Collection and treatment of samples

- Collect only young leaflets showing symptoms (retain petioles). Blot excess water using newspaper or any absorbent paper and place in plastic bags. Ideally, use 'Ziplock' plastic bags. If these are not available, use ordinary plastic bags sealed carefully, or securely closed with cellophane tape. Water transpired from leaflets helps to maintain humidity within the bag.
- Collect and bag the samples as close as possible to the day of despatch.
- Leaflets in sealed plastic bags can be stored for up to a week in a refrigerator without undue deterioration.
- If leaflets cannot be collected within a week before departure, rinse the leaflets in water containing 0.01% sodium azide, blot, and then store. This reduces rotting. **Caution—sodium azide is a poison; handle with care.**
- If fresh leaflets are not available, collect about 5 g of infected leaf material, cut it into small pieces, and place the bits directly into a plastic vial containing approximately 10 g calcium chloride ($CaCl_2$). Place a small amount of non-absorbent cotton wool on top of the $CaCl_2$ so that the chemical does not touch the plant material.

Transport of virus-infected material

- Use courier mail if possible. Send a fax or e-mail message to the addressee indicating the date on which the samples were despatched.
- Insulated covers (Jet Packs) or sheets of styrofoam are suitable for packing the material.

Evaluation of an Aphid-Resistant Groundnut Genotype (EC 36892) in China

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Aphids are major groundnut pests in China, causing direct damage and also acting as vectors for virus disease transmission (Li Yuanlian 1981, Xu Zeyong 1987). Three of the four major groundnut viruses in China are transmitted by aphids in a non-persistent manner; peanut stripe virus (PStV), cucumber mosaic virus (CMV), and peanut stunt virus (PSV). An aphid-resistant groundnut genotype, EC 36892, was introduced from ICRISAT Asia Center in 1990 and evaluated in China for resistance to aphids and PStV. Three local groundnut cultivars, Huohua No. 1 (spanish type), Hua 37, and Yihua No. 1 (both hybrids between virginia and spanish types), were also evaluated in the experiment.

Greenhouse tests. Five plants of each genotype were sown in 10 cm diameter pots in a greenhouse. Each plant was inoculated with two aphids (*Aphis craccivora*), and aphid population was recorded 3 days later. Aphid multiplication rates were much lower on EC 36892 than on two local varieties (Table 1). For example, in three tests in 1991 and 1992, the average number of aphids on EC 36892 was 0.6 aphid plant^{-1} compared with 6.9 aphids plant^{-1} on the local variety Huohua No. 1.

Field trials. Field trials were conducted at the farm of the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Wuhan, in 1991 and 1992. The trials were sown in a randomized block design with five replications. Each plot was 3.3 × 2.0 m, and contained 10 rows, with a spacing of 33 × 10 cm. Inspection of aphid population and PStV disease incidence was first conducted 5 days after emergence of groundnut, and continued at 10-day intervals. Virus-free seed was used in the 1991 trial. Two rows of a susceptible groundnut variety with high seed transmission of PStV were sown between plots, to provide the primary inoculum. PStV-infected seeds of the test genotypes were used in the 1992 trial.

Table 1. Aphid resistance in three groundnut genotypes in greenhouse tests, Oil Crops Research Institute, Wuhan, China, 1991–92.

Year	Genotype	Number of aphids plant^{-1} Test 1	Test 2
1991	EC 36892	0 (22)	1.5 (16)
	Huohua No. 1	6.7 (9)	10.0 (8)
1992	EC 36892	0.2 (20)	– (–)
	Yihua No. 1	1.2 (25)	– (–)
	Huohua No. 1	3.9 (18)	– (–)

Figures in parentheses show number of plants tested

Table 2. Aphid resistance in four groundnut genotypes in field trials, Oil Crops Research Institute, Wuhan, China, 1991–92.

Year	Genotype	First peak of aphid multiplication		Second peak of aphid multiplication	
		Percentage of plants with aphids	Number of aphids 100 plants^{-1}	Percentage of plants with aphids	Number of aphids 100 plants^{-1}
1991	EC 36892	4	4	4	27
	Hua 37	7	60	33	561
	Huohua No. 1	24	330	71	1104
1992	EC 36892	2	2	16	217
	Yihua No. 1	3	8	51	838
	Huohua No. 1	34	681	79	2448

Table 3. PStV resistance in four groundnut genotypes in field trials, Oil Crops Research Institute, Wuhan, China, 1991–92.

Year	Genotype	First inspection			Second inspection			Third inspection		
		PStV incidence (%)	Significance level 5%	1%	PStV incidence (%)	Significance level 5%	1%	PStV incidence (%)	Significance level 5%	1%
1991	EC 36892	10.5	a	A	24.2	b	B	77.1	b	A
	Hua 37	11.6	a	A	37.3	b	AB	87.6	ab	A
	Huohua No. 1	15.5	a	A	46.2	a	A	92.4	a	A
1992	Yihua No. 1	4.6	b	B	66.4	b	A	88.9	b	A
	EC 36892	8.8	b	B	71.4	b	A	95.4	ab	A
	Huohua No. 1	21.1	a	A	97.2	a	A	99.6	a	A

Significance level columns–genotypes with the same letter are not significantly different at the respective confidence levels.

EC 36892 showed high resistance to aphids in both years of field trials. In 1991, there were 4 aphids per 100 plants of EC 36892, compared to 60 aphids on Hua 37 and 330 aphids on Huohua No. 1, at the first aphid multiplication peak. At the second aphid multiplication peak in 1991, there were 27 aphids per 100 plants of EC 36892, compared to 561 on Hua 37 and 1104 on Huohua No. 1. Similar results were obtained in 1992 (Table 2).

PStV incidence in the genotypes varied during the 2 years of field trials. In 1991, EC 36892 showed the lowest PStV incidence of 24.2%, compared with 46.2% on Huohua No. 1, on 16 June. On 10 Aug, incidence was 77.1% on EC 36892, compared with 92.4% on Huohua No. 1. In 1992, PStV incidence on EC 36892 was 8.8% on 25 May and 71.4% on 6 Jun, compared with 21.1% and 97.2% on Huohua No. 1 on the same dates (Table 3).

In both years, EC 36892 gave yields higher than Huohua No. 1, but lower than those of Hua 37 and Yihua

Table 4. Yields of four groundnut genotypes in field trials at the Oil Crops Research Institute, Wuhan, China, 1991–92.

Year	Genotype	Yield (t ha^{-1})	Significance level 5%	1%
1991	Hua 37	3.93	a	A
	EC 36892	3.24	b	B
	Huohua No. 1	2.44	c	C
1992	Yihua No. 1	4.46	a	A
	EC 36892	3.80	a	A
	Huohua No. 1	1.84	b	B

Significance level columns–genotypes with the same letter are not significantly different at the respective (1%, 5%) levels.

No. 1. However, maturity duration was 160 days in EC 36892, longer than in any of the three local varieties (Table 4).

Conclusions. The genotype EC 36892 showed high resistance to aphids both in greenhouse tests and in field trials. However, this resistance (and low PStV incidence in this genotype) cannot be used directly by farmers because of its long duration. However, EC 36892 is a very good genotype for aphid resistance breeding. The results also showed that Huohua No. 1, a spanish type, was much more susceptible to aphids than the two spanish × virginia hybrids, Hua 37 and Yihua No. 1.

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Survey of Groundnut Virus Diseases in Pakistan

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In Jul 1995, a survey of virus diseases was conducted in the major groundnut-producing areas in Pakistan, including Attock, Chakwal, and Rawalpindi districts. In Pakistan, groundnut is sown mainly in Apr and May. Groundnut-fallow-groundnut or groundnut-fallow-wheat are the main rotations used. However, a few farmers sow groundnut after harvesting wheat if there is sufficient soil moisture. Some farmers in the Pothar area sow groundnut in Jul, at the onset of the monsoon. More than 98% of farmers grow the well-adapted spreading variety, No. 334. Soils in the major groundnut-producing areas are sandy or sandy loam. Sowing is done either by broadcasting the seed followed by moldboard plowing, or by using a tractor-driven drill in which seeds are dropped manually. Low plant population, drought stress in the month of Jun, weeds, lack of proper machinery for sowing and harvesting, and damage by boars and other wild animals are the major problems that groundnut farmers face in Pakistan.

It was apparent from the survey that diseases are not a major constraint to groundnut production. However, in one field near Dhudial (on the way to Chakwal from Mandhra), peanut clump virus disease (PCV) was observed, with incidence ranging from 4 to 10%. The diseased plants occurred in patches and were severely stunted, with typical symptoms of mottling on the younger leaflets; the lower leaves were dark green in color. The soil was sandy, and the crop had been sown in April. The variety was the local spreading type. In one field (sown by extension staff as a demonstration plot) 10 km from Fateh Jang en route to Talaganh, we observed 5–15% incidence of peanut bud necrosis virus (PBNV). The field had been sown early, in rows, with a semi-spreading variety mixed with a local spreading type. Virus-infected plants showed typical PBNV symptoms, with chlorotic lines and ring patterns. Some plants showed complete necrosis of the growing terminals. PBNV was observed in every field surveyed in the Pothar area, but always at a very low incidence (less than 1%). In many fields, we also found a few scattered stunted plants, which we suspected were affected by PCV; but mottling symptoms were not clear on young leaflets. PBNV was also observed in groundnut fields adjacent to the road at several places—Tarbela Dam, Hazro Tehsil, Kamra, Attock, Fateh Jang, and Chakwal. At Barani Agricultural Research Institute (BARI), Chakwal, and at the National Agricultural Research Centre (NARC) farm, Islamabad, a few plants suspected to be infected by PBNV and PCV were recorded. Due to the presence of severe iron deficiency, which causes yellowing of the leaflets, it was difficult to detect symptoms of virus diseases.

Samples were collected at all the places surveyed, and tested by ELISA with antisera raised against PBNV and PCV. The ELISA results confirmed the presence of PBNV and PCV in Pakistan. Two serotypes of PCV were identified. At BARI, two suspected plants reacted with an antiserum raised against the Ludhiana isolate of Indian PCV. Another plant (also collected at BARI) reacted with antiserum produced for the Talod isolate of Indian PCV. The plants from Dhudial and NARC also reacted with antiserum produced for the Talod isolate. None of the samples reacted with antisera raised against the Hyderabad or West African isolates.

On the basis of this survey, we conclude that two virus diseases—PBNV and at least two known serotypes of PCV—occur in farmers' fields in Pakistan. The overall