickpea



Period 1=79-82; 2=83-86; 3=87-91

1b.Biotech (Bi), Breeding (Br) and Chickpea (Ch)



Period 1=79-82; 2=83-86; 3=87-91

Figure 1. Publications AGRICOLA: 4 years' period.

key words Biotechnology, Breeding, and Chickpea, leads to some interesting results. There were almost no publications on Biotechnology for the period (1); there was a fair increase for period (2); and a further significant increase for period (3). The set of Breeding and Chickpea publications remained rather steady in number over the three periods (Fig. 1a). In combination, Biotechnology + Breeding flourished; Breeding + Chickpea did increase; but joint Biotechnology + Chickpea publications were notoriously absent (Fig. 1b). Singling out specific biotechnology applications that relate to genome mapping, Isozyme and Restriction Fragment Length Polymorphism (RFLP) analysis, it appears that both increased, but RFLP work only since 1987 (Fig. 1c). We found 9 publications on isozyme analysis, but none on RFLP analysis of chickpea!

During his sabbatic study leave in 1991, the author conducted isozyme and RFLP analyses at the University of California, Davis, USA and Washington State University, Pullman, USA, and Randomly Amplified Polymorphic DNA (RAPD) analyses at Pullman.For that purpose two sets of varieties were used as shown in Table 1. The protocols used for isozyme analysis resembled those described by Wendel and Weeden (1989) and Kazan and Muehlbauer (1991); the RFLP protocols resembled those described by Kochert et al. (1989); and the RAPD analysis was on the lines followed by Williams et al. (1990).

The set of 8 chickpea varieties was subjected to the analysis of the enzymes PGI, PRX, ACO, SKDH, ME,

Table 1. Sets of chickpea varieties used at the University of California, Davis, USA (1) and Washington State University, Pullman, USA (1 and 2) for isozyme, RFLP, and RAPD analysis.

Set 1		Set 2	
1. Surutatu 77	(k; FWR)	1. ICC 4951	(d; SRV)
2. ICCV 2	(k; FWR)	2. ICC 4958	(d; LRV)
3. UC 5	(k; FWS)	3. ICC 12257	(d; SRV)
4. UC 15	(k; FWR)	4. ICC 14196	(k; DRR)
5. JG 62	(d; FWS)	5. Surutatu 77	(k; DRS)
6. ICCV 10	(d; FWR)	6. ICC 12269	(d; DRR)
7. ICCV 88101	(d; FWR)	7. ICCV 88202	(d; DRS)
8. ICCV 88202	(d; FWR)	8. ICC 506	(d; PBR)
		9. ICC 5716	(d; PBS)
		10. ICCX 850786	(d)

k/d = kabuli/desi; FWR/S = Fusarium wilt resistant/susceptible; S/LRV = Small/large root volume; DRR/S = Dry root rot resistant/susceptible; PBR/S = Pod borer resistant/susceptible.

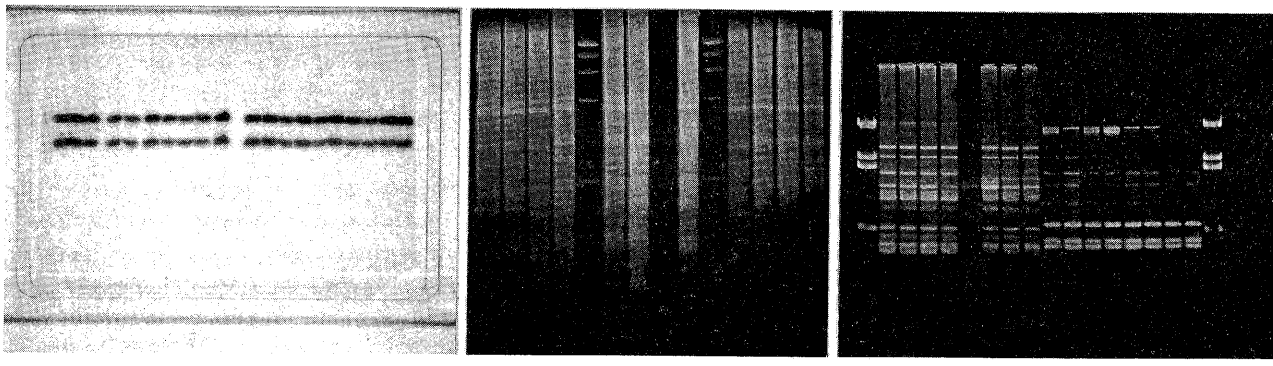


Figure 2. Isozyme, RFLP, and RAPD analyses for chickpea varieties of set 1 and 2 (see Table 1).
2a. 6 PGD on starch gel; set 2, from left to right varieties 1 to 10, replicated.
2b. DNA extracted, digested with EcoR 1 and separated on agarose gel; set 2, from left to right varieties 1-4, maker lane, varieties 5-8, marker lane, varieties 9, 10, and two extras.
2c. RAPD analyses: at extreme left and right: one marker lane.
eight test lanes on the left: primer CS 16.
eight test lanes on the right: primer CS 20.
for both primers: first 4 test lanes: varieties 1-4 of set 1, next 4-test lanes: varieties 5-8 of set 1.

Primers manufactured by Genosys Biotechnologies Inc.

MEST, LAP, MDH, and DIA, and the set of 10 varieties to the analysis of the enzymes AAT, PRX, ACO, ALDO, PGM, MEST, AAP, ADH, xEST, xGAL, 6PGD, ACP, and LAP. Only one polymorphism was detected, viz., for AAP (L. Alanyl × Naphthylamide Aminopeptidase): 9 varieties showed bands at distances 2.9 cm and 3.1 cm from the line of reference, but ICC 14196 missed the band at 2.9 cm. As also reported by other researchers, chickpea has shown little isozyme polymorphism (Fig. 2a).

Table 2. Proportion of polymorphic loci as examined by electrophoresis for chickpea and barley.

Varieties tested	Estimated number of genes examined	Proportion polymorphic	Author
		Chickpea	
20	27	0.15	Oram et al. 1987
1392	13	0.23	Tuwafe et al. 1988
25	31	0.00	Gaur and Slinkard 1990
8	19	0.00	Present
10	32	0.03	Present
		Barley	
28	?[1]	0.30	Weaver and Hedrick 1991

1. ? = Not known.

Our RFLP analyses failed to show polymorphism. For the set of 8 varieties we used the restriction enzymes EcoR I, EcoR V, Hind III, Hal III, BamH I, Xba I, and as probe R-DNA from pea; for the set of ten varieties we used EcoR I and Dra I and probed with genomic DNA from chickpea. Figure 2b shows the good results of DNA extraction and digestion by restriction endonuclease EcoR I.

The RAPD analyses were more successfully used to detect varietal differences, and gave sharp banding patterns (Fig 2c). The DNA of the set of 8 varieties was hybridized with 14 different primers containing 10 bases each, and 3 of these showed polymorphism. For the set of 10 varieties we selected 4 primers out of the 14 used earlier, and here all 4 showed polymorphism. Apparently these primers with short sequences were able to detect genomic variations at relatively high frequency.

Compared with, e.g., pea and lentil, chickpea seems to show little polymorphism at the single copy DNA and primary DNA product level. I have compiled some data on proportions of polymorphism of chickpea and barley (Table 2), and these seem to confirm the above observation. This phenomenon may have been caused by narrow

ancestry and reproduction by self-fertilization. The important thing is that breeders may achieve only limited success in genetic advancement, and may therefore have to resort increasingly to interspecific crossing and mutation induction to increase their chances of success.

Acknowledgement. The help and support by Dr Paul Gepts and his group, and Dr Fred Muehlbauer and his team in conducting the analyses are gratefully acknowledged.

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Entomology

Evaluation of Different Insecticides for the Control of Gram Pod Borer, *Helicoverpa armigera* (Hübner), on Chickpea

R.S. Giraddi, S.S. Patil, B.S. Goudreddy, and **B.V. Patil** (National Agricultural Research Project, Agricultural Research Station, Bidar 585 401, Karnataka, India).

Chickpea is one of the main pulse crops grown in Karnataka State covering an area of 155 000 ha, with an annual production of 62 000 tonnes and an average yield of 421 kg ha^{-1} (Directorate of Economics and Statistics 1988). The crop is attacked by insect pests at various stages of its growth, but the pod borer *Helicoverpa armigera* (Hübner) is the most destructive. This insect pest

Table 1. Efficacy of different insecticides in controlling the pod borer, *Helicoverpa armigera* and in increasing the seed yield of chickpea in Bidar, Karnataka, post-rainy season, 1987.

Treatment	Mean pod damage (%)	Mean yield (t ha^{-1})
Quinalphos 25 EC at 0.25 kg a.i. ha^{-1}	5.0 (12.8)[1]	1.07
Quinalphos 45 EC at 0.25 kg a.i. ha^{-1}	2.9 (9.9)	1.23
Quinalphos 45 EC at 0.12 kg a.i. ha^{-1}	6.9 (15.2)	1.10
Phosalone 35 EC at 0.35 kg a.i. ha^{-1}	4.3 (7.2)	1.12
Endosulfan 35 EC at 0.35 kg a.i. ha^{-1}	1.6 (7.2)	1.40
Penthoate 50 EC at 0.5 kg a.i. ha^{-1}	4.0 (11.6)	1.35
Malathion 50 EC at 0.5 kg a.i. ha^{-1}	6.1 (14.3)	1.13
Malathion dust 5% at 1.0 kg a.i. ha^{-1}	6.1 (14.4)	1.09
Quinalphos dust 1.5% at 0.3 kg a.i. ha^{-1}	3.3 (10.6)	1.26
Untreated control	12.2 (20.5)	0.67
SEm	± 0.91	±0.67
CV (%)	12	10

1. Figures in parentheses are angular transformation values.