How to accelerate the genetic improvement of a recalcitrant crop species such as chickpea

H. A. van Rheenen, R. P. S. Pundir and J. H. Miranda
International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India

Chickpea (*Cicer arietinum* L.) has shown little polymorphism in isozyme and Restriction Fragment Length Polymorphism (RFLP) studies which may be an indication of limited genetic variation. Mutagenesis and interspecific hybridization will increase the variation and can be useful for plant breeding purposes. Work conducted at ICRISAT Center in these fields is reported and reviewed.

CROP improvement literature abounds with success on breeding for resistance to biotic and abiotic stress factors, and chickpea (*Cicer arietinum*) forms no exception in this field[1]. However, strides made in breeding for yield improvement of chickpea seem to be less impressive. During a consultancy visit to ICRISAT Center, in 1988, Kenneth J. Frey, Iowa State University, Ames, Iowa, USA, called chickpea a 'recalcitrant' crop species, meaning that it was not very amenable to genetic yield improvement, in spite of the many efforts to breed for yield increase during the last three decades. This observation seems to agree with comments made by Saxena and Johansen[2] that two decades of chickpea breeding had failed to result in significant yield increases. Although another reference quoted an example of an annual yield increase[1] of 1.3%, in general, the expectations of yield improvement have probably been higher than the actual achievements. Also, the world mean yield records of chickpea show little increase from 1960 to 1990, compared to wheat and soybean (Figure 1). It needs to be realized though, that these yield data also reflect factors other than genetic yield potential.

Possible causes of recalcitrance

The expected genetic yield advance is mostly dependent on the available genetic variation of a character; and on its heritability (H), as expressed in the formula[3]: $G_s = (K)\ (\sigma A)\ (H)$, where G_s = genetic advance, K = selection differential and σA = phenotypic standard deviation.

The heritability for yield is generally low in agricultural crops, and we would not expect chickpea to be different from others in this respect. But the case may be different for genetic variation. In the literature we found nine references on isozyme studies in *Cicer*, and five observed the infrequent occurrence of polymorphism. Similarly, Restriction Fragment Length Polymorphism (RFLP) analyses revealed little polymorphism in the cultivated chickpea (Chuck Simon, pers. commun.). If limitation in genetic variation is the main cause for slow genetic yield advance, we may resort to methods that can increase the variation. In this article two such methods: mutation breeding, and interspecific hybridization have been discussed.

Mutation breeding of chickpea at ICRISAT

During 1981/82, seeds of chickpea cv. Chafa (a short-duration, desi type variety released in Maharashtra, India) were treated with ethyl methane sulphonate (EMS) mutagen, aiming to produce rare types and to increase the already-existing diversity for morpho-agronomic traits. Seeds of Chafa were given 12 different treatments of EMS soaking (combinations of concentrations 0.05, 0.10, 0.15, 0.20, 0.30 and 0.60%, and soaking periods of 2, 3, 4 and 6 h), and were sown in the field. Germination in all the treatments was normal, ranging from 85 to 91% (Table 1). The seeds harvested from the M_1 were advanced to the M_2 generation. Each

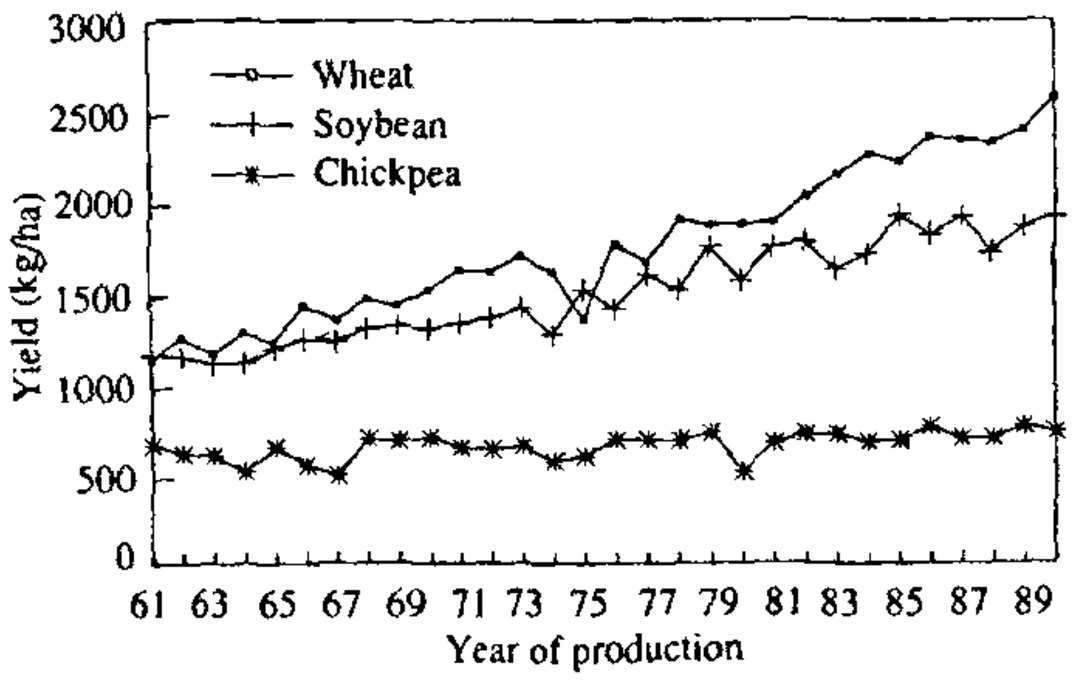

Figure 1. World productivity of wheat, soybean and chickpea (kg/ha).

Table 1. Frequency of morphological mutants in EMS-treated progenies of chickpea cv. Chafa, ICRISAT Center, post-rainy season, 1981/82

Treatment*	Mutagen dose**	M_1 germination (%)†	Morphological mutants from M_2‡			
			Albino	Nonalbino Sterile	Viable	Total
0.05%, 2h	0.10	91.4	0	4	1	5
0.05%, 4h	0.20	89.6	1	12	11	24
0.10%, 2h	0.20	90.0	1	1	6	8
0.10%, 4h	0.40	88.4	0	46	44	90
0.20%, 2h	0.40	87.8	1	8	19	28
0.15%, 3h	0.45	91.2	3	13	14	30
0.20%, 4h	0.80	89.2	2	23	28	53
0.15%, 6h	0.90	86.4	2	32	37	71
0.30%, 3h	0.90	90.4	3	23	39	65
0.30%, 6h	1.80	84.8	1	10	32	43
0.60%, 3h	1.80	89.2	3	16	13	32
0.60%, 6h	3.60	85.4	1	6	11	18
Control water, 6h	0	90.0	0	—	—	0

*Concentration of EMS and time.
**Product of concentration × duration.
†Each treatment with 500 seeds.
‡Each treatment had approximately 1200 plants.

treatment had about 1200 plants among which mutants were identified. The 0.10%, 4 h treatment gave the best result. The mutagen doses (EMS concentration × duration: an arbitrary measure) of 0.4–1.8 were equally effective, whereas the lower and higher doses were less effective. Some viable mutants identified from this work were: pale-green foliage, prostrate growth habit, entire leaflet margin, acuminate leaflet shape, narrow leaflets, brachytic leaves, large leaves, fewer leaflets, rectangular vexillum, open flower, short stature, thick stem, upright canopy, flattened pods, twin pods, large pods, glabrous stem and mutants for increased seed yield. Many of these morphological features were seen in the *Cicer* genus for the first time and some, e.g. glabrous stem, are extremely useful for basic studies[4].

Colchicine is commonly used to induce variability by increasing ploidy levels in plants. We achieved the same in chickpea using 0.25% colchicine for 4 h. However, in subsequent generations, some plants reverted back to the normal diploids. From the C_3 tetraploid progenies of cv. Annigeri, a unique plant was identified with twin pods. Subsequently, the mutant was verified to be normal-diploid, twin-podded and wilt-resistant (trait of parent cv. Annigeri). A useful mutant induced through colchicine was thus obtained[5]. It has been reported earlier[6] that, besides doubling the chromosomes, the colchicine treatment can induce gene mutations.

In 1986, we started a mutation breeding project by irradiating chickpea seed with gamma rays. We chose two released, high-yielding, fusarium wilt-resistant, small-seeded kabuli varieties, ICCV 2 and ICCV 6. Our main objectives were to increase the seed size, and to induce determinate plant growth habit. The moisture content of the seed ranged from 8.5 to 9.4%, and the three gamma ray doses applied were 15, 30 and 45 kR. The effect of the radiation treatments on germination, plant height and several other characters was tested in a field trial that had a randomized complete block design with four replications. Each plot had four plant rows of 4 m length, and accommodated 160 seeds at sowing. The trial results showed that the highest dose of 45 kR had not adversely affected the recorded plant characteristics (Table 2). The low plant stands at 30 kR were noted but could not be explained. In two experiments, we estimated the percentage of mutations for different morphological characters in M_2 populations, in a similar way as described by Filippetti and De Pace[7] for faba bean. We detected putative chlorophyll mutants, dwarf plants, early-flowering types, plants with determinate growth habit, with small and large leaf and seed sizes, and with other variations (Table 3, Figure 2). It seemed that higher radiation doses could have given greater effects. However, Mahto *et al.*[8] observed a decrease in the germination percentage and yield, when they gamma-irradiated the seed of two chickpea varieties with doses of over 15 kR and up to 75 kR. Haq *et al.*[9] treated chickpea seed of cv ILC 195 with 15 kR gamma rays and observed in the M_2, 0.04% chlorophyll mutants, 0.17% leaf mutants, and 0.24% other morphological mutants. Corresponding figures from our study were 0.53, 0.09 and 0.11%. Kharkwal *et al.*[10] reported 1.6% chlorophyll mutants.

A data survey of use of mutagens has been taken up and the results from a search in the AGRICOLA database covering 1979-91 are compiled in Figure 3; the keywords used were chickpea and breeding, and chickpea/*Cicer* and mutation/mutant. Although the numbers in the latter category suggest that mutation breeding in chickpea has not been widely applied, some remarkable successes have been achieved in respect of disease resistance and yield improvement[11].

Table 2. Effect of gamma radiation treatments of chickpea seed on seedling emergence, plant survival, days to flowering, plant height at harvest, yield and 100-seed weight

Variety* and treatment	Plant count (%)		Days to flowering	Plant height (cm)	Yield (t/ha)	100 seeds weight (g)
	25 days after sowing	At harvest				
ICCV 2						
A, 0 kR	36	33	35	28	1.0	23
A, 15 kR	46	43	31	25	1.1	25
A, 30 kR	26	24	36	27	0.8	23
A, 45 kR	48	43	33	28	1.6	23
B, 0 kR	25	22	33	30	1.0	24
B, 15 kR	25	24	32	29	1.0	26
B, 30 kR	16	13	35	31	0.8	26
B, 45 kR	34	30	33	29	1.2	26
ICCV 6						
A, 0 kR	51	45	57	33	1.5	19
A, 15 kR	54	53	62	34	1.6	18
A, 30 kR	26	25	62	32	0.7	19
A, 45 kR	49	51	62	31	1.6	18
B, 0 kR	48	48	58	31	1.8	18
B, 30 kR	36	34	57	31	1.4	19
Mean						
0 kR	40	37	46	31	0.3	21
15 kR	45	43	47	31	1.3	22
30 kR	26	24	48	30	0.9	22
45 kR	45	44	48	30	1.5	21
SE ±	6.1	6.4	0.7	1.9	0.2	0.5
Mean	37.1	34.9	44.8	29.8	1.2	21 9
CV	33 1	36.8	3 3	12.9	28.2	5.0

*A: Single plant progeny bulk; B: Breeders seed.

Table 3. Putative mutants observed in M_2 populations of chickpea plants derived from gamma-radiated seed

Variety* and treatment	Population size	Putative mutants (%)	
		Chlorophyll	Other**
ICCV 2			
A, 0 kR	1933	1.50	0.26
A, 15 kR	1782	0.56	0.11
A, 30 kR	1838	0.48	0.11
A, 45 kR	1915	0.84	0.16
B, 0 kR	1830	0.27	0.05
B, 15 kR	1890	0.37	0 48
B, 30 kR	1841	0 87	0.49
B, 45 kR	1820	0.66	0.33
ICCV 6			
A, 0 kR	2037	0.79	0.15
A, 15 kR	2000	0.65	0.00
A, 30 kR	1999	0.60	0.15
A, 45 kR	1995	0.85	0 00
B, 0 kR	2019	0 00	0.00
B, 30 kR	2057	0 44	0.15
Mean			
0 kR	7819	0.64	0 12
15 kR	5672	0.53	0.19
30 kR	7735	0.59	0.19
45 kR	5730	0.79	0.16
Overall mean	26956	0.63	0.17

*A: Single plant progeny bulk; B. Breeders seed.
**Dwarf, determinate growth, small and large leaf, large seed, early flowering, other abnormals.

Interspecific hybridization at ICRISAT: state of the art

Wide hybridization is one of the potential means of broadening the genetic base of a crop species. In the genus *Cicer*, nine annual species occur, of which one is cultivated (*C. arietinum* L.) and eight are wild species. Of these only *C. reticulatum* and *C. echinospermum* can be easily crossed with chickpea. Crosses of other species with chickpea have not yet been successful. However, some wild species can be crossed among themselves. The species *C. judaicum*, *C. pinnatifidum*, *C. bijugum* and *C. cuneatum* hold special significance, because they possess useful traits such as resistance to diseases and fast vegetative growth. At ICRISAT Center, we are attempting to introgress desirable traits from these species into chickpea through embryo rescue and tissue culture techniques.

Conclusions

Mutation breeding and interspecific hybridization may become increasingly important for the genetic improvement of chickpea because of indications that the chickpea genome is short of polymorphism in structural

Figure 2. Variant of chickpea, isolated from gamma rays irradiated progeny of chickpea cultivar, ICCV 6, showing smaller leaf and determinate growth habit.

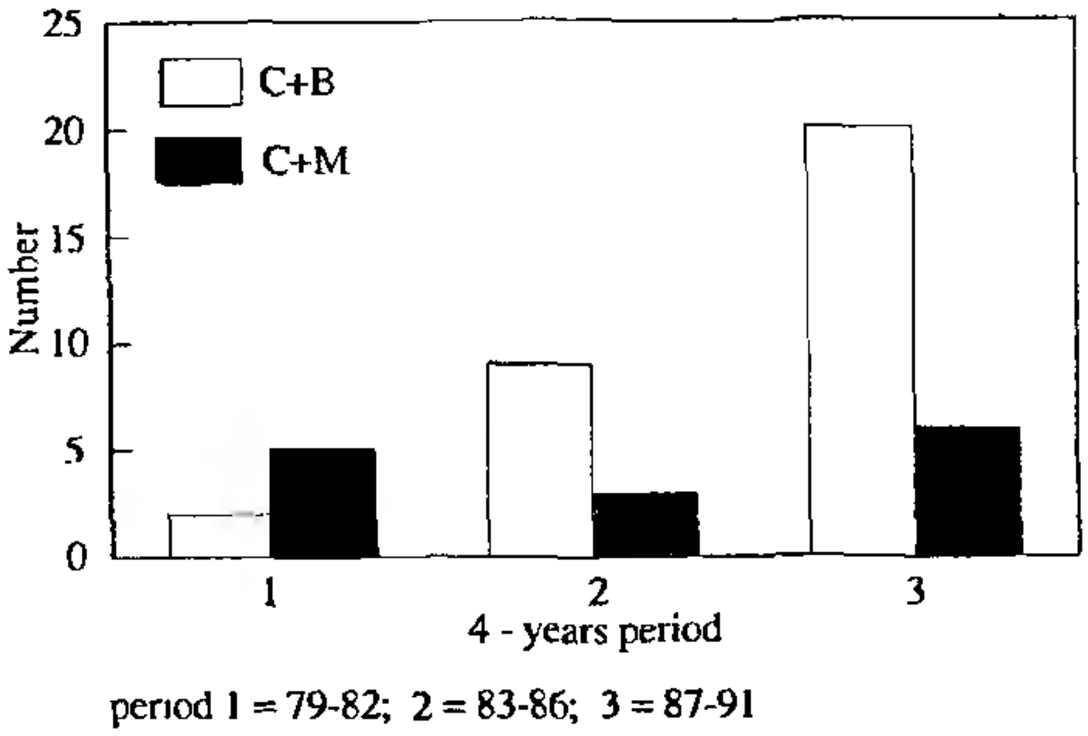

Figure 3. Number of *Cicer* publications found on searching the AGRICOLA Data Base 1979–1991, using the keywords: Breeding (B) and Mutations (M).

genes. This is supported by the successful results already achieved from the limited efforts of mutation breeding. It is suggested that not only high doses of gamma radiation are tested, but also that other mutagens are more widely applied. The use of wild species for interspecific hybridization will help to broaden the genetic base and their extensive utilization in chickpea improvement is suggested.

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Tent-roosting by the frugivorous bat *Cynopterus sphinx* (Vahl 1797) in Southern India

J. Balasingh, S. Suthakar Isaac* and R. Subbaraj*

Department of Zoology, St. John's College, Tirunelveli 627 002, India
*Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

The Indian fruit-eating bats *Cynopterus sphinx* make foliage tents using creeper plants *Vernonia scandens*. These foliage tents are made by chewing and clipping the twigs of the interior of the foliage. The number of bats roosting in a tent varied between 1 and 19. Here we report the tent making behaviour in *C. sphinx*.

Figure 1. A colony of *C. sphinx* roosting in a *V. scandens* tent.

SEVERAL species of bats are known to use modified leaves or 'tents' as day roosts. This behaviour has been reported for 14 species of phyllostomids in the genera *Artibeus, Ectophylla, Mesophylla, Uroderma* and *Vampyressa*[1,2], two species in the pteropid genus *Cynopterus*[3–5] and one species of vespertilionid, *Scotophilus kuhlii*[6]. This is the first record of the 'tent' making behaviour of *C. sphinx* by chewing the twigs of the common curtain creeper plant *Vernonia scandens*.

During field studies on the vespertilionid bat *Pipistrellus dormeri*, we came across a building at St. John's College in Tirunelveli (8° 44′ N; 77° 42′ E) with a thick patch of various species of creeper growing along its wall and trees close to the building. Long slender twigs of *V. scandens* with loosely arranged leaves were closely interwoven (at a height of ca. 5 m) drooping at their free ends. The crowns were hidden from open view by the surrounding vegetation. On 15 September 1991 at dusk we saw a chain of fruit bats *C. sphinx* emerging from this 'foliage tent'. Since then, subsequent detailed observation has been carried out between mid-September 1991 and late September 1992, to obtain more information about how the tents were made and used as day roosts.

The twigs of the interior of the foliage are chewed and clipped predominantly during pre-dawn and dusk hours to make four tents at intervals of less than half a metre. Approximately 200 twigs were cut from the undersurface of the crown either at one end or both ends to make a sort of dome-shaped tent (Figure 1). It was observed that only one bat started making a tent by cutting 10 to 20 twigs per day. In the initial period, the rate at which the 'tents' were made is higher compared to later days of tent making. However, periodically the tent's shape was maintained by cutting a few twigs by these bats. A maximum of 31 bats were recorded to be roosting in the four tents. 1 to 19 bats roosted in one tent over the seasons. The view of the bats in each tent was partly hidden by the dry and fresh drooping twigs all around.

Subsequently, we searched for other foliage roosts of *C. sphinx*. Evidently, this bat prefers palm trees (*Borassus flabellifer* – the palmyrah palm), *Areca catechu* (areca nut palm) and tall shady trees (*Polyalthia longifolia*) roosting very high up at the base of the fronds and leaves respectively.

Goodwin[3] reported palm frond modification by *C. sphinx* in Timor. *C. brachyotis* is also known to roost under palm fronds and is reported to modify roost sites[4]. Brosset[7] observed that when palm trees are not available in the vicinity, banyan trees (*Ficus bengalensis*) and Ficus trees (*Ficus religiosa*) are used for roosting. Although the number of *C. sphinx* using the palm fronds as roosts is undoubtedly greater, our observations indicate that tent-roosting behaviour using *V. scandens* is not fairly common among *C. sphinx*.

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