Drought Tolerance of Cowpea (*Vigna unguiculata* (L.) Walp.)
I. Method for the Evaluation of Drought Tolerance

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Abstract

In breeding programs for drought tolerance of cowpea (*Vigna unguiculata* (L.) Walp.), it is essential to develop methods of evaluation of drought tolerance both for the identification of gene sources of the tolerance and for the selection of segregating materials after crossing. Nine hundred cowpea accessions offered by the Genetic Resources Unit of IITA (International Institute of Tropical Agriculture) were evaluated for drought tolerance in the field during the dry season at Kano Station, IITA, northern Nigeria. In this evaluation, 22 germplasm lines were found to be highly tolerant. Since we could evaluate about 1,000 lines at a time, this method was very efficient. However, local differences in the residual soil moisture in the field seemed to adversely affect the accuracy of the evaluation. This method, therefore, was suitable for rough screening of a large number of materials. To improve the accuracy, a few replications are recommended. Another method was also tested, using potted seedlings. Soil moisture was kept constant by watering each pot on an electric balance. The optimum soil moisture for the determination of the tolerance was 3%(w/w) for sandy soil at Kano. For volcanic ash soil at Tsukuba, we mixed river sand in the ratio of 1:1 by volume to reach the 3%(w/w) optimum. In this method, 21 germplasm lines (for instance, TVu 11979, 14914) were found to be highly tolerant out of 90 lines/cultivars tested. Although this method was highly reliable, it was very laborious and allowed to handle about 100-170 lines at a time. Available root zone and available soil moisture, accordingly, for cowpea plants were very different between the two methods described above. However, tolerance scores rated in the two methods were very well correlated with a highly

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significant coefficient. Either of these two methods, therefore, can be adopted, depending on the objective of the evaluation.

Additional key words: breeding method, Sudan Savanna

Increasing drought tolerance of field crops may improve the food conditions in the semi-arid regions of the world, especially in the Sub-Saharan developing countries where the severity of food shortage has been increasing year by year for the past few decades[4]. As a result, crop yield may become stabilized, arable land and productivity may increase through the increase of the growth period. Although cowpea is one of the most tolerant field crops, it also experiences drought frequently in the Sudan Savanna, one of the most drought-prone regions in the world. International Institute of Tropical Agriculture (IITA) has made a remarkable contribution to agriculture in Africa through the breeding of crops, including cowpea. For further contribution, increasing drought tolerance of cowpea was one of the most important and urgent requirements in research. Therefore, studies on the identification of gene sources of tolerance and method of evaluation were performed partly at IITA Kano Station, northern Nigeria and partly at Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan.

Materials and Methods

1) Evaluation in the field during the dry season

In the beginning of the dry season, October 1990, 900 lines of cowpea germplasm, offered by the Genetic Resources Unit of IITA, were planted in the field of IITA Kano Station, northern Nigeria (12.0° N, 8.3° E). Seeds were sown densely (20cm between rows, 10 cm between hills and 3 seeds per hill). No fertilizer was applied. After germination, plants were thinned to 1 plant per hill, to obtain 10 plants per line without plot replication. To gain the information on the local differences in growth, 2 cultivars, IT 82D-716 and Dan Illa, were also planted with three replications at different spots in the field.

To secure germination, the field was watered as evenly as possible for 12 days after sowing. Thereafter until the beginning of February 1991, when the evaluation was performed, the field was left unwatered without any precipitation. In the beginning of February, drought tolerance of each line was scored from 1 to 5 by observing 10 plants as a whole, referring to a set of standard samples as follows. Score 1 (highly susceptible): plants died at the first trifoliate leaf stage with a plant height of around 10cm. Score 5 (highly tolerant): plants were still alive with 7th-8th trifoliate developing leaf showing a plant height of around 40cm. Scores 2 (susceptible), 3 (medium) and 4 (tolerant) were given to the lines showing intermediate degrees of tolerance based on defoliation, discoloration of leaves and plant height.

2) Evaluation of potted seedlings in a glasshouse

Another method was also tested, using potted seedlings in a glass house, because it enables to repeat the evaluation several times a year and the tolerance can be evaluated in areas lacking a dry season. The evaluation experiments were repeated three times. The first experiment was performed at IITA Kano Station, Kano, Nigeria (pot evaluation 1) and the other two (pot evaluations 2-A and 2-B) were performed at JIRCAS, Tsukuba, Japan.

(1) Pot evealuation 1

Based on the experimental results of the field evaluation described above, 5 lines from each score group from 1 to 5 (25 lines in all) were selected as materials. Six hundred gram of air-dried sandy soil at Kano was put in each small pot (about 10 cm both in diameter and in height). Each line was planted in nine pots, making 225 pots in all. No fertilizer was applied. In order to determine the

optimum soil moisture level for discrimination of the tolerance, soil moisture was treated at 3 levels, 2, 3 and 5 percent (w/w) with 3 replications each. A sufficient amount of water was given to all the pots before the completion of the development of the primary leaves. Thereafter, until the soil moisture decreased to the levels described above, pots were left unwatered. Afterwords, each pot was watered on a balance every morning to reach the initial soil moisture levels.

Two weeks after the beginning of the moisture treatment, differences in the tolerance among the lines were clearly revealed. Then, evaluation was performed for each plant, based on the following classifications. Score 1 (highly susceptible): the plant was dead, score 2: (susceptible): most leaves had fallen but the plant was still alive, score 3 (intermediate): leaves were yellow and/or wilting, score 4 (tolerant): leaves were slightly yellowish or partly yellow, score 5 (highly tolerant): leaves were green and stiff. Scores for each plant were averaged among the 3 replications.

(2) Pot evaluations 2-A and 2-B

To confirm the reliability of the pot-evaluation method, evaluation experiment was repeated two times in July and in August 1992 at Tsukuba, using the same materials, 87 germplasm lines and 5 cultivars (IT81D-994, IT81D-1228-4, IT82D-889, IT84S-2246 and Suvita 2). The method was the same as that used for pot evaluation 1 except for the following three points. ① The optimum soil moisture, 3%, was used exclusively. ② Volcanic ash soil, mixed with river sand (1:1 by volume), was used. ③ There were 5 replications instead of 3.

The scores for each plant were averaged first among 5 replications. Then, the two averages for 2-A and 2-B were averaged again. Based on the final average, materials were classified into 5 groups, using the following criteria. Highly susceptible: avg.< 2.0, susceptible: 2.0 ≦ avg.< 2.6, intermediate: 2.6 ≦ avg.< 3.6, tolerant: 3.6 ≦ avg.< 4.0, highly tolerant: 4.0 ≦ avg..

Results

1) Evaluation in the field during the dry season

Variation in the tolerance among the lines tested was large enough to expect successful breeding for drought tolerance. Highly susceptible lines were dead one or two weeks after the discontinuation of watering.

Highly tolerant lines, on the other hand, continued to grow and at 85 days after sowing, when the evaluation was performed, they were about 40cm in height with a 7th or 8th nodal main stem. Some lower leaves had fallen, but the upper ones remained green and new leaves were still developing. In some lines with an early maturity, plants bore several pods even at the high planting density applied in the experiment. It was suggested, therefore, that it might be worthwhile to cultivate these highly tolerant lines in the dry season, which was reported in the succeeding paper. In this evaluation, 22 lines were found to be highly tolerant as listed in Table 1, among which Tvu 7841, 11979 and 14914 were consistently evaluated as highly tolerant also in other trials.

2) Evaluation of potted seedlings in a glasshouse

The results of pot evaluation 1 are shown in Table 2. Stress under a 5% moisture level was not

Table 1. List of cowpea accessions found to be highly tolerant in field-evaluation (in Nigeria, 1990-91)

Accession* (TVu no.)	Origin (Country)	Accesion* (TVu no.)	Origin (Country)
91	South Africa	7866	Nigeria
111	Botswana	7929	Nigeria
617	Nigeria	8358	Tanzania
1548	Ghana	8565	Nigeria
3930	Nigeria	8713	Benin
4744	Niger	9178	Nigeria
4746	Niger	10460	Sierra Leone
4747	Niger	11414	Kenya
6914	Botswana	11979	Sudan
7320	Ghana	11984	Sudan
7841	Nigeria	14914	Niger

*At Genetic Resources Unit of IITA, accession of cowpea germplasm are designated by TVu (Tropical *Vigna unguiculata)* number.

Table 2. Drought tolerance scores* of cowpea seedings determined by pot-evaluation method at three levels of soil moisture (pot-evaluation-1, in Nigeria 1991)

Accession	Soil moisture (%)			Accession	Soil moisture (%)		
(TVu no.)	5	3	2	(TVu no.)	5	3	2
11982	5.0	4.7	1.0	127	5.0	2.0	1.0
14914	4.7	4.7	1.0	7878	3.0	2.0	1.0
11979	5.0	4.0	1.7	760	2.0	1.0	1.0
9167	5.0	4.0	2.0	8885	4.3	1.7	1.0
6914	4.3	3.7	1.0	7426	3.0	1.7	1.0
7841	4.5	3.0	1.0	8365	4.7	1.3	1.0
59	5.0	2.7	1.0	7778	3.7	1.3	1.0
7381	3.7	2.7	1.0	9357	3.0	1.3	1.0
8715	3.0	2.7	1.0	12355	4.7	1.0	1.0
8713	5.0	2.3	1.0	7758	3.7	1.0	1.0
433	5.0	2.3	1.0	8401	3.0	1.0	1.0
928	5.0	2.0	1.0	8048	3.0	1.0	1.0
85	5.0	2.0	1.0				

*Criteria of scores 1: Plant is dead and dry. 2: Plant is still alive, but most leaves have fallen. 3: Leaves are yellow and / or wilting. 4: Leaves are slightly yellowish or parrly yellow. 5: Leaves are green.
Scores of three replications were averaged.

strong enough for the discrimination, whereas a level of 2% was too strong and almost all the materials died simultaneously. The optimum soil moisture level was 3% and in this plot, tolerance was clearly discriminated among the lines tested. The optimum level of soil moisture depended on the characteristics of the soil used. In the case of volcanic ash soil at Tsukuba, since stress under a moisture level of 3% was too strong, we mixed an equal volume of river sand in the evaluation experiments 2-A and 2-B. Therefore, the optimum level of soil moisture with the soil to be used must be determined in advance.

The results of pot evaluations 2-A and 2-B are shown in Table 3. The scores marked in the two repeated experiments were nearly identical in most cases. Correlation coefficient between the two groups of scores which was 0.665^{**} (n=90) was highly significant. This method, therefore, seemed to be sufficientry reliable. All the 5 cultivars tested were classified into groups from intermediate and above. These cultivars had not been selected on account of their drought tolerance. However, during field trials, they might possibly have been selected for the stability of yield in drought-prone fields.

3) Correlation of the scores between the two methods and advantages and disadvantages of the methods

Twenty three lines were tested in all the evaluation experiments, field evaluation, pot evaluation 1 and pot evaluation 2. Tolerance scores were very similar between the two methods (Table 4). They were significantly correlated with correlation coefficients $r=0.666^{**}$ (between field evaluation and pot evaluation 1) and $r=0.561^{**}$ (between field evaluation and pot evaluation 2). Therefore, either method could be selected based on the objective of the evaluation and the advantages and disadvantages of the methods.

Field evaluation method is very efficient and labor-saving. When 10 plants are tested for a line with a planting density of 25 plants/m^2, as was the case in this experiment, 2,500 lines could be evaluated at the same time in a small field of 0.1 ha without replication. Dense planting leads to competition for water among lines and among hills. Competition between lines helps discrimination, but that between hills makes it useless to evaluate each plant. Tolerance scores of two cultivars, IT82D-716 and Dan Illa, which were planted with

Table 3. Drought tolerance scores* of cowpea seedlings determined by pot-evalution method at a soil moisture level of three percent (pot-evaluation-2A and -2B, in Japan 1992)

Accession (TVu no.) or Cultivar	Pot-evaluation			Accession (TVu no.) or Cultivar	Pot-evaluation			Accession (TVu no.) or Cultivar	Pot-evaluation		
	-2A	-2B	Avg		-2A	-2B	Avg		-2A	-2B	Avg
(highly tolerant)				(intermediate)				(susceptible)			
9157	5.0	4.9	5.0	7369	3.1	3.8	3.5	7326	2.0	3.0	2.5
14914	5.0	4.9	5.0	8719	3.5	3.4	3.5	8715	2.3	2.7	2.5
11986	5.0	4.7	4.9	12355	3.5	3.4	3.5	760	3.0	1.9	2.5
11979	4.7	5.0	4.9	9481	3.0	3.8	3.4	7883	2.5	2.4	2.5
7841	5.0	4.5	4.8	IT82D889	3.2	3.5	3.4	7753	2.5	2.3	2.4
12348	5.0	4.4	4.7	7149	2.3	4.3	3.3	7758	1.8	3.0	2.4
Suvita 2.	5.0	4.2	4.6	1350	3.4	3.2	3.3	7884	2.0	2.8	2.4
1463	5.0	4.1	4.6	4594	3.0	3.6	3.3	8033	3.0	1.8	2.4
14910	4.7	4.0	4.4	IT81D1228-4	3.0	3.5	3.3	7320	3.3	1.5	2.4
433	4.4	4.2	4.3	8331	2.2	4.3	3.3	7821	2.0	2.7	2.4
9178	3.5	4.8	4.2	8747	3.3	3.1	3.2	7267	2.3	2.3	2.3
3752	3.8	4.4	4.1	1645	3.9	2.5	3.2	85	2.0	2.6	2.3
7144	3.5	4.7	4.1	3662	3.1	3.3	3.2	9443	2.5	2.1	2.3
14915	4.3	3.9	4.1	8890	3.0	3.3	3.2	3916	2.0	2.6	2.3
IT84S2246	4.8	3.3	4.1	1549	3.0	3.3	3.2	7737	1.5	3.0	2.3
1469	3.9	4.2	4.1	7426	3.5	2.5	3.0	8265	1.9	2.6	2.3
1362	4.3	3.8	4.1	7879	3.0	2.8	2.9	7721	2.7	1.8	2.3
11982	3.5	4.5	4.0	8368	3.0	2.7	2.9	8465	1.4	2.9	2.2
2365	3.9	4.1	4.0	7368	2.9	2.8	2.9	8111	2.5	1.6	2.1
8885	3.3	4.7	4.0	7339	2.0	3.5	2.8	7340	1.8	2.3	2.1
4632	3.5	4.4	4.0	8892	3.0	2.5	2.8	7758	2.3	1.6	2.0
(tolerant)				8713	2.2	3.3	2.8	8401	1.9	2.0	2.0
11955	3.6	4.2	3.9	6914	1.8	3.7	2.8	(highly susceptible)			
59	3.1	4.4	3.8	10868	2.0	3.4	2.7	7719	2.0	1.8	1.9
IT81D994	3.3	4.1	3.7	7925	2.3	3.0	2.7	1366	1.0	2.5	1.8
1547	4.0	3.4	3.7	8868	2.3	3.0	2.7	1391	1.3	2.0	1.7
928	3.6	3.7	3.7	7146	2.5	2.7	2.6	7273	1.2	2.1	1.7
9167	3.8	3.5	3.7	9483	2.0	3.1	2.6	8294	1.3	1.7	1.5
1582	4.0	3.2	3.6					7778	1.3	1.6	1.5
7878	4.0	3.1	3.6					8256	1.0	1.8	1.4
8333	3.5	3.6	3.6					9357	1.0	1.6	1.3
127	4.7	2.4	3.6					8048	1.3	1.1	1.2

*Criteria of scores are the same as in Table 2. In each evaluation, scores of five replications were averaged. Using the average of the two evalutions, tolerance was classified into five categories as follows. Highly susceptible: average$<$2.0, susceptible: 2.0$\leqq$average$<$2.6, intermediate: 2.6$\leqq$average$<$3.6, tolerant: 3.6$\leqq$average$<$4.0, highly tolerant: 4.0$\leqq$average.

three replications at different spots in the field, differed by one rank in the score among replications, probably due to the uneven distribution of residual soil moisture and/or to the competition among lines. Based on this observation, a few replications was found to be necessary. Differences in the maturity of the materials tested adversely affected the accuracy of evaluation. Therefore, plants should be evaluated among lines with the same maturity, if information on the maturity of the materials is available in advance. Thus, field evaluation method is most suitable for a rough screening of a large number of materials in a breeding program involving the selection of genetic resources and mass selection in bulk breeding.

Pot evaluation method , on the other hand, is labour-intensive and enables to handle only about 500 pots at a time, or 100 lines with 5 replications. We can evaluate , however, each plant accurately. In this method, we can repeat the evaluation several times a year and it is also possible to transplant selected lines or plants to the field for further selections. Thus, the pot evaluation

Table 4. Drought tolerance scores of cowpea, determined by two different methods, field-evaluation method and pot-evaluation method (field-eveluation; in Nigeria 1990, pot-evaluation-1; in Nigeria 1991, pot-evaluation-2; in Japan 1992)

Accession (TVu no.)	Field-evaluation	Pot-evaluation -1	Pot-evaluation -2*	Average
14914	5	4.7	5.0	4.9
11979	5	4.0	4.8	4.6
7841	5	3.0	4.8	4.3
11982	4	4.7	4.0	4.2
6914	5	3.7	2.7	3.8
9167	3	4.0	3.7	3.6
433	4	2.3	4.3	3.5
8713	5	2.3	2.7	3.3
928	4	2.0	3.7	3.2
8885	4	1.7	4.0	3.2
59	2	2.7	3.8	2.8
85	4	2.0	2.3	2.8
8715	3	2.7	2.5	2.7
7426	3	1.7	3.0	2.6
7878	2	2.0	3.6	2.5
127	1	2.0	3.5	2.2
7758	3	1.0	2.0	2.0
760	2	1.0	2.5	1.8
12355	1	1.0	3.5	1.8
9357	2	1.3	1.3	1.5
8401	1	1.0	1.9	1.3
7778	1	1.3	1.5	1.3
8048	1	1.0	1.2	1.1

* Average of two evaluations, pot-evaluation-2A and -2B

method is most suitable for the selection of segregating materials in young generations after crossing or for genetic studies.

Discussion

For labor-saving in the screening process in breeding programs, screening at the seedling stage is suitable and often effective especially in the selection for disease resistance. For drought tolerance also, this method has been applied to some crops and found to be effective by Kilen and Andrew[2] and Williams et al.[5] for corn and by Wright and Jordan[6] for boer lovegrass. For rice, however, it was not effective. Drought-tolerant upland rice which has a well-developed root system, was not tolerant at the seedling stage[1]. Since the mechanism of the tolerance may be different among crops, discrepancies are likely to occur.

In this experiment, the tolerance scores rated by the two different methods were highly and significantly correlated even if the mechanism underlying each method may be different. In the field evaluation method, highly tolerant lines continued to grow by collecting residual soil moisture in deep soil layers, that is , by the mechanism of 'maintenance of water uptake'[3]. In the pot evaluation method, on the other hand, both root zone and soil moisture were strictly limited. Highly tolerant lines, therefore, remained alive for a longer period of time, presumably due to the 'reduction of water loss'[3] and/or 'dehydration tolerance'[3]. In the Sudan Savanna, crops experience two kinds of drought. One is the initial drought at the seedling stage, due to the erratic rainfall at the beginning of the rainy season. At this time of the year, little water remains in shallow soil. Seedlings whose roots are short yet, therefore, can survive until the next rain only by the 'reduction of water loss' and/or 'dehydration tolerance'. The other is the terminal drought at the ripening stage in the beginning of the dry season. At this time of the year, enough water remains in deep soil layers for deep-rooting crops to complete their life cycle by the 'maintenance of water uptake'. Therefore, it is likely that in the long history of adaptation of cowpeas to drought-prone Sudan Savanna, they have acquired both mechanisms mentioned earlier in order to produce seeds for the next generation. This might be the reason why the scores rated by the two methods were closely correlated.

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ササゲ（*Vigna unguiculata* (L.) Walp）の耐乾性
Ⅰ．耐乾性の評価方法

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摘　要

育種によりササゲ（*Vigna unguiculata* (L.) Walp）の耐乾性向上を図るためには，耐乾性が優れる交配親を特定し，交配後代の耐乾性を評価しつつ選抜する必要がある。このため，耐乾性の評価方法を確立するとともに，耐乾性極強の遺伝資源を見出そうとした。

NigeriaのKano市に存在するIITA（国際熱帯農業研究所）Kano支所の乾季の圃場を利用して，遺伝資源900系統の評価を行い，極強の22系統を特定した。乾季の圃場を利用するこの評価方法は省力的であり，試験精度の面でやや問題があるが，一回の供試量として1,000系統程度の大量の供試材料を粗選抜するのに好適する方法であると思われた。

次に，実生をポット栽培し，土壌水分を規制しつつ評価する方法を試みた。Kanoの現地で供試した砂質土壌の場合は，重量比で土壌水分３％が最適であった。つくばで追試した場合は火山灰土壌と川砂を容積比１：１で混合した土壌を用いることにより３％での評価が可能となった。この方法で90系統・品種を評価し，TVu 11979, 14914等の極強の21系統・品種を特定した。この評価方法は精度と再現性は高いが多労であるため一回の供試量として概ね100-170系統が限度であろうと思われた。

圃場での評価では下層土壌に土壌水分が残留するため，深根性であることが重要であろうと想定された。一方，ポットによる評価では，根圏と土壌水分が厳しく限定されているため，地上部の耐乾性が重要であろうと想定された。このため，評価結果は当然異なるもとの予想されたが，実際には良く一致し，これらの二つの方法で得られたスコアー間には１％レベルで有意な相関が認められた。したがって，これらの方法の長所と短所，及び評価の目的を考慮して，評価方法を選択することが出来る。

キーワード：育種方法，カウピー，ササゲ，スーダン・サヴァンナ，耐乾性，耐乾性評価法

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