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TRAITS ASSOCIATED WITH DROUGHT RESISTANCES IN COWPEA

SIFAT-SIFAT KACANG TUNGGAK YANG TERKAIT DENGAN KETAHANAN KEKERINGAN

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ABSTRACT

Progress in developing drought-resistant cowpea [*Vigna unguiculata* (L.) Walp.] cultivars has been hindered by the lack of efficient selection criteria. The purpose of this study was to explore simply measured plant characteristics that serve as selection criteria for screening drought-resistance in cowpea. The experiments involving two cowpea genotypes (Tvu 11986 and Tvu 7778) that were previously identified as contrasting in drought resistance were conducted during summer 1999, 2000, and 2001. Stepwise discriminant analyses performed on each year data as well as on the pooled data and linear discriminant functions were developed from the best discriminators selected by the stepwise discriminant analyses. This study suggested that delayed leaf senescence, stem diameter, and leaf temperature were potential trait as selection criteria for separating drought resistant from drought susceptible cowpea genotypes. Similarly, stem diameter could also be used as single selection criteria without losing too much accuracy.

Key words: drought resistance, stepwise discriminant analysis, linear discriminant analysis, delayed leaf senescence.

ABSTRAK

Kemajuan dalam pengembangan kultivar kacang tunggak [*Vigna unguiculata* (L.) Walp] banyak mengalami hambatan karena belum adalanya kriteria seleksi yang efisien. Tujuan dari penelitian ini adalah untuk menentukan sifat tanaman yang mudah diukur dan berguna sebagai kriteria seleksi dalam penapisan ketahanan terhadap kekeringan pada kacang tunggak. Percobaan dilaksanakan pada musim panas tahun 1999, 2000, dan 2001 dengan menggunakan Tvu 11986 (toleran kekeringan) dan Tvu 7778 (peka kekeringan). Analisis diskriminan bertatar dilakukan terhadap data dari masing-masing tahun maupun gabungannya dan fungsi-fungsi diskriminan linier disusun dari pembeda terbaik yang dihasilkan dari analysis discriminant bertatar tersebut. Hasil penelitian ini menunjukkan bahwa senesen daun, diameter batang, dan temperatur daun merupakan karakter yang potensial digunakan sebagai kriteria seleksi untuk membedakan genotipe-genotipe kacang tunggak yang resisten dari yang peka terhadap kekeringan.

Kata Kunci: tahan Kekeringan, analisis diskriminan bertatar, analisis diskriminan linear, senesens daun tertunda

INTRODUCTION

Like other crop species, the productivity of cowpea [*Vigna unguiculata* (L.) Walp.] is to a large extent determined by the growth limiting factors that exist in the area where it is grown. In low-rainfall areas, where cowpea is primarily cultivated, crop production is often hampered by drought during the growing season. Decreased soil water during periods of drought hinder plant growth and development, and eventually reduce crop yield. Although cowpea is a drought tolerant crop adapted to warm climates (Rachie,1985), productivity under drought stress will be less than when grown with an ample supply of water. Precipitation or irrigation can alleviate the effects of drought. If irrigation is not possible, breeding of plants that are resistant to drought stress is an alternative method to achieve higher, more stable yields.

Progress in breeding for drought resistance in cowpea is hindered by lack of reliable selection criteria. Drought stress in plants is a complex of morphological, physiological, and phenological characteristics and not a unique, heritable plant trait (Blum, 1988). There is no consensus regarding the traits that should be used as selection criteria. The empirical approach of yield testing of advanced lines in multiple locations and years results is complicated by genotype x environment interactions that can inhibit selection progress (Hall *et al.*, 1997). Similarly, the physiological traits are often effective in determining drought resistance but they are of limited value in a breeding project as the evaluation can only be made on small numbers of plants. The effectiveness of selection criteria is an important consideration, but simplicity of technique is desirable to allow for the evaluation of a large number of plants. In addition, there is a need to identify easily measured traits that are indicative of drought resistance which could be employed in breeding program with limited resources. The purpose of this study was to explore simply measured plant characteristics that potentially serve as selection criteria for screening drought-resistance in cowpea.

METODOLOGY

Studies were performed in a series of green-house pot experiments at the Mississippi Agricultural and Forestry Experiment Station (MAFES) Plant Science Research Center at Mississippi State, MS. The experiments were conducted during summer 1999, 2000, and 2001 using genotypes, Tvu 11986 and Tvu 7778, which have previously been identified as drought-resistant and drought-susceptible genotypes, respectively (Singh, 1999, personal communication). Plastic pots (25-cm diameter) filled with a mixture of spaghum peat moss and sand (1:1 v/v) were used as the planting media. Basal fertilizers (equivalent to 40 kg N, 80 kg P_2O5, 80 kg K_2O hectare^{-1}) and 2.2 kg m^{-3} dolomitic limestone were added to each pot. .Micro-nutrients were provided by adding 0.6 kg m^{-3} of Micromax® to each pot. The pots were randomly arranged on benches with 50 cm spacing between pots. Two seeds were sown in each pot but thinned to one pot^{-1} after emergence. All plant were irrigated on alternate days until they reached late vegetative stage. From flowering to early pod formation stages (15 d), plants were subjected to water-deficit. The moisture level was monitored daily using tensiometers and maintained by replenishing water at 3% of media weight.

Observations were made at the end of the water deficit period. The following traits were measured on all individual plants: a) Delayed leaf senescence, scored 1 to 5 (1=plant dead; 2= plant still alive, but most leaves abscised; 3= leave yellow and/or wilting; 4=leaves partially yellowed; and 5=leaves green); b) Stem diameter (mm), measured using a digital caliper at 1.5 cm above soil surface; c) Leaf temperature (^{o}C); d) Leaf relative humidity (%); e) Leaf diffusive resistance (s cm^{-1}); f) Transpiration rate (μg cm^{-2} s^{-1}); and g) Leaf water potential (atm) measured on the youngest, fully-expanded trifoliate leaf using a pressure bomb, and h) Leaf water content (%) measured on the youngest, fully-expanded middle trifoliate leaf. [Leaf temperature, relative humidity, diffusive resistance, and transpiration rate were measured on the youngest, fully-expanded middle trifoliate leaf using a Li-1600 steady-state porometer (Licor, Inc.)].

All data analyses were carried out using statistical package SAS v 7.0. (SAS Institutes. 1988). The TTEST procedure was performed on 1999, 2000, and 2001 data to compare the two genotypes. Analyses of variance on the pooled data were also performed. Variables that failed to show significant differences were eliminated from further consideration.

RESULTS AND DISCUSSION

In general, the performance of the resistant genotype (Tvu 11986) was observed more vigorous under water deficit conditions compared to the susceptible genotype (Tvu 7778). The means for the traits observed are presented in Table 1. Significant differences between the two genotypes were found in 1999 for delayed leaf senescence, stem diameter, leaf temperature, and leaf water potential. The resistant genotype tended to have prolonged leaf senescence, larger stem diameter, lower leaf temperature, and higher water potential than the susceptible genotype. Similar trends were observed in the following years, except that leaf temperatures did not differ; however, only stem diameter differed significantly in all 3 yr. Delayed leaf senescence differed only in 1999 and 2001. No significant differences were found for leaf temperature and leaf water potential in 2000 and 2001.

Table 2 presents the analyses of variance for pooled data over 3 yr. Delayed leaf senescence and stem diameter showed genotypic differences, and the differences were stable across environments, making these two traits good candidates for discrimination over different environments. The presence of a significant genotype x year interaction for leaf temperature indicated that this variable was sensitive to environmental conditions, and it may be suitable for discrimination only in particular environments. There was no consistence genotypic difference for leaf water potential as found in 1999; however, there were differences among years for this trait. Nevertheless, this trait was considered as having a possible association with drought resistance as implicated by t-test.

Stepwise discriminant analysis is a variable selection procedure which allows independent variables to enter into the discriminant function one at a time on the basis of their discriminating power (Johnson, 1998). At each step, inclusion of a new variable may result in the removal of some variables previously entered, provided that the information they hold is available in some combination of the other variables that are included. The results of stepwise discriminant analysis on the four variables suggested by univariate analyses are presented in Table 3. The STEPDISC procedure selected different subsets of variables for different sets of data. In 1999, the analysis resulted in the selection of delayed leaf senescence, stem diameter, and leaf temperature as the best predictors for revealing the differences in drought resistance among individuals. In 2000, the STEPDISC procedure identified stem diameter as the single best discriminator. For 2001 and the pooled data, stem diameter and delayed leaf senescence were selected as the best discriminators. The common feature among the subsets of variables was that stem diameter was included in all subsets, and it was the most influential variable in all subsets, as indicated by its squared partial correlations. In all cases, the estimated average squared canonical correlations (ASCC) were relatively high and significantly different from zero. This suggested that the discriminant functions developed from the selected subsets of variables should be effective in discriminating among individuals for drought resistance.

The linear discriminant functions formed using delayed leaf senescence, stem diameter, and leaf temperature are presented in Table 4. In all data sets, the 3-variable functions produced a high level of accuracy in differentiating resistant and susceptible individuals, with overall accuracy of 87.5 to 92.5%. With the functions developed using only delayed leaf senescence and stem diameter, the overall accuracies were generally reduced, except in 2001 where accuracy increased to 95.23%, and predicted equally well for both resistant and susceptible groups. The coefficients of the variables in the functions indicated the relative contribution of the associated traits in the discrimination. In all functions, stem diameter made the greatest contribution compared to the other variables. Moreover, the removal of delayed leaf senescence and leaf temperature did not necessarily reduce the accuracy of prediction as indicated by the functions formed using only stem diameter as a dicriminatory variables. These single variable functions had high overall accuracies comparable to the 2- and 3-variable functions in all data sets. The Press Q-statistics were significant for all functions, indicating that the classification accuracies for each function were higher than would be expected by chance (Hair *et al.*, 1995). Result of the present study demonstrated that drought-resistant and drought susceptible cowpea genotypes can be separated using delayed leaf senescence, stem diameter, and leaf temperature, although stem diameter can be used as single variable for separation without too much loosing the accuracy.

Table 1. Comparison between TVU 11986 and Tvu 7778 for the observed traits

Trait	1999		2000		2001	
	Tvu 11986	Tvu 7778	Tvu 11986	Tvu 7778	Tvu 11986	Tvu 7778
Delayed leaf senescence	3.97 a	2.82 b	3.38 a	2.89 a	3.88 a	3.02 b
Stem diameter	11.40 a	8.43 b	12.41 a	8.56 b	12.36 a	9.07 b
Leaf temperature	34.11 b	35.11 a	35.70 a	35.68 a	25.10 a	24.80 a
Leaf relative humidity	54.83 a	54.24 a	41.72 a	41.94 a	56.80 a	55.54 a
Leaf diffusive resistance	2.93 a	3.06 a	0.54 a	0.68 a	0.08 a	0.08 a
Transpiration rate	5.91 a	6.67 a	16.78 a	16.02 a	54.77 a	53.37 a
Leaf water potential	11.76 a	10.12 b	10.92 a	7.47 a	14.65 a	8.90 a
Leaf water content	81.44 a	82.07 a	83.34 a	84.09 a	82.53 a	82.83 a

a, b means within a row of the same year not followed by a common letter are significantly different according to the t-est ($P<0.05$).

Table 5. Analyses of variance across years for the traits showing genotypic difference in t-test

Source of variation	df	Mean squares			
		Delayed leaf senescence	Stem diameter	Leaf temperature	Leaf water potential
Year[¶]	2	1.065	7.261	1409.016 **	37.004 **
Rep (Year)	59	0.621	2.602	1.796 **	6.569
Genotype	1	20.481 **	333.366 **	0.775	0.803
Year x Genotype	2	1.119	1.530	5.174 **	5.647
Error	57	1.082	2.399	0.820	5.504

Year mean squares were tested using Rep(Year) mean squares, ** significant at the 0.01 level of probability

Table 3. Summary of variable selection from stepwise discriminant analyses

Year	Variable	Partial R-square	ASCC[†]
1999	Delayed leaf senescence	0.11	
	Stem diameter	0.28	
	Leaf temperature	0.11	0.59**
2000	Stem diameter	0.59	0.59 **
2001	Delayed leaf senescence	0.10	
	Stem diameter	0.48	0.58 **
1999 - 2001	Delayed leaf senescence	0.05	
	Diameter	0.53	0.55 **

[†] ASCC average squared canonical correlation, ** significantly different from zero at the 0.01 level of probability

Table 4. Discriminant functions using delayed leaf senescence, stem diameter, and leaf temperature as discriminatory variables

Year	Function†	Press Q	Overall accuracy (%)
1999	$D = 11.66 + 1.23 X_1 + 1.12 X_2 - 0.78 X_3$	22.50 **	87.50
	$D = -14.59 + 0.85 X_1 + 1.18 X_2$	25.60 **	80.00
	$D = -13.13 + 1.32 X_2$	14.40 **	82.50
2000	$D = -34.73 + 0.22 X_1 + 1.51 X_2 + 0.5 X_3$	28.90 **	92.50
	$D = -15.3 + 0.16 X_1 + 1.418 X_2$	25.60 **	90.00
	$D = -14.95 + 1.43 X_2$	25.60 **	90.00
2001	$D = - 44 + 1.1 X_1 + 1.3 X_2 + 1.05 X_3$	27.52 **	90.47
	$D = -17.58 + 1.06 X_1 + 1.3 X_2$	35.38 **	95.23
	$D = -13.84 + 1.29 X_2$	30.86 **	92.86
1999-2001	$D = -18.37 + 0.75 X_1 + 1.3 X_2 + 0.07 X_3$	78.72 **	90.16
	$D = -15.53 + 0.72 X_1 + 1.26 X_2$	66.39 **	86.88
	$D = -13.61 + 1.31 X_2$	75.54 **	89.34

† D = discriminant score; X_1 = delayed leaf senescence; X_2 = stem diameter; X_3 = leaf temperature

** significant at the 0.01 level of probability

CONCLUSION

Stepwise discriminant analyses performed on each year data as well as on the pooled data and linear discriminant functions were developed from the best discriminators selected by the stepwise discriminant analyses. This study suggested that delayed leaf senescence, stem diameter, and leaf temperature were potential trait as selection criteria for separating drought resistant from drought susceptible cowpea genotypes. Similarly, stem diameter could also be used as single selection criteria without losing too much accuracy.

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