undertaken with the objective of identifying the physiological attributes associated with drought tolerance that can be used as selection indices in breeding programs.

Twenty-five groundnut cultures [22 received from ICRISAT. and 3 from the Project Coordinating Unit (Groundnut), Junagadh] were evaluated during the 1990 rainy season in a randomized-block design with three replications. The trial was sown on 1 August in a sandy loam soil under totally rainfed conditions. Each genotype was sown in a 4.0 x 1.2 m^2 plot at a plant spacing of 30 x 10 cm. The following observations were recorded during die stress period (i.e., at 70 days after sowing): number of leaves per plant (LP), dry weight of leaves (DWL), total dry matter accumulation (DMA), relative water content of leaf (RWC), and percentage wilting of plants. The number of functional leaves, pod yield, and harvest index (HI) were recorded at the time of harvest. Simple correlation coefficients were worked out and are presented in Tablet.

A rainfall of 460 mm was received in 19 days during the crop growth period. The crop experienced severe drought during the pod development stage since there was no rain between 53 and 75 days after sowing. The soil moisture status during this drought period was 4.58%.

Highest pod yield was recorded in ICGV 86607 (5.46 g plant-') closely followed by ICGV 86635 (5.42 g plant-[1]) and ICG 3556 (5.2 g plant-')- These genotypes possessed many desirable attributes such as higher DWL, RWC, DMA, HL, and more functional leaves than other entries, and the first two also had lower wilting percentage.

Pod yield was positively and significantly correlated with DMA (r = 0.4270) and DWL (r = 0.5368) recorded on the 70th day. Upretty et al. (1979) also observed that DMA and pod yield were positively associated in cowpea. RWC on the 70th day was weakly correlated with yield (r * 0.2392) and functional leaves at harvest were positively but weakly correlated with yield (r = 0.2447). Percentage wilting of plants on the 70th day was negatively and significantly correlated with yield (r = -0.5545). Under drought stress conditions, the entire plant growth is ultimately affected in terms of DMA and partitioning efficiency. Therefore genotypes possessing higher DMA and LW will show drought tolerance and have greater productivity. Arjunan et al. (1988) also reported positive association of pod yield with DMA and LW under drought stress conditions.

The higher number of functional leaves at harvest observed in the tolerant genotypes ensured the plants a supply of photosynthates to the sink until maturity. For example, the genotypes ICG 3556 and ICGV 86635 had more functional leaves at the time of harvest (19.5 and 19.2) and recorded higher pod yield of 5.02 g and 5.42 g plant'[1] than other entries.

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Production of Monoclonal Antibodies to Bud Necrosis Virus

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The bud necrosis disease of groundnut *(Arachis hypogaea* L.) was first reported from India by Reddy et al. (1968). It was subsequently reported that bud necrosis disease was caused by tomato spotted wilt virus (TSWV) (Ghanekar et al. 1979). Recent studies using advanced serological techniques have shown that the causal agent of bud necrosis disease is distinct from TSWV and it has therefore been named bud necrosis virus (BNV) (Sreenivasulu et al. 1991, Reddy et al. in press). Hence BNV is considered to be a distinct member of the tospovirus group.

Following established facilities at ICRISAT Center, monoclonal antibodies (McAbs) against BNV were produced using the strategy initially described by Kohler and Milstein (1975). Purified BNV was used for immunization of Balb/c mice. Lymphocytes collected from mice spleen were fused with SP2/0 Ag.14 myeloma cells using polyethylene glycol as fusing agent (PEG m.w. 1300-1600, 40%). Seven hundred hybridoma cultures, deriving from three fusion experiments, were tested by direct antigen coating ELISA (DAC-ELISA) and protein-A coating ELISA (PAC-ELISA) for the production of

Table L Stable hybridoma cell lines secreting BNV-specific monoclonal antibodies.

	"type of McAb secreted		
McAb code	Heavy chain	Light chain	Ascitic fluid titer[1]
F15-4G8	**IgG3**	**Kappa**	**ND***
F16-3A7	**IgGl**	**Kappa**	**1/5 000 000**
F16-3A9	**IgGl**	**ND**	**1/50 000**
F16-3A11	**IgGl**	**Kappa**	**1/500 000**
F16-3C12	**IgGl**	**ND**	**1/500 000**
F16-4F12	**IgGl**	**Kappa**	**1/500**
F16-3C6	**IgGl**	**Kappa**	**1/500 000**
F16-3E11	**IgGl**	**Kappa**	**1/500 000**
F16-3G8	**IgGl**	**Kappa**	**ND**
F16-4G7	**IgGl**	**Kappa**	**1/50 000**

1. Ascitic fluid titer was determined by testing the fluids in DAC-ELISA (Hobbs et al. 1987) using purified virus as antigen.
2. Not determined.

virus-specific antibodies. Ten such cultures were selected, cloned by limit dilution, and used to induce ascite tumors in mice (Table 1).

All the 10 BNV-specific McAbs failed to react, either in ELISA or in dot immunobinding assay (DIBA) with the TSWV isolate that infects groundnut in the USA. In addition, 12 McAbs produced against a lettuce isolate of TSWV (Hsu et al. 1990) were tested for their ability to react in ELISA with the groundnut isolate of TSWV and BNV. Five of them did react with the groundnut isolate of TSWV but none with BNV. These results further support the proposal that BNV should be considered a distinct member of the tospovirus group as suggested by Reddy et al. (in press).

In another experiment, 10 BNV-specific McAbs were tested for their ability to distinguish eight isolates of BNV collected from various parts of India. All of them reacted to the eight BNV isolates, indicating that these McAbs are specific to common epitopes.

The BNV-specific McAbs were tested for virus detection. F16-3A7 McAb (Table 1) showed strong reaction in DAC-ELISA (Hobbs et al. 1987). A test was conducted to compare the potential of F16-3A7 McAb with rabbit polyclonal antibodies currently being used to detect the virus. The DAC-ELISA was performed by diluting the antigens (crude groundnut leaf extracts) in carbonate buffer and incubating for 1 h at 37'C. F16-3A7 McAb was used as ascitic fluid diluted 1/10 000 in PBS-Tween buffer and incubated for 1 h at 37* C. Polyclonal antibodies (antiserum diluted 1/3 000) were first cross-adsorbed for 1 h at 37'C with healthy plant extract and incubated for 1 h at 37*C with the antigens. Horseradish peroxidase (HRP) labelled anti-mouse immunoglobulins (1/4 000) and HRP labelled anti-rabbit immunoglobulins (1/1 000) were then incubated for 1 h at 37'C. The substrate, 3,3'^,5'-tetramethylbenzidine, was finally added and incubated at room temperature. As shown in Table 2, the F16-3A7 McAb was 10 times more sensitive than polyclonal antibodies in detecting the virus from infected plant tissue, thus improving the sensitivity of detection. Since the McAb can be produced in unlimited amounts, diagnostic tests utilizing the F16-3A7 McAb have the advantage of reproducibility, increased sensitivity, and specificity over the polyclonal antiserum. Further tests to detect the virus in thrips vectors are in progress.

Table 2. Detection of BNV **in** DAC-ELISA using F16-3A7 McAb and rabbit **polyclonal** antiserum.

		Optical density at 450 nm	
Dilution of plant extract	Type of extract	F16-3A7 McAb	Polyclonal antibodies
1/10	**P**	**>2**	**1.975**
	H[2]	**0.113**	**0.096**
1/100	**I**	**>2**	**1.958**
	H	**0.064**	**0.051**
1/1000	**I**	**>2**	**1.355**
	H	**0.050**	**0.037**
1/10 000	**I**	**0.826**	**0.287**
	H	**0.047**	**0.030**
1/100 000	**I**	**0.222**	**0.057**
	H	**0.048**	**0.027**
Antigen buffer		**0.044**	**0.033**

1. Infected plant extract.
2. Healthy plant extract.

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Seed Transmission of Peanut Stripe Virus in Groundnut

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A groundnut potyvirus distinct from peanut mottle virus has **been** reported from China (Xu et al. 1983). Demski et **al. (1984) reported** a similar virus naturally infecting groundnut in the USA and named it peanut stripe virus (PStV). These were later considered to be the same and **mere are** at present 24 isolates of PStV that induce different symptoms in groundnut (Wongkaew and Dollet 1990).

The spread of PStV to different countries is considered to be mainly due to its seed-transmitted nature in **groundnut. Thus the** supply of virus-free seed has assumed great importance for curtailing the spread of this virus. Studies conducted so far have shown that the rate of PStV seed transmission in groundnut is highly variable, depending on the PStV isolate and varieties of groundnut (Demski et al. 1984, Xu et all 1983, Xu et al. 1991, and Ohki et al. 1989). To determine the influence of PStV isolate on groundnut varieties, we tested 22 groundnut varieties with a single isolate of PStV and one groundnut variety with different PStV isolates.

Five isolates of PStV that induce stripe, necrotic, chlorotic ring mottle, blotch, or mild mottle symptoms were used to inoculate Tainan 9 groundnut in field trials at the Khon Kaen University. A replicate test with 56 plants per plot were established during the rainy season (May-August). Plants in one set were inoculated 2 weeks after emergence with each isolate separately and plants in the other set served as healthy controls.

In another test, seed from 22 groundnut varieties were sown in 5-m long rows with a spacing of 30 cm between rows. Seedlings were inoculated 2 weeks after emergence with a Thai isolate, T 2, that induces stripe symptoms. The percent PStV incidence in each variety was recorded 80 days after sowing.

Percentage seed transmission was determined by the seed test described previously by Demski and Warwick (1986). Seeds were also used in growing-out tests where the seedlings were observed for PStV symptoms.

The rate of seed transmission in Tainan 9 groundnut infected with five PStV symptom variants was between 6 and 16% (Table 1).

Percentage of seed transmission in 22 groundnut varieties infected with Thai isolate T 2 varied from 0 to less than 7% (Table 2).

These preliminary data suggest that the percentage of PStV seed transmission is highly variable. Further studies should be conducted under more controlled

Table 1. Effect of peanut stripe virus symptom variants on seed transmission frequencies for **Tainan** 9 **groundnut** grown **under** field conditions at Khon **Kaen, Thailand.**

Virus isolate	Seed transmission (%)'
Stripe (T 2)	13.30
Necrotic (T 6)	8.38
Chlorotic ring mottle CP-N (T 3)	10.91
Blotch (T 8)	6.32
Mild Mottle (T 1)	16.10

1. Based on 200-400 seeds using growing-out test combined with indexing by direct antigen coating ELISA.