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Heat Tolerance of Durum Wheat (*Tritcum durum* Desf.) Elite Germplasm Tested along the Senegal River

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Abstract

The Senegal River basin (Guinea, Mali, Mauritania, and Senegal) is a key agricultural production area in sub-Saharan Africa. Here, rice fields are left fallow during the cooler winter season, when the night temperatures reach 16 °C but the maximum daily temperatures remain above 30 °C. This season was used for the first time to conduct multi-environmental trials of durum wheat. Twenty-four elite breeding lines and cultivars were tested for adaptation during seasons 2014-15 and 2015-16 at two stations: Kaedi, Mauritania and Fanaye, Senegal. Phenological traits, grain yield and its components were recorded. Top grain yield was recorded at 5,330 kg ha^{-1} and the average yield at 2,484 kg ha^{-1}. The season lasted just 90 days from sowing to harvest. Dissection of the yield in its components revealed that biomass and spike fertility (*i.e.* number of seeds produced per spike) were the most critical traits for adaptation to these warm conditions. This second trait was confirmed in a validation experiment conducted in 2016-17 at the same two sites. Genotype × environment interaction was dissected by AMMI model, and the derived IPC values used to derive an 'AMMI wide adaptation index' (AWAI) to asses yield stability. The use of a selection index that combined adjusted means of yield and AWAI identified three genotypes as the most stable and high yielding: 'Bani Suef 5', 'DAWRyT118', and 'DAWRyT123'. The last two genotypes were also confirmed among the best in a validation trial conducted in season 2016-17. The data presented here are meant to introduce to the breeding community the use of these two research stations along the Senegal River for assessing heat tolerance of wheat or other winter cereals, as well as presenting two new ideal germplasm sources for heat tolerance, and the identification of spike fertility as the key trait controlling adaptation to heat stress.

Keywords: AMMI, genotype × environment interaction, selection index, short season, durum breeding, Mauritania

1. Introduction

The area along the Senegal River represents a major agricultural basin in Sub-Saharan Africa with the potential of 375,000 ha of arable and irrigated land. Today, a portion corresponding to approximately 200,000 ha is intensively cultivated with double seasons of rice (FAO, 2016). However, the cool season between middle of November to early March is not suitable for rice cultivation, and fields are mostly left at fallow. Preliminary results show that heat tolerant varieties of wheat could be cultivated in this area instead of the fallow season (Bado et al., 2010).

Here is presented an attempt to investigate further the suitability of durum wheat production as a replacement for the fallow season by means of full scale breeding trials conducted over locations and years. The adaptability of a variety over a diverse environment is usually tested by the degree of its interaction with it (Ashraf et al., 2001). The importance of genotype × environment (G×E) interactions in breeding programs has been demonstrated in many major crops, including wheat (Najafian et al., 2010; Zali et al., 2011). This interaction complicates the identification of superior genotypes for a range of environments and calls for the evaluation in multiple sites to determine their true genetic potential (Yaghotipour & Farshadfar, 2007). Various statistical methods have been proposed to study G×E interactions (Lin et al., 1986; Becker & Léon, 1988; Crossa, 1990; Lin & Binns, 1994; Mohammadi & Amri, 2008; Malosetti et al., 2013). The additive main effect and multiplicative interaction (AMMI) model was developed specifically for analysis of G×E interaction in multi-locations varietal trials (Zobel et al., 1988). It estimates the total G×E effect of each genotype and partitions it into interaction effects with environmental components (Malosetti et al., 2013).

Hence, the aim of this research was to identify stable and high yielding durum wheat genotypes well adapted to the Senegal River Basin through multi-year and multi-location trials, as well as pinpointing the main traits critical for adaptation to heat stress. To the best of our knowledge, this is the first time that such an effort is conducted for this region.

2. Materials and Methods

2.1 Argo-Environmental Conditions

The experiments were carried out in two irrigated Savanah-type experimental stations: Fanaye, Senegal (FAN: 16°53′ N; 15°53′ W) and Kaedi, Mauritania (KED: 16°14′ N; 13°46′ W) during winter seasons 2014-15, 2015-16 and 2016-17. FAN is located 150 Km inland from the Senegal River delta, while KED is 300 km further away from the coast and its mitigating effect, and therefore tends to be warmer (Figure 1). FAN has sandy-clay soil with higher organic matter and good water holding capacity, while KED has lighter sandy-loam-clay soils with intermediate water holding capacity. All soils are rich in phosphorus (P) and low in the other nutrient, as typical for the 'Sahara effect' (Boy et al., 2008).

2.2 Plant Materials and Experimental Design

Twenty-one durum wheat elites were selected from two ICARDA international nurseries, the 1st Afrique du Nord trials (AfN) and the 38th International Durum Yield Trials (IDYT38), and from CIMMYT 46th International Durum Yield Nurseries (IDYN46). In addition, the three cultivars 'Waha' (syn. 'Cham1', Syria and Algeria), 'Bani Suef5' (Egypt), and 'Miki3' (syn. 'Berdawni', Lebanon) were included as checks, thereby having 24 genotypes include in the 'discovery' trial conducted in seasons 2014-15 and 2015-16 (Table 1).

Table 1. Durum wheat genotypes used for field evaluation, their best linear unbiased estimator (BLUE) for grain yield (GY) across two sites in two seasons along the Senegal River and its summary statistics

Genotype	Pedigree	GY (kg ha^{-1})	
Icamoram7	IcamorTritArarat0472/Ammar7	2,931	a
Margherita	Terbol97-5/Geruftel2	2,861	ab
Bani Suef 5	Dupperez/Bushen3	2,858	ab
DAWRyT118	Mrb5/TdicoAlpCol//Cham1	2,825	abc
Icavert	Ter1/3/Stj3//Bcr/Lks4/4/Aghrass1/3/Mrf1// Mrb16/ Ru	2,762	abcd
DAWRyT123	Mrb5/TdicoAlpCol//Cham1	2,730	abcd
DurAM-196	Korifla/AegSpeltoidesSyr//Loukos	2,683	bcd
DWAyT217	Korifla/AegSpeltoidesSyr//Loukos	2,645	bcde
IDYN46-748	MXI12-13/C46IDYN/180129	2,639	bcdef
Icakassem1	Geromtel1/Icasyr1	2,604	cdefg
DAWRyT317	Korifla/AegSpeltoidesSyr//Mrb5	2,599	cdefg
Ouassara3	Ouasloukos1/5/Azn1/4/BEZAIZSHF//SD19539/Waha/3/Gdr2	2,595	cdefg
Icaverve	Azeghar1/4/IcamorTA0462/3/Maamouri3	2,524	defgh
Waha	Plc/Ruff//Gta/Rtte	2,438	efghi
Icarukus	Maamouri1/5/IcamorTA0462/4/Stj3//Bcr/Lks4/3/Icamor/6/Mgnl3/Ainzen1	2,404	fghi
DAWRyT208	Korifla/AegSpeltoidesSyr/Amedakul	2,378	ghi
DWAyT306	Korifla/AegSpeltoidesSyr//Heider	2,345	hi
IDYN46-742	MXI12-13/C46IDYN/180112	2,309	hij
Ouassara1	Ouasloukos1/5/Azn1/4/BEZAIZSHF//SD19539/Waha/3/Gdr2	2,304	hij
IDYN46-707	MXI12-13/C46IDYN/180006	2,250	ijk
DAWRyT110	Amedakul1/TdicoSyrCol//Cham1	2,094	jk
DAWRyT104	Amedakul1/TdicoJorCol//Cham1	2,028	kl
Bezaghras	Ossl1/Stj5/5/Bicrederaa1/4/BEZAIZSHF// SD19539/Waha/3/Stj/Mrb3/6/Mgnl3/Aghrass2	2,018	kl
Miki3	Stj3//Bcr/Lks4	1,797	l
Mean		2,484	
LSD		239	
Coefficient of variation (%)		9.6	
Heritability		0.77	

A second set of genotypes identified as 'validation' experiment was conducted only in season 2016-17. It included twenty durum wheat elites selected from the 39th International Durum Observation Nurseries (IDON39), the three best (DAWRyT123, DAWRyT118, Bani Suef 5) and the one earliest (Oussara3) genotypes from the two previous seasons used as checks (Table B1).

All experiments were performed in alpha lattice design with six sub-blocks of size four repeated two times. The genotypes were grown in experimental plots of 7.5 m^2 at a sowing density of 120 kg ha^{-1}. A total of 150 kg of nitrogen were provided in three equal split applications, while 50 kg of phosphorus and potassium were provided as base fertilization before planting.

Weeds were chemically controlled during season 2014-15 by using a tank mixture of Derby (DowAgroscience, florasulam and flumetsulam) and Cossack (Bayer, sulfonylurea and safener) applied at Zadoks stage 14 (Z14, Zadoks et al., 1974), followed by a tank mixture of Derby and Pallas (DowAgroscience, pyroxsulam) at tillering stage (Z23). Mechanical weeding was also conducted as needed to ensure clean paddocks. For seasons 2015-16 and 2016-17 only mechanical weeding was conducted due to the unavailability of the chemical herbicides.

During 2014-15 season nine gravity irrigations were performed at intervals of 7-10 days in KED and in FAN for a total estimated of 320 mm and 410 mm of water provided, respectively. During 2015-16 season, the same number of gravity irrigations were performed in FAN, but reducing the quantity of water to approximately 360 mm total, while the number of irrigation was increased to 13 in KED for a total of approx. 380 mm of water. For season 2016-17 a total of approx. 380 mm of water were provided at the two stations via at intervals of 7-10 days.

2.3 Data Recording

The days to heading (DtH) was recorded as the number of days elapsed from sowing to the moment that 50% of the plot showed spikes emerging from the flag leaf (Z59). Before maturity (Z83-87), the number of fertile spike

per meter square (Spk/m^2) were counted. Days to maturity (DtM) was recorded when 50% of the spikes turned yellow (Z91-92). A proxy of grain filling period (GFP) was then computed as the difference between DtM and DtH. Plant height (PLH) was measured in cm from the ground to the top of a representative ear excluding its awns. For each plot, only the middle rows were harvested for a total surface of 4.5 m^2, dried and the biomass (Biom) weighted before threshing. The weight of the threshed grains was converted into yield (GY) expressed as kg ha^{-1}. The ratio between GY and Biom was expressed as harvest index (HI). One thousand grains were weighted in grams as 1000-kernels weight (TKW). The number of grain per meter square (Gr/m^2) was imputed using the weight of the grains harvested from 4.5 m^2 area and the average weight of one kernel derived from the TKW value, as per:

$$Gr/\mathrm{m}^2 = \frac{Harvested\ weight\ of\ plot}{4.5\ \mathrm{m}^2 \times \frac{TKW}{1000}} \quad (1)$$

The number of grains per spike (Gr/spk) was derived from dividing the imputed number of grains per unit area by the number of spikes recorded for the same area, as follows:

$$Gr/spk = \frac{Gr/\mathrm{m}^2}{Spk/\mathrm{m}^2} \quad (2)$$

DtM, GFP, and Spike/m^2 were not recorded for season 2014-15.

2.4 Data Analysis

Both genotypes and environments were considered as fixed effects. Best linear unbiased estimators (BLUEs) of all traits were obtained using META-R (Multi Environment Trial Analysis with R for Windows) version 5.0 (Alvarado et al., 2015). Analysis of variance was computed for each environment using R version 3.2.1 (R Core Team, 2015), while combined ANOVA was obtained with GEA-R (Genotype × Environment Analysis with R for Windows) version 2.0 (Pacheco et al., 2015). Heritability was calculated based on the modified method suggested by Burton and Devane (1953) as follows:

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p} = \frac{\frac{MS_g - MS_e}{r}}{MS_e + \frac{MS_g - MS_e}{r} + \frac{MS_{gxe} - MS_e}{r \cdot e}} \quad (3)$$

Where, $\sigma^2 g$ is genotypic variance, $\sigma^2 p$ is phenotypic variance, *MSg* is the mean square for the genotype, *MSe* is error mean square, *MSG×E* is the mean square of the interaction, *r* is the number of replicates and *e* is the number of environments considered.

The ratio of variance accounted by each source of variations (G, E, and G×E) was calculated dividing the sum of square of each for the total sum of square of the experiment.

For grain yield, G×E was partitioned by additive main effects and multiplicative interaction 2 (AMMI) model using R software (version 3.2.4) on R Studio. The 'AMMI wide adaptation index' (AWAI) was calculated using the following formula:

$$AWAI = \Sigma_i s_i \cdot |PC_i| \quad (4)$$

Where, *i* is the number of significant IPCs determined by classical Gollob F-test in R Studio corresponding to 4 IPC in this specific case, si is the percentage of total G×E variance explained by each IPC, and PC is the actual IPC value. AWAI values close to '0' are obtained for the most widely adapted and stable germplasm (Bassi & Sanchez-Garcia, 2017). A performance index was generated by simultaneously selecting the best one third of the genotypes based on stability (AWAI) and one third best for average yield (BLUE). Genotypes that met both criteria were selected as the most suitable for cultivation along the Senegal River.

3. Results

3.1 Heat-Prone Field Stations along the Senegal River

Temperatures along the Senegal valley varied across sites and years with much warmer temperatures during the season 2015-16 (Figure 1) mainly at the flowering windows. Planting was completed on the 6th of December in FAN15, then the 17th of December in FAN16, and further delayed to the 24th December in FAN17. Sowing occurred on the 3rd December in KED15, 10th December in KED16, and 18th December in KED17. The delay of sowing at both sites were due to late harvesting of rather long rice seasons.

During all growing seasons in FAN, average minimum night temperatures oscillated between 14 °C and 18 °C, while in KED the minimum night temperatures rarely descended below 22 °C. Maximum day temperatures oscillated between 30 °C and 33 °C in FAN15, while reached between 34 °C and 37 °C in FAN16 and FAN17. In KED16 the maximum temperatures remained constant between 33 °C and 35 °C while reached 37 °C during the

period of grain filling in KED17. Temperature data for KED15 could not be recorded due to the unavailability of a weather station at the time.

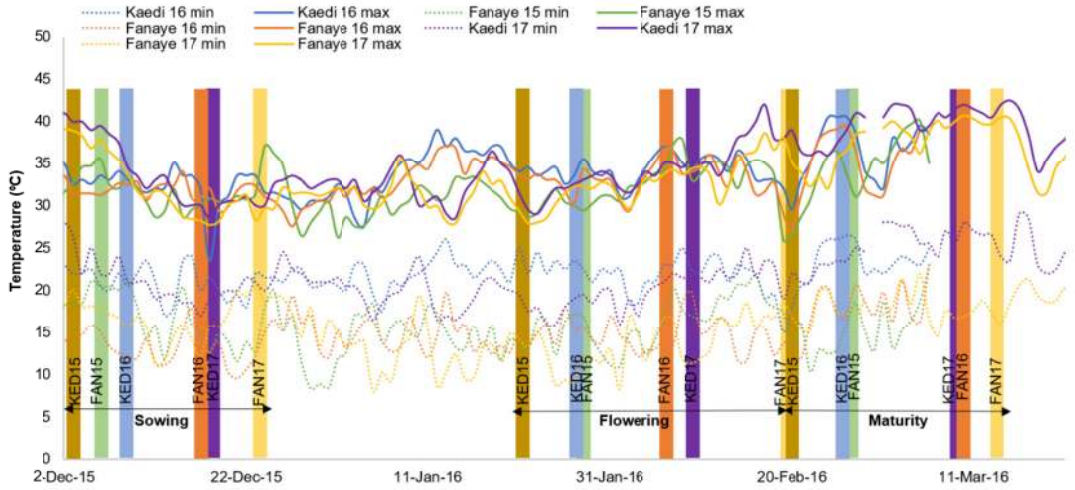

Figure 1. Maximum and minimum temperatures during seasons 2014-15, 2015-16 and 2016-17 at Fanaye and Kaedi with indications on the date of planting, average flowering and average maturity dates. Temperature data for Kaedi 2014-15 are not available

3.2 Effect of Phenology

The ANOVA (Table 2, Table A1 and Table A2) for DtH and PLH revealed statistically significant differences ($p < 0.01$) for the environments (E), genotypes (G), and their interaction (G×E). The DtH mean across 4 environments of the two first seasons was 52 days with experimental coefficient of variation (CV) acceptably low (2.0%). The 'validation' experiment of 2016-2017 for two environments had a DtH mean of 56 days with a CV of 1.6 %. The E explained 33.7% of the total variation in heading across the four environments, while G accounted for the largest part (35.8%), and G×E for 20.0%. The broad sense heritability (H^2) was measured at 0.85 (Table 2). PLH mean across environments was 72 cm and its CV 4.0%. E explained 41.0% of the variance, G and G×E accounted for 30.1% and 20.7%, respectively, with a broad sense heritability of 0.84. The ANOVA (Table 3 and Table A7) for DtM revealed statistically significant differences ($p < 0.05$ at FAN16 and $p < 0.01$ at KED16, FAN17 and KED17) for the genotype effect (G). The overall DtM across the two environments (FAN16 and KED16) was 80 days with a CV of 1.1% while it was 86 days for the validation set with a CV of 1.3%.

Table 2. Combined analysis of variance for days to heading (DtH), plant height (PLH), grain yield (GY), 1,000-kernels weight (TKW), harvest index (HI) and biomass (Biom) expressed as ratio of the total variance (% var.), significance (p), and broad sense heritability (H^2) across two sites in two seasons along the Senegal River

SOV	df	DTH		PLH		GY		TKW		HI		Biom	
		p	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.
E	3	(0.001)	33.70	(0.001)	41.00	(0.001)	77.70	(0.001)	57.77	(0.001)	66.39	(0.001)	59.11
G	23	(0.001)	35.78	(0.001)	30.06	(0.001)	7.29	(0.001)	23.20	(0.001)	8.61	(0.001)	14.22
G×E	69	(0.001)	19.95	(0.001)	20.70	(0.001)	12.63	(0.001)	11.43	(0.001)	17.16	(0.001)	17.84
PC1[a]	25	(0.001)	52.95	(0.001)	58.68	(0.001)	83.48	(0.001)	64.87	(0.001)	66.01	(0.001)	62.76
PC2[a]	23	(0.001)	34.41	(0.001)	34.77	(0.01)	11.32	(0.05)	28.72	(0.01)	25.88	(0.01)	27.03
PC3[a]	21		12.64		6.55		5.20		6.41		8.11		10.21
Error	96		10.57		8.25		2.39		7.60		7.84		8.82
H^2		0.85		0.84		0.77		0.84		0.59		0.70	

Note. SOV, source of variation; df, degrees of freedom; E, environment; G, genotype; PC, principal component of G×E from AMMI model; DtH, days to heading; PLH, plant height; GY, grain yield; TKW, thousand kernel weights; HI, harvest index; Biom, biomass.

[a] Partitioning of the G×E.

Table 3. Analysis of variance for days to maturity (DtM), grain filling period (GFP), spike per m^2 (Spk/m^2) and number of grain per spike (Gr/spk) expressed as ratio of the total variance (% var.), significance (*p*), and broad sense heritability (H^2) at two sites during season 2015-16 along the Senegal River

SOV	df	Fanaye 2015-16 (FAN16)								Kaedi 2015-16 (KED16)							
		DtM		GFP		Spk/m^2		Gr/spk		DtM		GFP		Spk/m^2		Gr/spk	
		p	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.	*p*	% var.
G	23	(0.05)	86		81	(0.05)	86	(0.01)	89	(0.01)	95		77	(0.01)	90	(0.01)	94
Error	96		14		19		14		11		5		23		10		6
H^2		0.54		0.40		0.55		0.65		0.82		0.32		0.66		0.80	

Note. SOV, source of variation; df, degrees of freedom; G, genotype; DtM, days to maturity; GFP, grain filling period; Spk/m^2, number of spikes per meter square; Gr/spk, number of grain per spike.

3.3 Grain Yield Related Traits

The ANOVA for GY and TKW was significant ($p < 0.01$) for E, G, and their interaction (G×E). The GY mean across four environments (FAN15, FAN16, KED15 and KED16) was 2,484 kg ha^{-1} and the CV was 9.6%, while it was 3,078 kg ha^{-1} for the 'validation' trial in 2016-17 with a CV of 15.5%. The E explained the largest part of the total variation (77.7%), G accounted for 7.3%, and G×E for 12.6% (Table 2 and Table A6.1). The broad sense heritability was estimated at 0.77. The variation in GY among genotypes at each environment is presented in Figure 2, while their average performance across four environments is presented in Table 1. In FAN15, GY averaged 4,047 kg ha^{-1} and ranged from a minimum of 2,076 kg ha^{-1} to a maximum of 5,330 kg ha^{-1} for line 'Icamoram7'. In FAN16 average GY dropped by 60% to 1,633 kg ha^{-1}, ranging from 1,113 to 2,259 kg ha^{-1}. In KED15, the average GY was 2,492 kg ha^{-1}, with a minimum of 1,748 kg ha^{-1} and a maximum of 3,091 kg ha^{-1} reached by the same top yielding line of FAN15 ('Icamoram7'). In KED16 the average GY was reduced by 29% to 1,771 kg ha^{-1}. GY average in the "validation" trial was 2,871 kg ha^{-1} at FAN17 and 3,286 kg ha^{-1} at KED17.

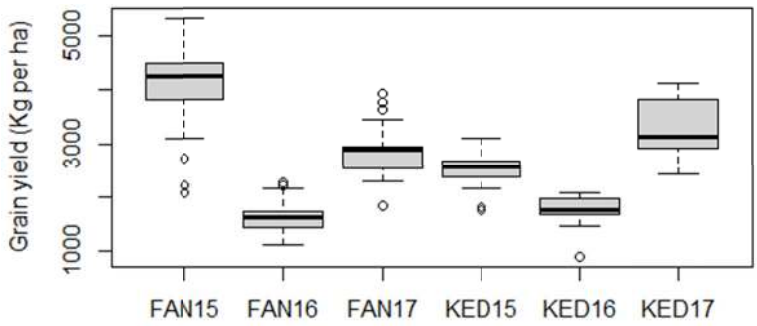

Figure 2. Grain yield distribution at two stations (KED-Kaedi, FAN-Fanaye) over seasons: 2014-15 (15), 2015-16(16) and 2016-17 (17). Thick dark horizontal lines show the averages, the box provides the total variation, whiskers have the length of one standard deviation, and empty circle indicate outliers

The combined analysis of genotypes performances across four environments (FAN15, FAN16, KED15, and KED16) identified 'Icamoram7', 'Margherita', 'Bani Suef 5', 'DAWRyT118', 'Icavert', 'DAWRyT123', 'DurAM-196' and 'DWAyT217' as the one third top yielders (Table 1), with performances from 6 to 18% above the grand mean. 'DAWRyT118' and 'DAWRyT123' were also 2nd and 8th top yielders of the 'validation' set with values non-significantly different than the top yielding line. Phenotypic variation was also identified for all other yield-related traits, and full details for each genotype at each site is provided as supplementary Dataset.

3.4 Stability Analysis

Combined ANOVA showed significance ($p < 0.01$) for G×E interaction for all agronomic traits. For GY, G×E effect accounted for 12.6% of the total variance (Table 2) and was further partitioned by AMMI into three PCs, each explaining 83.5%, 11.3%, and 5.2% of the G×E variation, respectively. The AWAI score was calculated, where a smaller value is indicative of genotypes falling closer to all PC axis and therefore more stable. Since G×E stability is also reached by genotypes that have low average yield performances, a selection index was designed to combine performances and stability in a bi-plot graph between the BLUE of GY and the AWAI

(Figure 3). The top one third of the genotypes were selected from both axis. Two ICARDA elites ('DAWRyT118' and, 'DAWRyT123') and the Egyptian cultivar 'Bani Suef 5' were the top yielding and most stable performers along the Senegal River. The two ICARDA's elites were also confirmed as top yielders in the 'validation' set.

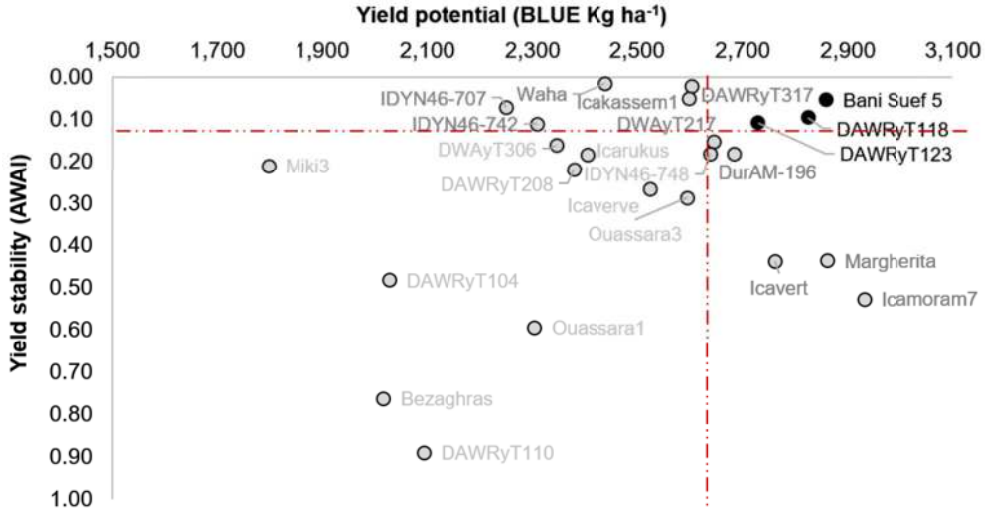

Figure 3. Biplot graph between the best linear unbiased estimator (BLUEs) for grain yield (kg ha^{-1}) and the additive main effects multiplicative interaction (AMMI) model's wide adaptation index (AWAI). Dashed lines were placed in correspondence to 1/3 best performing entry for each axis. The genotypes falling within the top right dashed square are the best yielding and most stable among those tested

3.5 Interactions among Traits

To understand the strategy utilized by the genotypes to adapt to the conditions of Senegal River Basin, a correlation matrix was developed to investigate associations between traits (Table 4). DtH was positively and highly correlated to DtM ($r = 0.91$, $p < 0.01$). PLH affected positively TKW and Biom ($r = 0.52$, $p < 0.05$ and $r = 0.57$, $p < 0.01$, respectively) and was negatively associated with HI ($r = -0.52$, $p < 0.05$). The same negative association was observed between Biom and HI ($r = -0.44$, $p < 0.05$). Gr.spk and Biom were the only traits that affect positively GY ($r = 0.48$ and $r = 0.49$, respectively, $p < 0.05$).

Table 4. Matrix of correlations among traits combined across locations and years. Those underlined were either significant (underlined once) or highly significant (underlined twice)

Traits	DtH	DtM	PLH	Spk/m^2	Gr/spk	TKW	Biom	GY
DtM	0.91							
PLH	0.23	-0.17						
Spk/m^2	-0.23	-0.25	-0.13					
Gr/spk	0.34	0.35	0.09	nv				
TKW	-0.19	-0.19	0.52	-0.28	-0.13			
Biom	0.35	-0.25	0.57	0.25	0.18	-0.03		
GY	0.02	0.13	-0.02	0.27	0.48	-0.17	0.49	
HI	-0.23	0.37	-0.52	-0.04	0.28	-0.22	-0.44	0.20

Note. Critical value Pearson's correlation for 23 df: 0.53 ($p < 0.01$), 0.41 ($p < 0.05$). DtH, days to heading; DtM, days to maturity; PLH, plant height; Spk/m^2, spikes per m^2; Gr/spk, grains per spike; TKW, 1,000, kernels weight; Biom, biomass; GY, grain yield; HI, harvest index; nv, not valid correlation.

A second analysis was conducted to further refine the identification of the traits used for adaptation. The top three (Top) and worst three (Worst) yielders at each location were singled out and compared for their performances across traits at each site (Table 5). The average GY was significantly different between the Top and

Worst in all environments. Top and Worst genotypes had significant difference for Gr/spk and Biom at two 'discovery' environment, and at one of the two 'validation' sites. PLH and HI showed significant differences only in one environment. All other traits had no significant differences between Top and Worst yielders during the two first seasons. Instead, in the 'validation' trials in 2016-17 DtH and DtM became significant at both sites.

Table 5. Top 3 and worst 3 yielding genotypes at each environment and comparison between their key traits for adaptation to heat stress

Traits	Fanaye 2014-15			Kaedi 2014-15			Fanaye 2015-16		
	LSD	Top	Worst	LSD	Top	Worst	LSD	Top	Worst
GY	673	5,176 *	2,337	383	2,934 *	1,900	487	2,242 *	1,116
DtH	2	54	52	2	51	51	2	53	52
DtM	.	.	.	.	.	.	2	83	81
PLH	7	72	76	5	63	68	6	78	75
Spk/m^2	.	.	.	.	.	.	63	352	330
Gr/spk	.	.	.	.	.	.	4	14 *	8
TKW	4	43	44	3	34	35	5	43	44
Biom	1,350	10,241 *	8,422	969	7,405	6,719	2,668	7,801	6,179
HI	7	51	51	6	40 *	31	12	28	20
Traits	Kaedi 2015-16			Fanaye 2016-17			Kaedi 2016-17		
	LSD	Top	Worst	LSD	Top	Worst	LSD	Top	Worst
GY	415	2,069 *	1,280	1,310	3,776 *	2,179	529	4,080 *	2,565
DtH	3	51	49	2	59 *	63		56 *	52
DtM	1	80	80	3	89 *	93	2	84 *	82
PLH	5	65 *	59	6	77	76	8	68	65
Spk/m^2	73	314	252	114	325	342	66	370	319
Gr/spk	3	19 *	15	13	28	19	7	33 *	26
TKW	2	35	34	10	45	37	4	34	32
Biom	1,395	6,189 *	3,339	3,375	10,023	7,884	1,539	9,188 *	6,374
HI	8	35	39	13	38	27	7	45	40

Note. GY, grain yield; DtH, days to heading; DtM, days to maturity; PLH, plant height; Spk/m^2, spikes per m^2; Gr/spk, grains per spike; TKW, 1,000, kernels weight; Biom, biomass; HI,harvest index. * More than one LSD significant difference between Top and Worst genotypes.

4. Discussion

4.1 Two New Wheat Experimental Stations for Discriminating Heat Tolerance

The stations of Fanaye, Senegal and Kaedi, Mauritania were selected to represent the agro-environmental diversity that occurs along the Senegal River, with a particular focus on the delta and middle valley, respectively. The E effect of the experiment captured 77.7% of the total variance for GY, suggesting that these two stations are adequately contrasting to conduct significant multi-locations breeding selection for heat tolerance (Figure C). The three seasons used for testing, 2014-15, 2015-16 and 2016-17 had clear differences in temperatures during the phase of flowering, mostly caused by the delay in sowing. In fact, GY at FAN16 and KED16 were 60% and 29% lower compared to the timely sown season 2014-15 at the same sites, respectively. In FAN16 the germplasm was exposed to the highest temperatures (37 °C) during the time of flowering time, which in turn caused a severe drop in productivity. The following season (FAN17) planting was further delayed, but a drop in temperature to 34 °C occurred at the time of flowering, and this pushed the average GY to nearly double of what achieved in FAN16. This result shows the level of damage that the increase of just 3 °C in temperature can cause to the productivity of durum wheat if it occurs at the time of heading.

4.2 Selecting the Most Heat Tolerant Genotypes

The two stations over the two first seasons generated significant ($p < 0.01$) G×E interaction for GY, indicating that the tested genotypes did not respond equally to the changes in temperatures and sowing time. However, several genotypes were found to be stable and high yielding regardless of these changes, such as 'DAWRyT118',

'Bani Suef 5' and 'DAWRyT123'. These lines were among the top yielders in all environments and their AWAI score showed good stability (AWAI < 0.11). In particular, 'DAWRyT118' and 'DAWRyT123' were also confirmed as best performers in the 'validation' trials, which indicates that these lines carry heat tolerant traits capable of maintaining GY performance under stressed conditions. The two entries are in fact sister lines derived from top crossing the two most successful cultivars of the ICARDA durum program ('Om Rabi 5' and 'Cham 1') to *Triticum dicoccoides* collected in the surroundings of Aleppo (Table 1). Zaim et al. (2017) already described the usefulness of using *T. dicoccoides* in breeding durum elites, and identified 'DAWRYT118' as a top performer across drought prone sites in North Africa, with strong disease resistance, and good industrial processing qualities. Hence, their use in crossing schemes by durum breeders targeting heat tolerance is highly advised.

4.3 Traits to be Targeted by Durum Wheat Breeders to Increase Tolerance to Heat Stress

Heat stress has many detrimental effects on wheat at its various growth stages. Phenology traits (DtH and DtM) interacted among one another, but did not affect grain yield. Also, there was no significant difference between Top and Worst yielding lines for phenology in the two first season. Only the 'validation' set showed sufficient phenological variation to identified significant differences between Top and Worst. This would suggest that rather phenology is not an important characteristic for heat tolerance, when temperatures are constantly hot throughout the growing cycle as instead previously suggested by Hossain et al. (2012). Or more likely, the difference in results could be due to a limited amount of variation expressed for phenology by the 'discovery' set, while it was sufficient in the 'validation' set. Hence, phenology would instead represent a critical target that must be fixed through breeding first in order to then identify additional useful traits for heat tolerance.

High temperatures also shorten the tillering phase, resulting in poor setting of fertile tillers (Baldy, 1984). In addition, when heat occurs at the time of flowering it can reduce the vitality of the pollen and fertilization during pollen formation (Barlow et al., 2015; Draeger & Moore, 2017). Instead, during the grain filling period, heat stress reduces grain size and its weight (Dias & Lidon, 2009). Therefore, all these yield components appear of interest for improving heat tolerance. The number of Gr/spk and Biom showed positive correlation to GY, and also scored as significantly different among Top and Worst genotypes in two 'discovery' and one 'validation' environments. This is in good agreement with previous research that has also shown that Biom plays a decisive role in favoring GY (Mekhlouf & Bouzerzour, 2000; Abbassene et al., 1997; Masoni et al., 2007; Bahlouli et al., 2008). FAN16 was the environment with the most severe temperatures extremes during the flowering phase, and Gr/spk was identified as the only trait significantly different between Top and Worst elites at this location. Therefore, the ability of the best genotypes to maintain good fertilization under the severe heat resulted in better seed setting (Gr/spk) and ultimately higher yields. This is in agreement with Barnabas et al. (2008), and Hatfield and Prueger (2015), who found that the moment of fertilization is one of the most heat sensitive phase. Gr/spk represents therefore the single most appealing target trait for breeding better heat tolerance. The genotypes 'DAWRyT118' and 'DAWRyT123' were selected for their performances and stability. Their strategy for adaptation in fact relied mostly on the capacity of maintaining high spike fertility (Gr/spk) regardless of the temperatures, and to produce more Biom early in the cycle. Conversely, *T. dicoccoides* has been already praised by other authors for its capacity to produce vast biomass as well as for the fertility of its spikes (Merchuk-Ovnat et al., 2016a, 2016b; Merchuk-Ovnat et al., 2017). It is therefore not surprising that the two genotypes derived from it maintained these positive traits and used them to maximize heat tolerance.

5. Conclusion

The results presented here suggest that Senegal Valley provides ideal conditions for testing heat tolerance in wheat. A total of three genotypes identified as stable and well performing under these conditions ('DAWRyT118', 'DAWRyT123' and 'Bani Suef 5') showed good heat tolerance through the production of large biomass and maintenance of spike fertility. Breeders targeting improvement for this or similar regions should then focus on these traits, and possibly combining it with better harvest index. The Senegal River basin is regarded as a key place to bring social stability and food security to sub-Saharan Africa. Our results indicate that durum wheat is a suitable replacement of the fallow cycle and monoculture of rice. The area of possible expansion of wheat cultivation corresponds to the 200,000 ha currently grown as rice. Multiplying this area by the average yield of 3 t ha^{-1} reached by the three best lines, suggests the potential of producing 600,000 t of new food in sub-Saharan Africa, a potentially life-changing impact.

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Appendix

Appendix A. ANOVA tables of all traits in all environments

Table A1. ANOVA tables of days to heading

Table A1.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	393.80729	37.6851	37.6851	3	131.2691	102.03914	0
GEN	418.11979	40.01166	77.69676	23	18.17912	14.13114	0
ENV*GEN	233.06771	22.30324	100	69	3.37779	2.62565	0.00001
PC1	124.78853	52.95196	52.95196	25	4.99154	4.28955	0
PC2	81.08349	34.40644	87.3584	23	3.52537	3.02958	0.00047
PC3	29.79165	12.6416	100	21	1.41865	1.21914	0.27522
PC4	0	0	100	19	0	0	1
Residuals	123.5	0	0	96	1.28646	NA	NA

Table A1.2. ANOVA of days to heading in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	307.597	13.374	16.635	2.69E-06***
Residuals	13	10.451	0.804		

Table A1.3. ANOVA of days to heading in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	392.31	17.0571	30.324	6.86E-08***
Residuals	13	7.31	0.5625		

Table A2. ANOVA tables of plant height

Table A2.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	4485.55729	44.68208	44.68208	3	1495.18576	159.04469	0
GEN	3288.45312	32.75734	77.43942	23	142.97622	15.20855	0
ENV*GEN	2264.81771	22.56058	100	69	32.82345	3.49147	0
PC1	1363.46279	58.68321	58.68321	25	54.53851	7.77204	0
PC2	807.84054	34.76932	93.45253	23	35.1235	5.00529	0
PC3	152.12579	6.54747	100	21	7.24409	1.03232	0.44491
PC4	0	0	100	19	0	0	1
Residuals	902.5	0	0	96	9.40104	NA	NA

Table A2.2. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	555.24	24.1407	3.0619	0.02015*
Residuals	13	102.5	7.8843		

Table A2.3. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	1144.21	49.748	4.9531	0.002244**
Residuals	13	130.57	10.044		

Table A3. ANOVA tables of biomass

Table A3.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	458010479	64.8325	64.8325	3	152670160	214.44405	0
GEN	110200240	15.59911	80.43161	23	4791314.78	6.72999	0
ENV*GEN	138241296	19.56839	100	69	2003497.05	2.81416	0
PC1	83686108	62.75976	62.75976	25	3347444.32	5.64576	0
PC2	36037580.5	27.02611	89.78588	23	1566851.32	2.64263	0.00189
PC3	13619877.5	10.21412	100	21	648565.597	1.09386	0.38322
PC4	0	0	100	19	0	0	1
Residuals	68345730.5	0	0	96	711934.693	NA	NA

Table A3.2. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Gnotypes	23	54645387	2375886	1.4045	0.2661
Residuals	13	21991330	1691641		

Table A3.3. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	26443797	1149730	3.7886	0.007991**
Residuals	13	3945148	303473		

Table A4. ANOVA tables of thousand kernel weight

Table A4.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	2776.04167	62.51994	62.51994	3	925.34722	243.379	0
GEN	1114.75	25.10557	87.62551	23	48.46739	12.74759	0
ENV*GEN	549.45833	12.37449	100	69	7.96316	2.09442	0.00041
PC1	353.32486	64.86914	64.86914	25	14.13299	3.91812	0.00002
PC2	156.44081	28.72196	93.5911	23	6.80177	1.88567	0.0297
PC3	34.90758	6.4089	100	21	1.66227	0.46084	0.97288
PC4	0	0	100	19	0	0	1
Residuals	365	0	0	96	3.80208	NA	NA

Table A4.2. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	472.9	20.561	1.0862	0.4523
Residuals	13	246.07	18.928		

Table A4.3. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	156.667	6.8116	1.7956	0.1373
Residuals	13	49.315	3.7935		

Table A5. ANOVA tables of harvest index

Table A5.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	12408.6823	72.03777	72.03777	3	4136.22743	270.95042	0
GEN	1610.11979	9.34744	81.38521	23	70.00521	4.58581	0
ENV*GEN	3206.44271	18.61479	100	69	46.47018	3.04411	0
PC1	2116.53764	66.00889	66.00889	25	84.66151	5.4112	0
PC2	829.78342	25.87863	91.88752	23	36.07754	2.30592	0.00644
PC3	260.12212	8.11248	100	21	12.38677	0.79171	0.71605
PC4	0	0	100	19	0	0	1
Residuals	1465.5	0	0	96	15.26562	NA	NA

Table A5.2. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	2209.53	96.067	2.2609	0.06472
Residuals	13	552.37	42.49		

Table A5.3. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	581.73	25.2925	2.6473	0.03612*
Residuals	13	124.2	9.5542		

Table A6. ANOVA tables of grain yield

Table A6.1. Combined ANOVA across four environments (FAN15, FAN16, KED15 and KED16)

SOV	SS	PORCENT	PORCENAC	df	MS	F	PROBF
ENV	176873915	79.59798	79.59798	3	58957971.8	1040.00694	0
GEN	16591033.2	7.46641	87.06439	23	721349.271	12.72446	0
ENV*GEN	28744097.4	12.93561	100	69	416581.121	7.34841	0
PC1	23995126.1	83.47845	83.47845	25	959805.046	16.65322	0
PC2	3254626.87	11.32277	94.80121	23	141505.516	2.45521	0.00373
PC3	1494344.34	5.19879	100	21	71159.2541	1.23466	0.26358
PC4	0	0	100	19	0	0	1
Residuals	5442238	0	0	96	56689.9792	NA	NA

Table A6.2. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	15604475	678455	3.661	0.009322**
Residuals	13	2409163	185320		

Table A6.3. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	9307709	404683	8.4176	0.0001384***
Residuals	13	624988	48076		

Table A7. ANOVA tables of days to maturity

Table A7.1. ANOVA in KED16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	98	4.2609	10.226	4.64E-05***
Residuals	13	624988	48076		

Table A7.2. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	392.31	17.0571	30.324	6.86E-08***
Residuals	13	7.31	0.5625		

Table A7.3. ANOVA in FAN16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	97.323	4.2315	3.3357	0.01403*
Residuals	13	16.491	1.2685		

Table A7.4. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	510.48	22.1947	9.2639	8.12E-05***
Residuals	13	31.15	2.3958		

Table A8. ANOVA tables of grain filling period

Table A8.1 ANOVA in KED16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	39.979	1.73822	1.9403	0.1081
Residuals	13	11.646	0.89583		

Table A8.2 ANOVA in FAN16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	35.25	1.53261	2.35	0.05638
Residuals	13	8.478	0.65217		

Table A9. ANOVA tables of spikes per m^2

Table A9.1. ANOVA in KED16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	135662	5898.4	4.8826	0.002408**
Residuals	13	15705	1208		

Table A9.2. ANOVA in KED 17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	26360	1146.1	1.118	0.4298
Residuals	13	13327	1025.2		

Table A9.3. ANOVA in FAN16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	71610	3113.49	3.4107	0.01274*
Residuals	13	11867	912.87		

Table A9.4. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	35327	1535.9	0.7023	0.7781
Residuals	13	28432	2187.1		

Table A10. ANOVA tables of grains per spike

Table A10.1. ANOVA in KED16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	452.48	19.673	8.9934	9.58E-05***
Residuals	13	28.44	2.1875		

Table A10.2. ANOVA in KED17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	425.57	18.5031	1.8773	0.1199
Residuals	13	128.13	9.8564		

Table A10.3. ANOVA in FAN16

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	346.51	15.0656	4.7061	0.002883**
Residuals	13	41.62	3.2013		

Table A10.4. ANOVA in FAN17

SOV	df	Sum Sq	Mean Sq	F value	Pr(> F)
Genotypes	23	961.19	41.791	2.1358	0.07883
Residuals	13	254.37	19.567		

Appendix B

Table B1. Durum wheat genotypes used for 'validation' trial, their best linear unbiased estimator (BLUE) for grain yield across two sites in season 2016-17and its summary statistics

Genotypes	Pedigree	BLUE	
AfN_14	Korifla/AegSpeltoidesSyr//Loukos	3,881	a
DAWRyT0118	Mrb5/TdicoAlpCol//Cham1	3,559	ab
AfN_19	Younes/TdicoAlpCol//Korifla	3,539	ab
ADYT14_29	IcamorTA041/4/IcamorTA0469/3/Bcr/Gro1//Mgnl1/5/MIKI2	3,514	abc
IDON38-38	Terbol975/Geruftel2 = Margherita	3,506	abc
AfN_05	Korifla/AegSpeltoidesSyr//Amedakul	3,387	abcd
IDON38-25	CandocrossH25/BEZAIZAHF//Adnan2	3,356	abcd
DAWRyT0123	Mrb5/TdicoAlpCol//Cham1	3,356	abcd
Ouassara3	Ouasloukos1/5/Azn1/4/BEZAIZSHF//SD19539/ Waha/3/Gdr2	3,278	abcde
ADYT14_2	Adnan1//Mgnl3/Ainzen1	3,239	abcde
ADYT14_55	Ossl1/Stj5/5/Bicrederaa1/4/BEZAIZSHF//SD19539/Waha/3/Stj/Mrb3/6/Stk/Hau//Heca1	3,222	abcde
IDON38-32	Azeghar2/Murlagost2//Bicrederaa1/Azeghar2	3,067	bcde
ADYT14_27	IcamorTA0471//IcamorTA0459/Arislahn10/3/Mgnl3/Ainzen1	2,983	bcdef
ADYT14_50	Mgnl3/Ainzen1/3/Bcr/Gro1//Mgnl1	2,878	bcdef
ADYT14_26	IcamorTA0471//IcamorTA0459/Ammar8/4/Stj3//Dra2/Bcr/3/Ter3	2,832	cdef
IDON38-96	Maamouri1/5/IcamorTA0462/4/Stj3//Bcr/Lks4/3/Icamors/6/Mgnl3/Ainzen1	2,831	cdef
AfN_18	Amedakul1/TdicoJCol//Cham1	2,823	cdef
IDON38-09	Icamilmus1/Waha/4/Icasyr1/3/Bcr/Sbl5//Turartu	2,781	def
Bani Suef 5	Dupperez/Bushen3	2,739	def
ADYT14_58	Azeghar1//Blrn/Mrf2/3/Bicrederaa1/Azeghar2	2,739	def
IDON38-42	Bicrederaa1//Ossl1/Stj5/3/Ammar8	2,722	def
ADYT14_19	Mrb3/Tdicoccoides601116//IcamorTA0463/Zna4/4/Stj3//Bcr/Lks4/3/ Ter3/6/Ossl1/Stj5/5/Bicrederaa1/4/BezaizSHF//SD19539/Waha/3/Stj/Mrb3	2,690	def
ADYT14_73	Ouasloukos1/5/Azn1/4/BEZAIZSHF//SD19539/Waha/3/Gdr2	2,646	ef
IDON38-49	Bcr/Lks4//Mrf1/Stj2/3/Mrf2/NormalHamari//Bcr/Lks4	2,311	f
Grand Mean		3,078	
LSD		698	
CV		16	

Appendix C

Figure C1. Experimental plots used for conducting yield trials in 2015/16, left Fanaye, Senegal and right Kaedi, Mauritania