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EFFECTS OF WATER STRESS ON THE LEAF PHYSIOLOGY AND GRAIN YIELD OF SOME DROUGHT RESISTANT COWPEA GENOTYPES (*VIGNA UNGUICULATA* (L.) (WALP)

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ABSTRACT
Cowpea adapts to drought through escape, avoidance or tolerance mechanism. The aim of the present study was to screen for drought resistance through analysis of leaf physiological traits (stomatal conductance and chlorophyll content) and grain yield of cowpea subjected to different water stress conditions. Field experiments were conducted at IITA farm centre in Wasai (longitudes 8º 37º E and latitude 12º 11º N) located in Minjibir local government area of Kano State, Nigeria, during the terminal rainy season from 27th September to 3rd December 2008. The experimental material comprised of twenty cowpea lines arranged in randomized complete block design with three treatment regimes, which include wet (unstressed), moderate (pod-filling stages) and severe (vegetative stages) water stress condition. Soil moisture was monitored using Neutron Moisture Meter. Physiological traits measured include leaf stomatal conductance and chlorophyll content (SPAD) measured at 17 and 33 days after water stress induction and agronomic components. The results showed that, at 33 days after water stress induction, reduction in chlorophyll content (SPAD) was 100% under vegetative and pod-filling stages. However 99% of the genotypes respond to water stress by reducing their stomatal conductance at vegetative and pod-filling stress, variety IT00K-1217 and IT00K-898-2 had an increase in their conductance (1.22% and 2.54%). The results revealed that drought stress reduced grain yield component more at vegetative (76.25%) than at pod-filling stage (64.88%).

Keywords: Drought Resistance, Leaf stomatal conductance, chlorophyll content (SPAD), Water stress and Cowpea (Vigna unguiculata (L.) (Walp)

INTRODUCTION

Environmental stresses have adverse effects on the growth and yield of field crops. Water stress is one of the environmental stress factors that limit the production of main grain legumes such as Cowpea This grain legumes is the most important African indigenous legume crop (Langyintuo *et al*., 2003). Cowpea is one of the most ancient legumes cultivated in semiarid West Africa, where rainfall resources are characteristically low (300-600mm) (Ogbonnaya *et al*., 2003) The region produces about 80% of the world production. The main producers of Cowpea in the region are Nigeria, Niger and Senegal. The yield in the Sahelian region of West Africa is lower than what is obtained in the USA and else where in the world (Quin, 1997). This is because of the constraint of biotic and abiotic factors that limit the production of cowpea in the region.

Cowpea has strong stomatal regulation of water loss. This is due to the adaptive mechanism to tolerate low soil moisture as reported by Lawan (1983) and Boyer (1996). Other mechanism that cowpea adopts for protection consist of chlorophyll concentration changes in order to reduce the extent of the absorbed light (Giardi *et al*., 1996; Murchie and Horton, 1997); chloroplast movement reducing the organelle and photosynthetic complexes exposure to light (Haupt,1990), and leaf movement or paraheliotropism (light-avoidance), causing the leaves to be cooler and thus transpire less (Shackel and Hall 1979), which help to minimize water loss and maintain water potential (Agbicodo *et al*., 2009).

Drought limits the agricultural production by preventing the crop plant from experiencing their full genetic potential. The mechanisms namely drought escape, drought avoidance and drought tolerance are involved in drought resistance. Mitra (2001) reports that the various morphological, physiological and biochemical characters confer drought resistance.

Screening for drought tolerance in cowpea is based on empirical or performances approach that utilizes grain yield and its component as the main criteria, and by analytical or physiological approach that identifies a specific physiological or morphological trait that will enhance growth and yield in the event of drought (Agbicodo *et al*., 2009). The aim of the present study is to screen for drought resistance through analysis of leaf physiological response and grain yield of cowpea to different water stress conditions.

MATERIALS AND METHODS

Field trial was conducted at the Institute of Agricultural Research/International Institute of Tropical Agriculture Farm, Minjibir located in Sudan Savanna ecological zone of Nigeria (lat. 12^0 08N', long. 8^0 33E', 500m above sea level), during the terminal end of the rainy season between 27th September to 3rd December 2008 (early dry season).

The experimental set up comprised of three treatment regimes i.e. unstressed (continuous irrigation), moderate (water stress at pod-filling stage) and severe water stress (water stress at vegetative growth stage) in complete randomized block design with three replications.

The land was ploughed, harrowed and ridged (0.75cm between ridges). The plot size consisted of 4 ridges, the experimental field comprised of three blocks, each having 60 plots of 4×3m with 180 plots.

Three seeds selected from each variety was planted in all 180 plots, with distance of 20cm between plants. Fertilizer (NPK) was applied uniformly at the point of planting, measured quantity of 0.6kg was broadcasted to 4m×20 rows across the plots. Each 20 rows received a basal application of the fertilizer (0.6kg) Fertilizer applications were done uniformly.

Moisture Stress Treatments

The experimental set up comprised of three treatment regimes i.e. unstressed (continuous irrigation) which served as control, vegetative stage of water stress (stopping irrigation at vegetative growth stage) and pod-filling stages of water stress (stopping irrigation when 50% of the plant started pod-filling/grain filling). Water stress treatment was imposed under vegetative stage at 46 days after planting, and 56 days for pod-filling stages.

Measurement of Soil moisture

Soil moisture at the moderate and severe plots were measured with Neutron moisture Meter (NMM) at prefixed depth of 10, 20, 30, 40, 60 and 100cm. PVC Tubes were installed to the depth of 1m into the soil, and measurements were carried at weekly intervals starting from the day when stress was imposed at water stress plots.

Measurement of Leaf Stomatal Conductance

Stomatal conductance measures the maximum amount of either the passage of air or water vapor across the stomatal opening. The measure of stomatal behaviors under plant stress is imperative because it is a character leading to water regulation in plants.

Measurements of Stomatal conductance were made between 10:00am to 12:00 non under a sunny day. The measurements were carried out on fully matured leaflets and were conducted in two plants per plot. The Decacon Leaf Porometer was left to acclimatize in the field for 30min. before the measurements. The measurement takes about 4min. in each plot; the data were saved in the memory of the Porometer and was latter download in the computer. Data was reported in three replicates in all the three treatments. The conductance was taken at 17 and 33 days after water stress induction.

Measurement of Chlorophyll Content (SPAD)

Chlorophyll contents of each genotype were estimated with Minolta chlorophyll SPAD 502 meter. Ten fully expanded sunlit leaves were used for the measurement in each 180 plots, ten SPAD readings were recorded and an average SPAD values were reported in three replications in all the plots. The chlorophyll content (SPAD) was measured at 17 and 33 days after water stress induction.

Agronomic Yield

The grain yield components were obtained from harvesting of dry pods at first picking and second picking. Number of pods (PN), pod weight (PW), seeds weight (SW) and

100 seed weight were reported in all the plots.

Grain Yield (GY)

Plots were harvested when 95% of the pods were dry and brown. The harvest areas were identified and determined before harvest, border rows and plants within the harvest plots were clearly marked.

The harvest area was determined as: Harvest plot (m^2) = Total length of harvested row (m) × space between rows (m) The grain yield was calculated using the formula as suggested by Amede *et al.* (2004)

Grain yield (g/m^2) = Seed weight/ Harvest area

RESULTS AND DISCUSSION

Leaf Stomatal Conductance

The result for leaf stomatal showed that, genotypes showed significant differences at 17 days after water stress induction, water stress treatments at 17 and 33d were significant but association between treatment and genotypes were not significant (Table 2).

The first physiological response to water stress is reduction in stomatal conductance and chlorophyll content. At 17 days after stress induction, the leaf stomatal conductances of stressed genotypes were similar to unstressed conditions. As the water stress increases to 33d, there was general reduction in conductance at vegetative and pod-filling stages (Table 1). At vegetative stage 99% of the genotype responds to water stress by reducing their conductance. Genotypes at vegetative stress that physiologically respond to water deficit by reduction in their stomatal conductance include IT98K-555-1, IT00K-835-45, IT98K-1111-1 and IT97K-568-18. Reduction in conductance at pod-filling stress ranged from 54.45% in IT96D-610 to 0.26% (IT97K-819-118) , this finding agrees with that of Rahman Khan *et al.* (2007) in *Vicia faba*. Stomatal behaviors have been suggested as a potential useful trait in developing crop plants with improved water use efficiency (De Michele and Sharpe, 1974; Shawcroft *et al.*, 1974). Muchow and Sincliar (1989) reported that the ability of sorghum plant to survive severe water deficits depends on its ability to restrict water loss through leaf epidermis, by stomata attaining minimum aperture size. Closure of stomata to reduce water loss through transpiration and cessation of growth (drought avoidance), osmotic adjustment and continued growth (drought tolerant) have been suggested as the possible mechanism for drought tolerance in cowpea (Lawan 1983; Boyer 1996).

Table 1: Soil moisture (mmol) measurement at different soil depths and at different days after water stress induction at the water stress plots

Pre-fixed depth (cm)	10d	17d	24d	42d	51d
10	0.0	0.0	0.0	0.3	0.3
20	45.3	40	29.0	22.0	19.0
30	101.0	91.7	78.3	70.7	65.0
40	143.7	131.3	120.7	105.3	103.7
60	124.7	109.7	108.0	100.0	99.0
100	204.7	181.0	166.3	335.3	276.0
Mean	103.2	92.3	83.7	10.56	93.8
s.e.d.	1.912	2.224	1.833	1.876	1.598

s.e.d, standard error of differences of means

Table 2: Leaf Stomatal Conductance (mmolm^{-2}s^{-1}) at 17 and 33 days after water Stress Induction of Cowpea Genotype Grown under Different Moisture Stress Condition

	STC at 17d			STC at 33d		
Genotypes	**Unstr**	**Vgt**	**Pdf**	**Unstr**	**Vgt**	**pdf**
IT00K-1217	190.8	145.8	230.6	195.8	85.6	198.2
IT00K-835-45	98.7	104.1	219.8	143.6	49.6	89.8
IT00K-898-5	149.8	173.4	241.6	145.1	148.8	128.3
IT00K-901-5	255.1	195.9	332.2	231.2	87.3	133.5
IT03K-378-4	178.2	186.4	273.5	254.8	146.4	177.7
IT04K-405-5	256.2	222.2	206.6	224.3	97.1	167.4
IT84S-2246-4	311.1	182.6	249.7	280.7	121.8	186.4
IT89KD-288	146.5	98.6	245.6	160.5	108.5	115.2
IT95K-238-3	292.6	226.8	247.5	150.0	104.5	146.5
IT96D-610	213.3	86.0	185.8	175.2	91.0	79.8
IT97K-499-35	229.5	249.5	225.2	201.1	89.7	141.5
IT97K-568-18	151.2	166.1	296.5	198.3	80.6	183.8
IT97K-819-118	181.6	90.4	198.1	113.8	112.2	113.5
IT98K-1093-1	221.5	225.9	294.5	228.4	137.6	157.9
IT98K-1111-1	261.7	187.2	280.9	222.8	95.4	129.3
IT98K-205-8	188.7	156.4	248.9	264.9	132.2	152.2
IT98K-555-1	241.3	107.3	173.0	240.3	77.3	147.7
IT99K-216-44	276.1	295.8	255.1	152.5	71.7	103.6
IT99K-241-2	211.4	176.2	201.2	202.5	106.8	121.8
IT99K-377-1	204.5	228.4	256.8	165.6	64.0	137.1
Mean	213	175.3	243.1	197.6	100.4	140.6
SE	42.63	32.82	33.67	29.17	23.13	36.51
mean square	STC17d			STC33d		
WTrt	69404**			14301		
Geno	13724**			6966**		
WTrt*geno	4932NS			2308NS		

Key: SE, standard error of means; WTrt, water stress treatment; Geno, genotypes; WTrt*geno, water stress treatment and genotypes interaction; Unstr, unstressed; vgt, vegetative; pdf, pod-filling stress; NS, not significant

Chlorophyll content (SPAD)

Mean total chlorophyll content (unit SPAD) at 17 and 33 days after water stress induction are presented in Table 2. Genotypes and water stress treatment showed significant differences for total chlorophyll content (SPAD) at 17 and 33 days after water stress induction respectively. The results (table 3) showed that the chlorophyll content of the stressed genotypes at 17d were similar to non stress genotype. At 33 days after water stress induction, there was a reduction in the chlorophyll content at vegetative and pod-filling stages of water stress

Reduction in total chlorophyll content (SPAD) at vegetative stages ranged from 56.87% recorded by IT99K-241-2 to 18.55% in IT00K-1217 respectively. Reduction of chlorophyll content (SPAD) under pod-filling stage ranged from 59.45% in IT99K-241-2 to 10.30% recorded by IT00K-901-5. Similar findings were reported in Soybean (Paknejad *et al.* (2009), Corn (Jun and Junying, 1996) and Wheat (Andres *et al.*, 1989).

The reduction in total chlorophyll concentration by water stress leads to chlorophyll proxidation and thereby its break-down (Jabari *et al.*, 2006). Castrillo and Trujillo (1994) assert that the more retention in chlorophyll concentration under drought the more stability in photosynthesis, selection for chlorophyll stability index will enhance in improving drought resistance in cowpea. Phenotypic correlation coefficient among stomatal conductance and chlorophyll content (SPAD) showed a positive correlation at vegetative stage of water stress (r= 0.49) and unstressed plants (r= 0.544) the positive correlation between these two parameters suggest the possible use of both measurements in selecting drought- tolerant genotypes.

Table 3: Total chlorophyll content (SPAD) for cowpea genotype at 17 and 33 days after water stress induction grown under different moisture stress condition

	SPAD at 17d			SPAD at 33d		
Genotype	**Unstr**	**vgt**	**pdf**	**Unstr**	**Vgt**	**pdf**
IT00K-1217	57.97	53.7	59	44.53	36.27	29.3
IT00K-835-45	52.8	35.63	51.13	32.4	21.9	23.9
IT00K-898-5	55.2	40.7	62.6	35.37	22.63	27.3
IT00K-901-5	61.0	48.47	60.67	39.13	22.23	35.1
IT03K-378-4	63.03	55.93	66.07	56.17	28.03	36.1
IT04K-405-5	56.3	47.13	49.77	52.8	24.77	28.8
IT84S-2246-4	66.5	49.63	58.2	44.57	18.0	27.4
IT89KD-288	52.4	40.53	55.33	43.17	19.23	26.1
IT95K-238-3	57.17	49.63	57.77	47.07	23.67	25.7
IT96D-610	55.33	45.77	62.93	29.43	14.73	24.2
IT97K-499-35	61.57	56.07	62.93	46.13	22.73	39.9
IT97K-568-18	53.17	41.2	57.0	36.2	24.07	25.6
IT97K-819-118	57.07	41.13	49.5	29.9	18.03	24
IT98K-1093-1	55.4	46.57	54.77	53.07	25.07	38.6
IT98K-1111-1	59.53	41.23	58.23	39.83	19.13	18.9
IT98K-205-8	56.9	59.87	62.43	54.27	23.73	34.1
IT98K-555-1	52.9	30.1	41.57	24.07	18.47	20.2
IT99K-216-44	63.47	50.43	63.67	44.5	23.1	29.9
IT99K-241-2	62.5	57.03	60.2	58.2	25.1	23.6
IT99K-377-1	64.4	49.37	57.7	50.17	31.23	39.1
Mean	58.23	47.01	57.57	43.05	23.11	28.9
SE	3.912	2.996	2.298	3.764	3.182	4.79
mean square	SPAD17d			SPAD33d		
WTrt	2380.4*			6315.4**		
Geno	244.50**			308.58**		
WTrt*geno	45.49			80.26		

Key: SE, standard error of means; WTrt, water stress treatment; Geno, genotypes; WTrt*geno, water stress treatment and genotypes interaction; Unstr, unstressed; vgt, vegetative; pdf, pod-filling stress.

Grain Yield

The present study showed that water stress reduced yield component more especially number of pods at harvest, resulting in decrease in grain yield (Table 4). Drought stress significant reduced grain yield at vegetative stages than at pod-filling stages. At vegetative stage, the grain reduction ranged from 47.23% to 76.25%, the lowest reduction in grain yield was exhibited by IT00K-898-5 having 47.23%. The highest grain yield reduction was recorded in IT04K-405-5 with 73.53%. At pod-filling stages of water stress, there was an increase in grain yield as exhibited by IT00K-1217 with 3.64%, IT95K-238-3 6.58%, IT99K -214-2 8.83% and IT99K-377-1 2.86%. However, varieties IT04K-405-5 had the highest percent reduction in grain yield (64.88%) followed by IT98K-555-1 (56.90%) at vegetative stages. The result concur with that of Abayomi and Abidoye (2009) in cowpea, that water stress reduced grain yield more under severe stress than moderate stress. Decrease in the grain yield was due to reduced stomatal conductance which ultimately reduced the physical transfer of carbon for photosynthesis and reduced sink. Flower abortion in drought stress genotypes may be attributed to high reduction in grain yield.

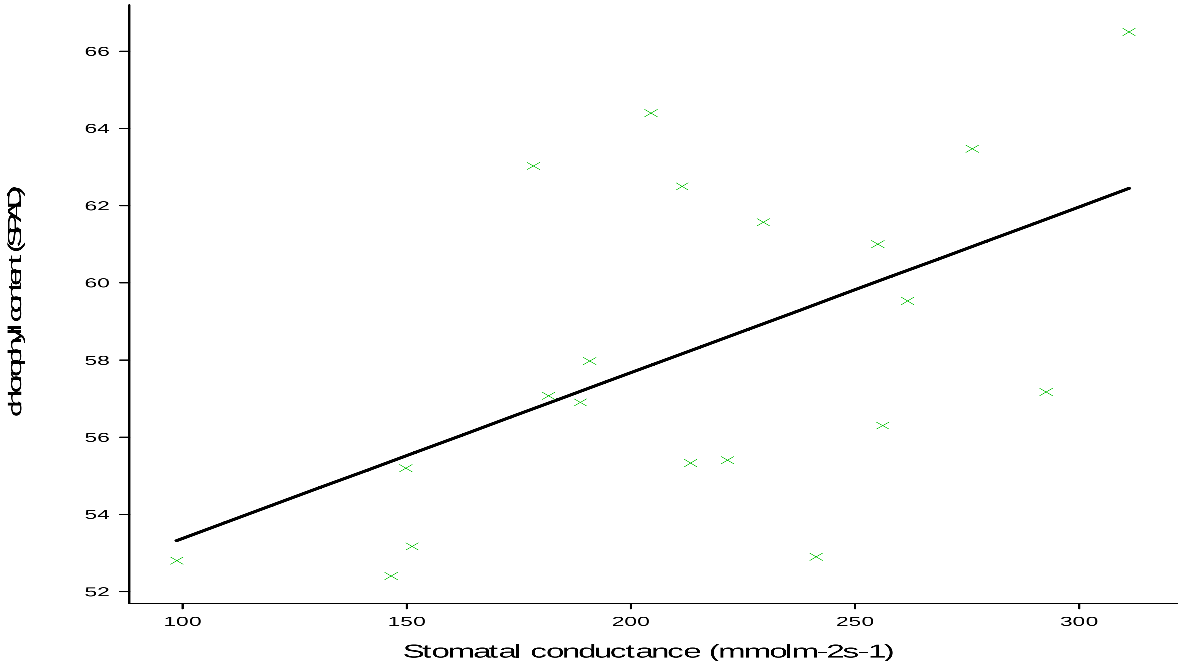

Figure1: Linear regression of stomatal conductance versus chlorophyll content (SPAD) at non stress water condition

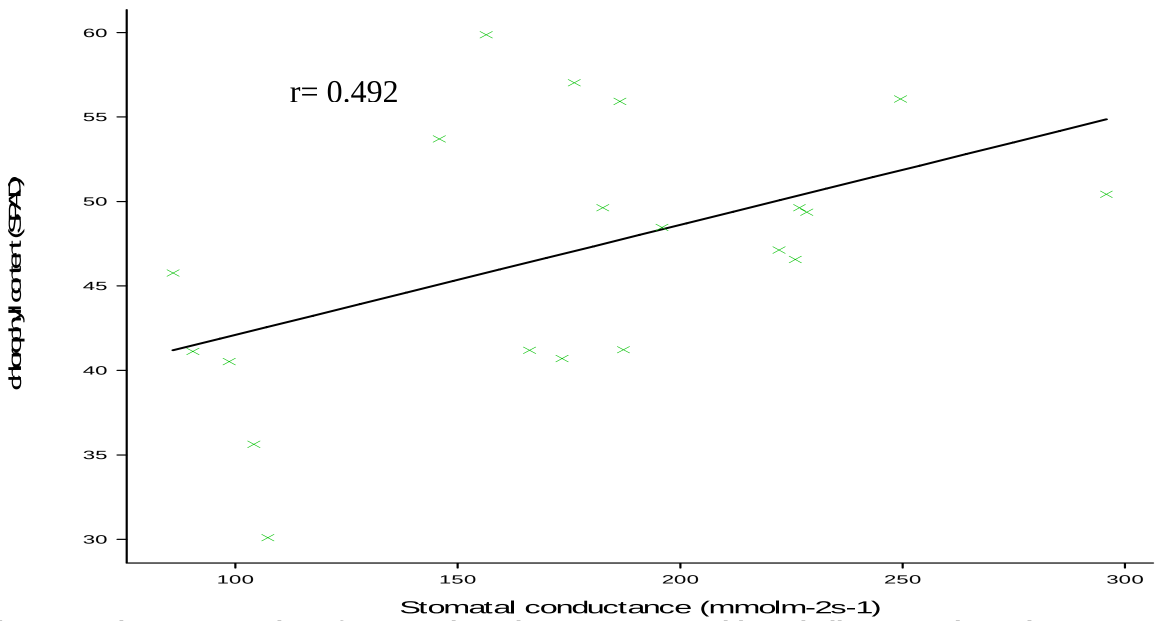

Figure 2: Linear regression of stomatal conductance versus chlorophyll content (SPAD) at vegetative water stress condition

Water stress reduced grain size and weight under both water stress conditions, reduction in 100 seed weight may be due to shortened assimilates as a result of limitation of physical transfer of carbon in to the stomatal pores (reduction in stomatal conductance) , similar findings were reported in Soybean (Abayomi, 2008).

The result showed that genotypes exhibiting higher reduction in grain yield and stomatal conductance were recorded in IT04K-405-5, IT98K-555-1, IT99K-241-2 and IT99K-216-44 under vegetative stages of water stress. These genotypes exhibited lower reduction in grain yield and stomatal conductance at pod-filling stages except in IT04K-405-5, which recorded an increase in grain yield. Genotypes with low reduction in GY and moderate reduction in STC were recorded in IT98K-205-5, IT00K-898-5 and IT00K-901-5 at vegetative stages. Moderate reduction in stomatal conductance, which is associated with lower reduction in grain yield, is a desirable trait for selection of genotypes under water stress conditions.

CONCLUSION

The present study concluded that vegetative stages of water stress significantly reduced stomatal conductance and chlorophyll content, similarly reduction in agronomic components under vegetative water stress were higher than pod-filling stress. Selection for genotypes under water stress based on physiological as well as agronomic traits indicates that IT04K-405-5, IT98K-555-1, IT99K-241-2 and IT99K-216-44 recorded higher reduction in GY and stomatal conductance. On the other hand, genotypes IT98K-205-5, IT00K-898-5 and IT00K-901-5 showed low grain yield reduction and moderate reduction in stomatal conductance.

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Table 4: Mean Pod Number, Pod Weight (g plant-1), Grain Yield (g/ m2) and 100 Seed Weight (g) of cowpea genotypes at different moisture stress

	Pod number			**Pod W(g)**			**grain yield(g/m2)**			**100SW(g)**		
Genotype	**Unstr**	**vgt**	**Pdf**	**unstr**	**Vgt**	**pdf**	**unstr**	**vgt**	**Pdf**	**unstr**	**vgt**	**pdf**
IT00K-1217	390	259	496	529	291	596	66	34.4	68.4	15.53	13.81	13.78
IT00K-835-45	570	448	328	462	265	328	65	34.3	43.1	10.53	10.21	9.69
IT00K-898-5	607	424	508	647	380	598	87.6	47.8	83.2	17.37	16.36	17.17
IT00K-901-5	495	357	527	799	414	730	106.6	56	93.5	17.04	14.83	16.23
IT03K-378-4	577	279	335	1159	524	629	154.9	70.3	82.1	22.73	21.9	21.94
IT04K-405-5	620	267	297	2398	585	807	285	67.7	100.1	21.91	19.7	18.46
IT84S-2246-4	577	359	433	693	391	506	95.6	45.1	60.8	15.35	13.87	14.02
IT89KD-288	457	241	486	818	315	702	109.4	40.6	93.8	18.78	17.18	16.84
IT95K-238-3	299	160	353	406	132	416	44.2	11.7	47.1	20.78	18.41	18.69
IT96D-610	245	120	194	308	143	293	42.6	18.6	38.6	18.7	14.55	15.67
IT97K-499-35	424	276	362	784	394	562	95.9	47.4	71.3	17.59	14.89	15.85
IT97K-568-18	591	318	390	941	372	585	121.8	47.1	75.7	18.38	15.62	15.50
IT97K-819-118	352	179	210	336	188	244	48.4	24.5	32.3	15.16	13.69	14.22
IT98K-1093-1	734	418	480	1492	673	910	194.1	83.3	114.9	13.51	12.6	12.53
IT98K-1111-1	490	265	413	648	258	512	89.4	32.5	68.6	15.92	13.82	13.95
IT98K-205-8	288	273	289	468	328	378	59.1	34.6	45.5	16.21	14.65	14.95
IT98K-555-1	368	223	218	668	251	301	77.5	24.6	33.4	17.63	12.84	15.38
IT99K-216-44	336	153	280	644	272	505	82.1	32.5	62.8	25.74	24.27	23.90
IT99K-241-2	394	217	547	946	376	996	123.5	48.6	134.4	27.66	23.66	23.31
IT99K-377-1	515	341	561	1168	538	1016	119	61.5	122.4	14.51	13.22	13.00
Mean	466	279	385	816	355	581	103.4	43.1	73.6	18.05	16	16.25
SE	87.7	60.3	69.1	177.1	63.2	100.7	20.71	7.58	12.82	0.526	0.775	0.62
mean squares	NPD		Fpr	PDW		Fpr	GY		Fpr	100SW		Fpr
T	584711		< 0.001	31658		< 0.001	54015.7		< 0.001	92.369		< 0.001
G	8328		< 0.001	59698		< 0.001	9172.4		< 0.001	125.114		< 0.001
T*G	17327		0.384	14469		< 0.001	2042.8		< 0.001	1.494		0.181
Covariate	39292		0.122	6995		0.69	288.5		0.496	0		0.988

SE, standard error of means, Lsd; least significant difference at 5%, unstr, unstressed; vgt, vegetative stage; pdf, pod-filling stage; G, genotype; WT, water stress treatment; WT*G, water stress treatment and genotype interaction; **, significant at p<0.001