



## Original Research Article

# Leaf and stomata characteristics and tolerance of cowpea cultivars to drought stress based on drought tolerance indices under rainfed and irrigated conditions

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## ABSTRACT

### Keywords

*Vigna unguiculata*;  
cowpea;  
Irrigated;  
Leaf,  
stomata;  
Drought  
indices;  
rainfed;  
Seed yield.

In this study, leaf and stomata characteristics, and drought tolerance indices such as stress tolerance, mean productivity, geometric mean productivity, stress susceptibility index, stress index, stress tolerance index, harmonic mean, yield index and yield stability index were studied in cowpea (*Vigna unguiculata* (L.) Walp.) cultivars Karagoz-86 and Akkiz-86. Experiments were setup based on completely randomized design (CRD) with factorial arrangement of the treatments with five replications under rainfed (non-watered) and irrigated (well-watered) conditions in 2005 and 2006 years. Correlations among leaf characteristics (leaf number, leaf length, terminal leaflet length and width, petiolule length and leaf fresh weight), stomata characteristics (stoma number, stoma width and length on lower and upper epidermis) and seed yields of Karagoz-86 and Akkiz-86 under rainfed and irrigated conditions were determined. Plant seed yield positively and significantly correlated with leaf number, leaf length, terminal leaflet width, terminal leaflet length, petiolule length, leaf fresh weight, plant height and pod number. Based on drought tolerance indices, Karagoz-86 was found more tolerant to drought stress than Akkiz-86 and it could be recommended for rainfed condition. Supplemental irrigation, particularly at flowering, pod setting and seed filling stage to improve plant growth and guarantee sufficient seed yield should be performed if it is possible.

## Introduction

Nowadays, drought is a wide-spread problem seriously influencing agricultural production and crop quality. Blum (1988) reported that drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought

stress. Drought tolerance indices, based on yield reduction under drought conditions in comparison to normal conditions, were defined to provide a measure of drought constraint and to screen the most drought-tolerant genotypes (Mitra, 2001).

Cowpea is usually better adapted to drought, high temperatures and other biotic stresses compared with other crop plant species because of that it is primarily grown in drier regions of the world where is one of the most drought-resistant food legumes (Dadson *et al.*, 2005). Cowpeas are grown under both irrigated and non-irrigated regimes (Davis *et al.*, 1991), but irrigation increases cowpea yield (Peksen, 2007; Abayomy and Abidoye, 2009). Reductions in plant development and seed yield due to water deficit show differences depending on plant development stages and some other factors. The most sensitive growth stages of cowpea to water deficit or water stress reported by the different researchers are just prior to and during bloom (Davis *et al.*, 1991), seed filling stage (Cordeiro *et al.*, 1998) and vegetative stage, followed by the flowering and fruiting stages (Carvalho *et al.*, 2000). Developing of cowpea cultivars with special focus on irrigated conditions for different environments (Santos *et al.*, 2000) and more tolerant to water deficit or more efficient in water use (Anyia and Herzog, 2004) are necessary.

Stomatal function is important on the physiology, adaptation and productivity of plants. The number and distribution of the stomata in unit leaf area have an important role in these processes by adjusting CO<sub>2</sub>, O<sub>2</sub> and moisture exchange between the leaves and the atmosphere (Brownlee, 2001). There are large variation in the number and structure of the stomata in per unit among plant species, cultivars and leaves with different architecture.

Cowpeas are fairly unique among crop plants in exhibiting very small changes in leaf water potential when subjected to drought and very little osmotic adjustment (Shackel and Hall, 1983). Cowpeas also

have stomata that are very sensitive to soil drying, partially closing before any changes in leaf water potential were detected (Bates and Hall, 1981). When cowpea plants are subjected to drought in field conditions, their leaves do not usually wilt but tend to orient more vertically, tracking the sun in a manner that minimizes the interception of solar radiation (Shackel and Hall, 1979). These mechanisms contribute to the unique ability of cowpeas to survive extreme vegetative-stage droughts that kill most other crop plants (Hall, 2012).

The objectives of this study were to determine relationships among leaf and stomata characteristics, and seed yield under rainfed and irrigated conditions, to evaluate Karagoz-86 and Akkiz-86 cowpea cultivars for drought tolerance based on some indices such as stress tolerance, mean productivity, geometric mean productivity, stress susceptibility index, stress index, stress tolerance index, harmonic mean, yield index and yield stability index under rainfed and irrigated condition.

## **Materials and Methods**

### **Experimental site description, experimental design and planting**

Field trials were carried out at the experiment field of Faculty of Agriculture, Ondokuz Mayıs University of Samsun (41.3°N longitude, 36.3°E altitude, 150 m above sea level), Turkey during 2005-2006 years.

The experiments were laid out as a completely randomized design (CRD) in a factorial arrangement with 5 replications. Factors that investigated in the study were two cowpea cultivars (Akkiz-86 and

Karagoz-86) and two irrigation treatments consisted of a full irrigation (well-watered at 100% field capacity, FC) and no irrigation (rainfed). In well watered treatment, plants were watered at 100% FC during early vegetative growth stage, just prior to flowering, at flowering and seed filling stage to avoid plants from drought stress.

Seed sowing was performed by hand on May 16, 2005 and May 24, 2006, respectively. Spacing between and within the rows were 0.6 and 0.1 cm, respectively.

Physical and chemical properties of the soil were very similar for both study year. Soil was low in lime and phosphorus, rich in potassium and high in organic matter and heavy clay in texture.

During the field experiments, average air temperatures were determined as 20.4 and 20.6°C for 2005 and 2006 years, respectively. Total rainfall was 338.2 and 229.2 mm for the same periods. Monthly total rainfall noted for the study years were evidently different from that of the long-term period, while average air temperature values were very similar (Figures 1 and 2).

### **Leaf and stomata measurements**

Leaf fresh weight, number of leaves, terminal leaflet length and width, the number of stomata, stomata length and width were on both lower and upper epidermis were determined. Slides from the clear nail polish impression on both lower and upper epidermis of the leaflets were prepared to determine stomata number and size.

Stomata characters measured at leaf samples taken from the sixth/seventh nodes of the main stem at the full

flowering stage between the hours of 9:00 and 10:00 am. Stomata size was defined as the length and width in micrometers ( $\mu\text{m}$ ) between the junctions of the guard cells at each end of the stoma, including the opening.

The sizes of five randomly chosen stomata from a view field were determined under microscope (Nikon, YS2-H) under microscope at 10x40 magnification. Stomata counts were done in randomly selected five plants in three different fields from either side of the mid-rib of the leaflets.

Stoma length and width was measured in three stomata from three different fields for five plants with ocular micrometer. Terminal leaflet width was measured from tip to tip at the widest part of the lamina and leaf length was measured from lamina tip to the point of petiole intersection along the lamina midrib. Leaf characters and plant height were noted at the flowering stage. Correlations among leaf characteristics, stomata size and number, and seed yield were determined.

### **Drought tolerance indices**

Drought tolerance indices such as Stress Tolerance (TOL), Mean Productivity (MP), Geometric Mean Productivity (GMP), Stress Susceptibility Index (SSI), Stress Index (SI), Stress Tolerance Index (STI), Harmonic Mean (HAM), Yield Index (YI) and Yield Stability Index (YSI) were estimated for each cowpea cultivars based on seed yield under stress and non-stress environment.

Drought tolerance indices based on seed yield obtained from rainfed and irrigated conditions were calculated as in the following.

Stress Tolerance Mean Productivity Geometric Mean Productivity Stress Susceptibility Index Stress Index	TOL = $Y_p - Y_s$ (Rosielle and Hamblin, 1981) $MP = (Y_p + Y_s) / 2$ (Rosielle and Hamblin, 1981) $GMP = (Y_p * Y_s)^{1/2}$ (Fernandez, 1992) $SSI = [(1 - (Y_s / Y_p)]$ (Fischer and Maurer, 1978) $SI = 1 - (\tilde{Y}_s / \tilde{Y}_p)$ (Fischer and Maurer, 1978)
Stress Tolerance Index	$STI = (Y_p * Y_s) / (\tilde{Y}_p)^2$ (Fernandez, 1992)
Harmonic Mean	$HAM = [2 * (Y_p * Y_s)] / (y_p + Y_s)$ (Kristin <i>et al.</i> , 1997)
Yield Index	$YI = Y_s / \tilde{Y}_s$ (Lin <i>et al.</i> , 1986)
Yield Stability Index	$YSI = Y_s / Y_p$ (Bousslama and Schapaugh, 1984)

$Y_p$  and  $Y_s$ : Seed yield of each genotype under non-stress and stress conditions, respectively.

$\tilde{Y}_p$  and  $\tilde{Y}_s$ : Mean seed yield of all genotypes under non-stress and stress conditions, respectively

### Statistical analysis

Data from two years experiments were subjected to analysis of variance (ANOVA) and means were separated by least square difference (LSD) test at the 0.05 probability level using Statistical Package for the Social Sciences (SPSS) for Windows Version 15.

### Results and Discussion

Means and ANOVA results for stomata number and size in cowpea Karagoz-86 and Akkiz-86 growth under irrigated and rainfed conditions in 2005 and 2006, are presented in Table 1.

Significant differences were found for stomata number on upper epidermis (USN) and stomata length on lower

epidermis (LSL) among study years and cultivars, respectively. Years, irrigation treatments and cowpea cultivars did not show any differences regarding the rest of stomata characteristics determined in both lower and upper surface of the leaves (Table 1).

The effect of year on all determined leaf characters were statistically significant, except for pod number per plant (PN) (Table 2). PN and seed yield (SY) obtained in irrigated condition (7.20 pods plant<sup>-1</sup> and 6.36 g plant<sup>-1</sup>) were significantly higher than in rainfed conditions (7.20 pods plant<sup>-1</sup> and 3.55 g plant<sup>-1</sup>). Water has play a vital role in plant growth, development and crop productivity. Plant growth and yield performance of the cultivated plants are limited in the case of permanent or temporary water deficit. Limiting affect of water deficit more than any other environmental factors (Lobato *et al.*, 2008; Shao *et al.*, 2009). It has been demonstrated that cowpea seed yield increased when irrigation applied (Peksen, 2007; Pejic *et al.*, 2013).

Cowpea cultivars were differed for TLW and SY, while cultivars were not different for the rest of the other characteristics (Table 2).

Relationships among plant seed yield and stomata number, size, leaf characteristics are given in Table 3. Plant seed yield positively and significantly correlated with LN, LL, TLW, TLL, PL, LFW, PH and PN. LSN positively and significantly correlated with LSW, LSL, USN, USW and USL (Table 3).

Taghian and Abo-Elwafa (2003) informed that different quantitative criteria have been proposed for selection of genotypes

**Table.1** Means and ANOVA results for stomata number and size in cowpea cvs Karagoz-86 and Akkiz-86 growth under irrigated and rainfed conditions in 2005 and 2006

Treatments		LSN	LSW	LSL	USN	USW	USL
Y	2005	372.81	35.19	39.97	153.33a	40.34	44.35
	2006	339.90	35.54	39.31	129.80b	40.99	44.04
IT	Irrigated	340.83	35.50	39.44	139.58	41.07	44.90
	Rainfed	371.88	35.23	39.84	143.54	40.27	43.49
C	Karagoz-86	364.90	34.62	38.64b	140.94	40.11	43.80
	Akkiz-86	347.81	36.10	40.64a	142.19	41.23	44.59
	Degree of freedom	Significance level					
Y	1	ns	ns	ns	**	ns	ns
IT	1	ns	ns	ns	ns	ns	ns
Y*IT	1	ns	ns	ns	*	ns	ns
C	1	ns	ns	*	ns	ns	ns
IT*C	1	ns	ns	ns	**	ns	ns
Y*C	1	ns	ns	ns	ns	ns	ns
Y*IT*C	1	ns	ns	ns	ns	ns	ns

Y: Year, IT: Irrigation treatment, C: Cultivar, LSN: Stomata number on lower epidermis (stomata mm<sup>-2</sup>), LSW: Stomata width on lower epidermis (µm), LSL: Stomata length on lower epidermis (µm), USN: Stomata number on upper epidermis (stomata mm<sup>-2</sup>), USW: Stomata width on upper epidermis (µm), USL: Stomata length on upper epidermis (µm)  
 \*: Significant at 0.05 level, \*\*: Significant at 0.01 level, ns: non-significant

based on their yield performance in stress and non-stress environments. Genotypes are compared in irrigated and rainfed conditions or in different levels of irrigations based on these indicators.

In the present study, grain yield of cowpea cultivars under well watered and rainfed (stressed) conditions were measured for calculating of different sensitivity and tolerance indices. Drought tolerance indices for Karagoz-86 and Akkiz-86 growth under irrigated and rainfed

conditions over two years are shown in Table 4. Karagoz-86 had higher values for all drought tolerance indexes, when compared with Akkiz-86. Although reduction in seed yield of Karagoz-86 depending on water deficit were more than Akkiz-86 in both 2005 and 2006, Karagoz-86 gave higher seed yield than Akkiz-86 due to having high seed number per plant and high 100-seed weight (Peksen, 2007). Similar results were found regarding seed yield per plant in the present study.

**Table.2** Means and ANOVA results for leaf characteristics in cowpea cvs. Karagoz-86 and Akkiz-86 growth under irrigated and rainfed conditions in 2005 and 2006

Treatments		LN	LL	TLW	TLL	PL	LFW	PH	PN	SY
Y	2005	7.40b	17.77b	4.93b	7.76b	1.88b	0.70b	38.20b	5.75	3.49b
	2006	10.90a	24.62a	7.16a	9.88a	2.34a	1.28a	71.15a	5.91	6.43a
IT	Irrigated	9.35	21.98	6.15	8.77	2.13	1.06	63.25	7.20a	6.36a
	Rainfed	8.95	20.41	5.94	8.87	2.09	0.92	46.10	4.46b	3.55b
C	Karagoz-86	9.50	21.51	6.37a	9.05	2.23	1.06	53.90	5.75	6.50a
	Akkiz-86	8.80	20.88	5.72b	8.59	1.99	0.92	55.45	5.91	3.41b
	Degree of freedom	Significance level								
Y	1	**	**	**	**	**	**	**	ns	*
IT	1	ns	ns	ns	ns	ns	ns	ns	*	*
Y*IT	1	ns	**	ns	ns	*	ns	ns	ns	ns
C	1	ns	ns	*	ns	ns	ns	ns	ns	*
IT*C	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*C	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*IT*C	1	ns	ns	ns	ns	ns	ns	ns	ns	ns

Y: Year, IT: Irrigation treatment, C: Cultivar, LN: Leaf number (leaf plant<sup>-1</sup>), LL: Leaf length (cm), TLW: Terminal leaflet width (cm), TLL: Terminal leaflet length (cm), PL: petiolule length (cm), LFW: Leaf fresh weight (g), PH: Plant height (cm), PN: Pod number (pods plant<sup>-1</sup>), SY: Seed yield (g plant<sup>-1</sup>)

\*: Significant at 0.05 level, \*\*: Significant at 0.01 level, ns: non-significant

Determined drought tolerance indexes showed that Karagoz-86 was more tolerant to drought stress than Akkiz-86. The effect of drought stress on the yield of cowpea depends on genotype, intensity and duration of stress and the growth stage exposed to water stress.

Irrigation at the critical growth stages including flowering, pod setting and seed filling can be compensate seed yield losses on a large scale. Therefore, irrigation

should be performed at least during these stages to decrease seed yield losses due to water deficit. Karagoz-86 can be recommended for dry seed production whereas water limited or under rainfed conditions as it is more tolerant to water stress than Akkiz-86. Comprehensive studies including new cowpea cultivars/lines should be carried out to obtain more information on behavior of the cowpea cultivars.

**Table.3** Correlations among stomata number, size, leaf characteristics and seed yield of cowpea cvs. Karagoz-86 and Akkiz-86 growth under irrigated and rainfed conditions over two years

	LSW	LSL	USN	USW	USL	LN	LL	TLW	TLL	PL	LFW	PH	PN	SY
LSN	- 0.361*	- 0.414**	0.616**	- 0.515**	- 0.487**	0.228	-0.112	0.077	0.122	0.084	0.069	0.052	-0.078	0.024
LSW	-	0.825**	-0.043	.498**	.495**	0.159	0.072	0.029	0.007	-0.084	0.090	0.107	0.156	-0.011
LSL	-	-	-0.071	.632**	.590**	0.058	-0.054	-0.217	-0.175	-0.187	-0.138	-0.048	0.187	-0.146
USN	-	-	-	-0.232	-0.239	0.064	-0.229	-0.252	-0.203	-0.159	-0.184	-0.040	0.105	0.006
USW	-	-	-	-	.796**	0.017	0.126	-0.113	-0.057	0.061	-0.019	-0.126	0.222	-0.049
USL	-	-	-	-	-	-0.103	0.064	-0.206	-0.165	-0.063	-0.103	-0.313*	0.195	-0.129
LN	-	-	-	-	-	-	.526**	.627**	.602**	.437**	.706**	.670**	.462**	.597**
LL	-	-	-	-	-	-	-	.787**	.831**	.784**	.819**	.453**	0.340*	.418**
TLW	-	-	-	-	-	-	-	-	.916**	.668**	.940**	.634**	0.201	.527**
TLL	-	-	-	-	-	-	-	-	-	.699**	.915**	.488**	0.192	0.400*
PL	-	-	-	-	-	-	-	-	-	-	.638**	0.312*	0.230	.409**
LFW	-	-	-	-	-	-	-	-	-	-	-	.646**	.403**	.587**
PH	-	-	-	-	-	-	-	-	-	-	-	-	0.395*	.668**
PN	-	-	-	-	-	-	-	-	-	-	-	-	-	.681**

LSN: Stomata number on lower epidermis (stomata mm<sup>-2</sup>), LSW: Stomata width on lower epidermis (µm), LSL: Stomata length on lower epidermis (µm), USN: Stomata number on upper epidermis (stomata mm<sup>-2</sup>), USW: Stomata width on upper epidermis (µm), USL: Stomata length on upper epidermis (µm), LN: Leaf number (leaf plant<sup>-1</sup>), LL: Leaf length (cm), TLW: Terminal leaflet width (cm), TLL: Terminal leaflet length (cm), PL: petiole length (cm), LFW: Leaf fresh weight (g), PH: Plant height (cm), PN: Pod number (pods plant<sup>-1</sup>), SY: Seed yield (g plant<sup>-1</sup>)

\*: Significant at 0.05 level, \*\*: Significant at 0.01 level

**Table.4** Drought tolerance indices over two years in cowpea cvs. Karagoz-86 and Akkiz-86 growth under irrigated and rainfed conditions

	MISY	MRSY	TOL	MP	GMP	SSI	STI	HAM	YI	YSI
Karagoz-86	9.73	4.64	5.09	7.18	6.40	0.84	1.05	5.75	1.01	0.58
Akkiz-86	4.19	2.28	1.91	3.24	2.90	0.56	0.20	2.66	0.50	0.72

MISY and MRSY: Mean seed yield under irrigated and rainfed conditions, respectively. TOL: Stress Tolerance, MP: Mean Productivity, GMP: Geometric Mean Productivity, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, HAM: Harmonic Mean, YI: Yield Index, YSI: Yield Stability Index

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