Indian J. Genet., 59(2): 211-220 (1999)

A SIMPLE SCREENING METHOD FOR DROUGHT TOLERANCE IN COWPEA

B. B. SINGH*, Y. MAI-KODOMI AND T. TERAO

International Institute of Tropical Agriculture (IITA), Kano Station, P M B 3112, Kano, Nigeria

(Received: December 26, 1998; accepted: April 20, 1999)

ABSTRACT

A simple screening method was developed that accurately discriminates between drought tolerant and susceptible cowpea {*Vigna unguiculata* (L.) Walp} varieties. Wooden boxes (130 cm length, 65 cm width, 15 cm depth) made of 2.5 cm thick planks were lined with polythene sheets, filled with a 12 cm layer of soil and sand mixture (1:1) and kept on the table top in a screenhouse. Test materials were planted in the boxes with 10 cm between rows and 5 cm between plants within the row and watered daily until the partial emergence of the first trifoliate, after which watering was stopped. Percent permanent wilting was recorded at various intervals for each line until all the plants of the most susceptible lines were dead. Watering was then resumed and percent plant recovery in each line recorded. Based on the days taken to permanent wilting and percent recovery, cowpea lines IT90K-59-2, Kanannnado, Dan Ila, TVu 11979 and TVu 11986 were drought tolerant while TVu 7778 and TVu 8256 were susceptible., Field and pot testing of these lines gave a close correspondence between drought tolerance in seedling stage and reproductive stage. The method has additional advantage that seedlings from the wooden boxes can be saved and transplanted for further progeny testing and selection.

Key word : Cowpea, *Vigna unguiculata*, drought tolerance

Cowpea {*Vigna unguiculata* (L.) Walp.} is widely grown in the semi-arid regions of the tropics covering Asia, Africa and South America where drought is a major production constraint [1, 2]. Among the popular crops grown in these zones, cowpea is inherently more drought tolerant, but it still suffers considerable damage due to frequent drought in the Sahelian region where rainfall is scanty and irregular. Early maturing cowpea varieties escape terminal drought [3] but if exposed to intermittent moisture stress during the vegetative or reproductive stages, they perform very poorly. Therefore, efforts are being made to develop cowpea varieties with enhanced drought tolerance.

*Corresponding author mailing address: Dr. B.B. Singh, IITA Kano, c/o L.W. Lambourn & Co. Caroyln House, 26, Dingwall Road, Croydon, CR9 3EE, England.

Recent reviews [4-6] have brought together available knowledge on different aspects of drought tolerance in crop plants and ways and means to minimize yield losses due to drought. Major differences among and within crop species have been reported and different strategies to breed drought tolerant varieties have been suggested [7-10]. However, the success in breeding for drought tolerance has not been as pronounced as for other traits. This is partly due to lack of simple, cheap and reliable screening methods to select drought tolerant plants/progenies from the segregating populations and partly due to complexity of factors involved in drought tolerance.

Several methods have been used to estimate drought tolerance and water use efficiency that involve measurement of water potential, relative turgidity and diffusion pressure deficit, chlorophyll stability index and carbon isotope discrimination etc. [11-14]. However, most of these methods are expensive and time consuming and therefore, are not very efficient for screening a large number of plants in segregating populations. Also, field screening is difficult due to uncertain rainfall and different photoperiod and temperatures in the dry season. Traditionally, drought tolerance is defined as the ability of plants to live, grow and yield satisfactorily with limited soil water supply or under periodic water deficiencies [4]. Since several factors/mechanisms (in shoot and root) operate independently or jointly to enable plants to cope with drought stress, drought tolerance appears as a complex trait. However, if the factors/mechanisms contributing to drought tolerance can be separated and studied individually, the components leading to drought tolerance will appear less complex and may be easy to manipulate by breeders. Singh [15], and Watanabe *et al.*, [16] have reported preliminary work on screening cowpea at seedling stage for shoot drought tolerance without the effect of roots. This paper describes the details of a simple screening method for shoot drought tolerance in cowpea which eliminates the effects of roots and permits non-destructive identification of drought tolerant plants in seedling stage.

MATERIALS AND METHODS

The experiments were conducted at IITA Kano Station, Nigeria located at 12°3'N latitude, 8°32' E longitude and 476 m altitude. Twelve cowpea varieties representing a range of plant types, maturity and seed types were used for the study. These were screened for drought tolerance in wooden boxes, in plastic pots and in the field. The details of the procedures used are given below.

Box screening

Wooden boxes of 130 cm length, 65 cm width and 15 cm depth made of 2.5 cm thick planks were kept on benches in a rain protected screenhouse. The boxes were lined with polythene sheets and filled with a 1:1 mixture of top soil and sand which averaged about 7.5% clay, 84% sand, 8.5% silt and 0.8% organic matter. The boxes were filled to a 12 cm depth, leaving about 3 cm space on the top for watering. The polythene lining along the sides and bottom of the boxes ensured even distribution of water in the boxes. A spirit level was used to ensure a flat soil surface on the boxes after these have been watered. Equidistant holes were made in straight rows 10 cm apart with a hill to hill distance of 5cm within the rows. A specially made wooden guide was pushed in the soil up to a stopper (2 cm from its bottom end) in order to make holes uniformly at 2 cm depth. Two hand picked healthy seeds were sown on January 6, 1994 in each hole and after germination, thinned to one plant per hill. Each box contained one row of each of the 12 cowpea varieties with 12 plants and constituted one replication. Treatments were arranged in three randomized complete blocks. The boxes were watered daily using a small watering can until the partial emergence of the first trifoliate after which watering was stopped (January 22, 1994). Thereafter, a daily count of permanently wilted plants in each variety was made until all the plants of the susceptible lines appeared dead. Watering was then resumed to ascertain regeneration percentages for each variety. Based on the days taken to wilting and percent recovery, the varieties were rated as drought tolerant or drought susceptible.

Field screening

To compare seedling screening for drought tolerance and field performance under drought stress, the same 12 varieties were planted in the field towards the end of rainy season on September 19, 1994, after which little or no rain was expected but there was adequate moisture for germination. The trial was planted in a randomized block design with 3 replications. Each plot consisted of 4 rows, which were 4 m long and 75 cm wide with a hill-to-hill distance of 20 cm within the rows with 2 plants/hill.

Days to 50% flowering, days to 50% leaf senescence, pod yield, seed yield and total biomass were recorded from the middle 2 rows of each plot. Observations were also made on the degree of premature senescence due to drought stress. Based on the degree of premature senescence, biomass production and seed yield, the varieties were rated as drought tolerant or susceptible.

Pot screening

To further verify the results of box screening and field screening, five selected varieties representing different levels of drought tolerance and susceptibility, based on the box and field screening, were grown in plastic pots and subjected to drought stress at the onset of reproductive stage. Five seeds of each variety were planted on April 6, 1995 in 10 pots (10 litre size) kept on benches in a screenhouse and arranged in split-plot design with five replications. The main plots were i) normal watering and ii) no watering at reproductive stage and the sub- plots were the 5 varieties. After germination, plants were thinned to 2 plants/pot. At bud initiation stage, watering was stopped in the stress treatment. Seventeen days later, differences between susceptible and drought tolerant plants in the stress treatment were quite pronounced. At this stage, one plant from each pot (control as well as stressed) was cut from the ground level and its moisture content was determined as a difference between fresh and dry weight. The other plants were left in the pots, and watering was resumed. Percent recovery was recorded 2 weeks later.

RESULTS AND DISCUSSION

Box screening

Seed germination and initial growth of plants of all the varieties were normal. About 7 days after the termination of watering, the stress effects started appearing in the seedlings of susceptible varieties, and differences among varieties became visible and progressively more pronounced with advancing days of moisture stress. The stress effects were first seen on the unifoliate leaves, which became wilted, followed by the emerging trifoliates and finally the growing tip itself dried. The most susceptible lines were TVu 8256 and TVu 7778, which sowed wilting much before other lines (Table 1). Interestingly, the unifoliate leaves of TVu 7778 turned to deep yellow color in response to moisture stress and then dried; whereas the plants of TVu 8256 and other varieties showed different shades of yellow, brown and green. The differences among varieties with respect to drought tolerance were very clear (Fig 1a). The data on wilting percentage at different days after termination of watering indicated TVu 8256 and TVu 7778 to be the most susceptible to drought (Table 1) and others with different levels of drought tolerance. For example on 8th day after withholding of water, 50% plants of TVu 8256 and 72% plants of TVu 7778 had wilted while IT90K-59-2 and Dan IIa had zero wilting and others ranged from 6 to 31%. On day 14, Kanannado and Dan II a had 29% and 49% wilting

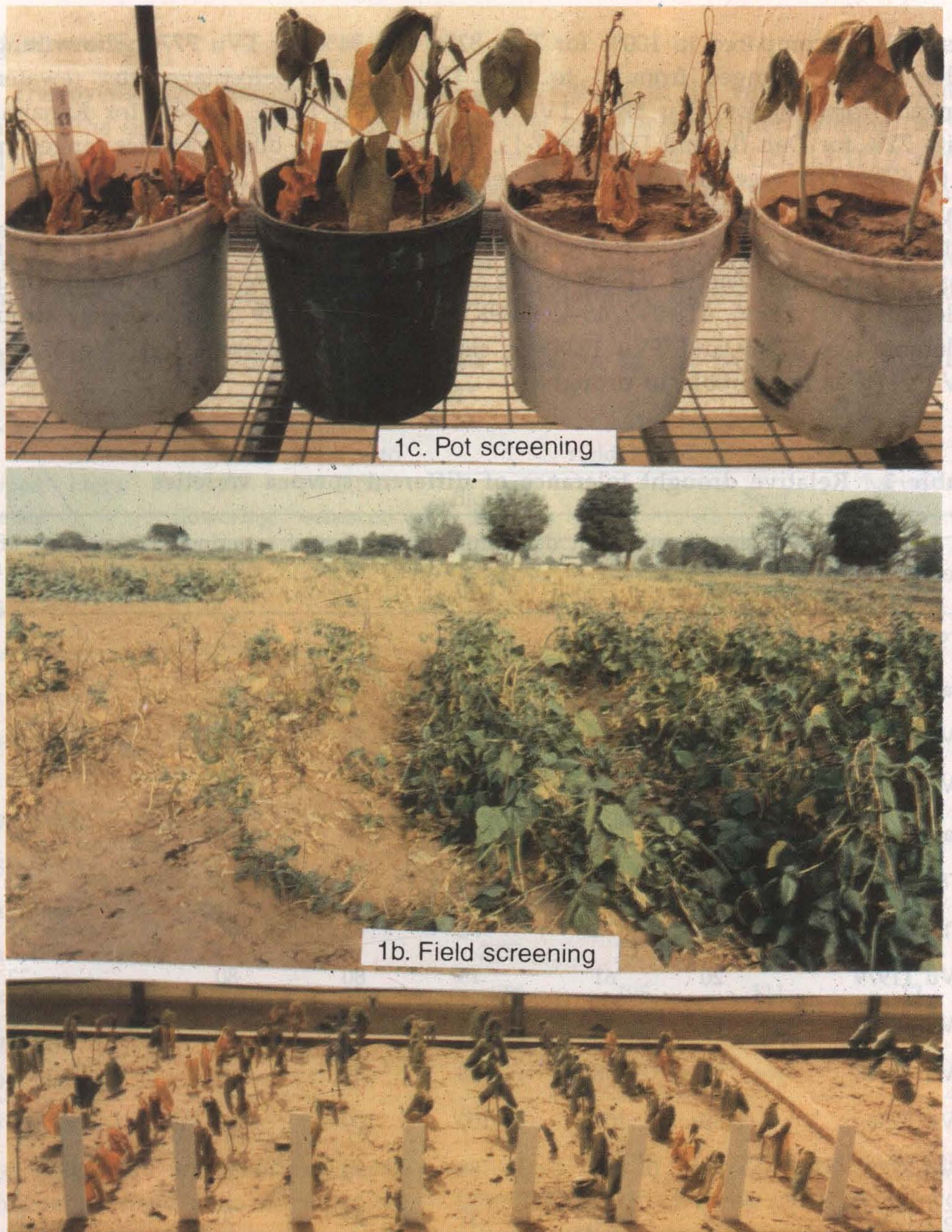

Fig. 1. Drought tolerant and susceptible plants of cowpeas in box screening, field screening and pot screening

respectively compared to 100% for TVu 8256 and 94% for TVu 7778. The wilting in other varieties ranged from 61 to 88%. The recovery percentage after rewatering ranged from 0% for TVu 8256, TVu 7778 and TVu 12349, to 89% for Kanannado with 72% for Dan IIa and IT88D-867-11. The recovery in other varieties ranged from 3 to 67%. The regenerated plants developed mainly from the growing tips as the unifoliates, and the first trifoliate dried out during the moisture stress. Based on the actual counts of wilted plants/regenerated plants and visual assessment on the row basis, Kanannado, Dan IIa, IT88D-867-11 and IT90K-59-2 were rated as highly drought tolerant; TVu 11986, IT84S-2246-4, IT89KD-288, TVu 11979 as moderately drought tolerant; TVu 12349 and TVu 12348 as slightly drought tolerant and TVu 8256 and TVu 7778 as susceptible to drought.

Table 1. Relative drought tolerance of different cowpea varieties

Cowpea variety	Percent wilting at days after termination of watering*					% recovery after rewatering
	D8	D10	D12	D14	D15	
IT90K-59-2	0	11	28	67	78	61
Dan IIa	0	23	36	49	73	72
TVu 11986	6	42	68	74	86	8
TVu 13464	10	31	51	61	86	55
Kanannado	11	29	29	29	47	89
IT88D-867-11	11	51	69	69	76	72
IT89KD-288	11	72	72	83	100	67
TVu 11979	20	61	75	80	80	22
TVu 12349	27	51	71	79	92	0
TVu 12348	31	56	78	88	100	4
TVu 8256	50	100	100	100	100	0
TVu 7778	72	83	89	94	100	0
LSD (5%)	31	39	33	29	30	30

*Planted on 6-01-94, water stressed from 22-01-94 and rewatered on 9-02-94

Field screening

The overall seed germination and initial growth of all the varieties were quite normal in the field planting also. Three light rains were received after planting (Sept. 19, 1994). These were on Sept. 29 (17 mm), Oct. 3 (20 mm) and Oct. 13 (11.7 mm) and no rain thereafter. Thus, there was no moisture stress in the seedling stage but quite severe stress during reproductive stage. The stress symptoms started appearing about 50 days after planting when most of the varieties had flowered. Thus, the drought stress affected the plant growth and development mostly at reproductive

Table 2. Performance of selected cowpea varieties in the field under drought stress at Minjibir, Nigeria 1994*

Cowpea varieties \ Characters	Days to 50% flowering	Days to 50% senescence	Yield kg/ha			Percent harvest index
			Biomass	Pod	Seed	
TVu 12349	52	102	3248	1371	847	26
T90K-59-2	44	75	2036	1121	751	37
Kanannado	47	77	2053	1012	703	34
TVu 11979	52	84	2267	1042	653	29
TVu 12348	48	80	3032	761	455	30
Dan Ila	46	72	1874	841	586	31
IT89KD-288	46	73	2472	853	547	23
IT88D-867-11	46	70	1737	828	561	32
TVu 13464	54	80	3032	761	455	15
TVu 11986	54	80	2331	737	403	17
TVu 7778	47	64	958	195	111	12
TVu 8256	44	60	1414	168	84	6
LSD (5%)	3	7	973	381	271	12

*Planted Sept 19, 1994

stage. Premature senescence of leaves was noticed first in TVu 7778 and TVu 8256 with characteristic yellow coloration in TVu 7778. Both of these varieties showed 50% senescence at 60 days after planting whereas others were still green and setting pods. The data on total biomass produced and pod and seed yields showed TVu 7778 and TVu 8256 to be most drought susceptible and TVu 12349, IT90K59-2 and Kanannado to be most drought tolerant (Table 2). Others were moderately tolerant to drought. Dan IIa showed less growth, probably because of its photosensitivity and short day lengths in October-November. Thus, except for TVu 12349, there was a close correspondence between drought tolerance at seedling and at reproductive stage. TVu 12349 retained green leaves for the longest period (Fg. 1b), which may be due to its intermediate level of drought tolerance as noted in box screening combined with probably, a deep root system enabling the plants to follow the receding water table.

Pot screening

The results of pot screening reconfirmed the results of box screening and field screening. The plants of TVu 7778 and TVu 8256 were completely wilted 17 days after withholding of watering at reproductive stage, whereas the plants of Dan IIa, TVu 11986 and TVu 12349 were still alive and showed only minor stress (Fig. 1c). The plant moisture content after 17 days of drought stress showed major differences among the 5 varieties (Table 3). The reduction in plant moisture content of TVu 11986, Dan IIa and Tvu 12349 were 16% and 19% and 29% respectively compared to 48% and 49% of TVu 778and Tvu 8256 confirming the drought susceptibility of Tvu 7778 and TVu 8256. This was further evident from poor recovery of these plants when watering was resumed (Table 3).

Table 3. Percent moisture content of pot-grown cowpea plants with and without moisture stress*

Cowpea variety	Percent	Moisture	Content	Percent recovery
	Control	stress	reduction	
Dan IIa	82.5 a	66.6 cd	19	60
TVu 11986	85.9 a	71.8 bc	16	60
TVu 12349	86.4 a	61.1 d	29	40
TVu 7778	80.7 ab	41.9 a	48	20
TVu 8256	82.2 a	42.3 e	49	0

*value with the same letter do not differ significantly

The results of box screening indicate that varietal differences for plants response to drought stress can be assessed in seedling stage in cowpea. Also, the close correspondence between the results of seedling screening, field screening and pot screening further indicate that the phenomenon responsible for drought tolerance in seedling stage is also manifested at reproductive stage in cowpea. Therefore, screening cowpea varieties at seedling stage is quite reliable method to identify drought tolerant varieties. Since the results of the box screening, field screening and pot screening are similar, box screening is more practical because of ease of handling, possibility of controlled environment and ability to screen large number of lines/plants. Also, field screening for drought tolerance may be complicated due to differences in length and root architecture of test material. The shallow box method described herein eliminates the effects of roots and thereby permits identification of plants with enhanced ability for shoot drought tolerance.

The box method is simple and non-destructive for drought tolerant plants and offers flexibility in terms of size of operation as boxes can be larger or smaller depending upon the need. The test materials can be homozygous lines or segregating populations and the drought tolerant plants can be saved and transplanted for further progeny testing and selection. This method is routinely being used in cowpea breeding and now being tested for other crops. The preliminary results indicate that by modifying the clay content of the soil used in the boxes, the moisture stress can be made more gradual to suit screening of other crops like millet, sorghum, maize and soybean etc. Also, the number of days taken to permanent wilting varies with the prevailing temperature and humidity but the relative differences among varieties/crops remain the same.

REFERENCES

1. B. B. Singh. 1994. Breeding suitable cowpea varieties for West and Central African savanna. *In*: J. M. Menyonga, J. B. Bezuneh, J. Y. Yayock and I. Soumana (Eds.) Progress in Food Grains Research and Production in Semi-Arid Africa OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso. pp. 77-85.
2. B. B. Singh, O. L. Chambliss and B. Sharma. 1997. Recent advances in cowpea breeding. *In*: B. B. Singh, D. R. Mohan Raj, K. E. Dashiell and L.E.N. Jackai (Eds.) 1997. Advances in cowpea research. Copublication of Intl. Inst. of Trop. Agriculture (IITA) and Japan Intl. Res. Centre for Agri-Sciences (JIRCAS). IITA, Ibadan. Nigeria. pp. 30-49.
3. B. B. Singh. 1987. Breeding cowpea varieties for drought escape, *In*: J. M. Menyonga, Taye Bezuneh and A. Youdeowei (Eds). Food Grain Production in Semi-Arid Africa. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso. pp. 299-306.
4. John Ashley. 1993. Drought and crop adaptation. *In*: J. R. J. Rowland (Ed.) Dryland Farming in Africa 1993. Macmillan Press Ltd, London. pp. 46-67.
5. G. V. Subbarao, C. Johansen, A. E. Slinkard, R. C. Nageswara Rao, N. P. Saxena and Y. S. Chauhan. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences., **14**: 269-523.
6. John S. Boyer. 1996. Advances in drought tolerance in plants. Advances in Agronomy., **56**: 187-218.
7. A. Blum. (1985). Breeding crop varieties for stress environments. CRC Critical Reviews in Plant Sciences., **2**: 199-238.

8. M. A. Arraudeau. 1989. Breeding strategies for drought resistance *In*: W. G. Baker (Ed.) Drought Resistance in Cereals. CAB Intl. Wallingford, London. pp. 107-116.

9. E. Acevedo and S. Ceccarelli. 1989. Role of physiologist-breeder in a breeding program for drought resistance conditions. *In*: W. G. Baker (Ed.) Drought Resistance in Cereals. CAB Intl. Wallingford, London. pp. 117-139.

10. D. W. Walker and J. C. Miller Jr. 1986. Intraspecific variability for drought resistance in cowpea. Scientia Horticulturae., **29**: 87-100.

11. L. S. Bates, R. P. Waldren and I. O. Teare. 1973. Rapid determination of free proline in water stress studies. Plant and Soil., **38**: 205.

12. K. J. Turk and A. E. Hall. 1980. Drought adaptation of cowpea II. Influence of drought on plant water status and relations with seed yield. Agron J., **72**: 421-427.

13. R. B. R. Yadava and B. D. Patil. 1984. Screening of cowpea (*Vigna unguiculata* L.) varieties for drought tolerance. Z. Pflanzenzuchtg., **93**: 259-262.

14. A. E. Hall, R. G. Mutters, K. T. Hubick ad G. D. Farquhar. 1990. Genotype differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci., **30**: 300-305.

15. B. B. Singh. 1993. Cowpea Breeding. Archival Report (1988-92) of Grain Legume Improvement Programme, Intl. Inst. of Trop. Agriculture, Ibadan, Nigeria pp. 24 and 64-69.

16. I. Watanabe, S. Hakoyama, T. Terao and B. B. Singh. 1997. Evaluation methods for drought tolerance in cowpea. *In*: B. B. Singh, D. R. Mohan Raj, K. E Dashiell and L. E. N. Jackai (Eds.) 1997. Advances in cowpea research. Copublication of Intl. Inst. Of Trop. Agriculture (IITA) and Japan Intl. Res. Centre for Agri sciences (JIRCAS). IITA, Ibadan. Nigeria. pp. 141-146.