Seventh framework programme
Food, Agriculture and Fisheries, and Biotechnology

Specific International Co-operation Actions
Small or medium scale focused research project

Sweet Fuel

Sweet Sorghum an alternative energy Crop

Grant Agreement n° 227422

Deliverable 4.3:

Information on expression of desirable traits (biomass, sugar, grain yield, delayed leafand root senescence through stay green, membrane integrity) under droughtin the field

Composition of the consortium

CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP

For this particular deliverable we worked on the parameters most related with sugar production (height, biomass, stem-stem-juice weight, brix) and membrane integrity as an indicator of resistance to stress. We did not work with root senescence due to the difficulty to analyze under field situation and leaf senescence because of other factors that affect the characteristics of leaf such as disease or soil fertility.

The following tasks were conducted :

1. *Characterize response to reproductive-stage and terminal drought for the reference pool of contrasting accessions (population will be completed with breeders' key materials).*
2. *Evaluate varietal tolerance to drought in terms of membrane integrity (electrolyte leakage);*
3. *Identify candidate genes/gene sequences for high sugar content from association mapping using SCAR markers.*

1. Characterize response to reproductive-stage and terminal drought for the reference pool of 12 contrasting accessions (population will be completed with breeders' key materials).

Materials and methods:

We evaluated 26 genotypes using a Split Plot Design. The experimental unit was four rows with 0.80 m between them and 5 m long. The water treatments were applied as shown in Figure 1.

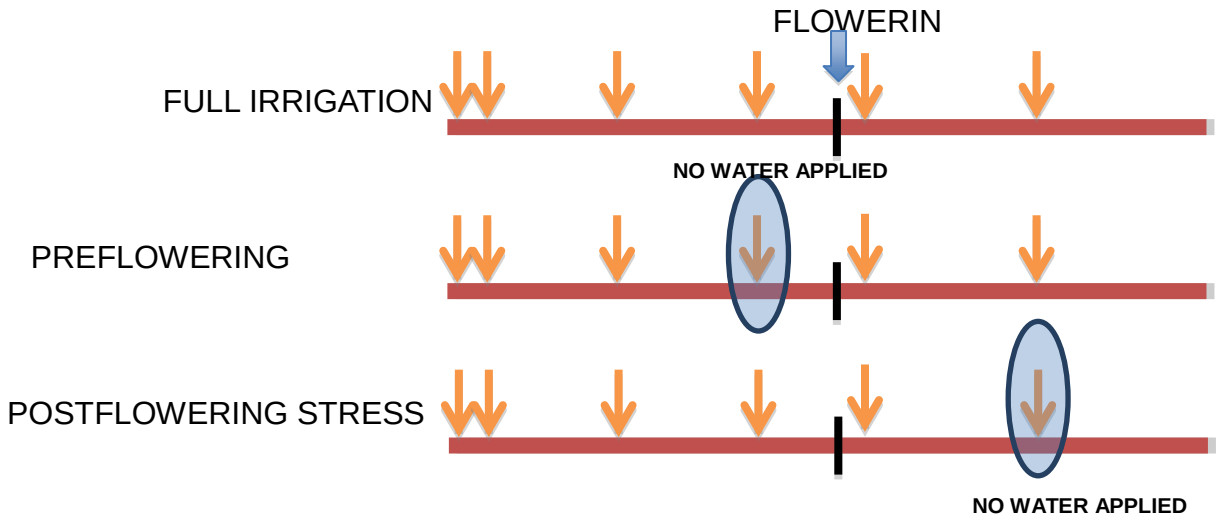


Figure 1.Water stress in the field for Preflowerig and posflowering stress treatments and full irrigation.

The following characteristics were measured: Plant height (AP m), Fresh weight (PV Kg), Stem weight (PT Kg), Juice weight (PJ Kg) and ºBrix (BRIX).

Results and discussion:

The analysis of variance for all characteristics is presented in Table 1.

Table 1: Analysis of variance for all characteristics

	AP	PV	PT	PJ	BRIX
rep	4.253	6.627	4.094	0.626	36.928
trat	**1.036***	0.041	**0.493***	0.029	7.730
rep*trat	0.346	0.089	0.063	0.011	10.547
genotype	**0.439***	**0.952***	**0.249***	0.034	**12.493***
trat*genotype	0.315	0.460	0.161	0.050	6.508
Error	0.215	0.516	0.134	0.043	5.710

Base on the analysis of variance, the mean comparison for the water treatment is presented in Table 2 for plant height.

Table 2: Mean comparison for water treatments

	m	treatments
A	2.1	POSTFLOWERING STRESS
B A	2.0	FULL IRRIGATION
B	1.8	PREFLOWERING STRESS

The table shows the effect of water stress during preflowering stage. In this period of growth the stem of the plant is still elongate and the water stress affects the height by limiting the stem to express the maximum value.

One of the main purpose of this experiment was to find the genotypes with better expression in height; this is because the stem is considered as the place to store the juice and therefore better chances to have the opportunity to hold juice for higher bioethanol production.

Based on the results of the analysis of variance, the mean comparison for the genotypes is presented in Table 3.

Table 3: Mean comparison for genotypes based on plant height (m)

		mean	Genotype
A		2.7	FAUANL-3
B	**A**	2.3	ICSA89002 X SPV422
B	**A**	2.3	AVRT2070-1
B	**A**	2.2	AVRT2015-3
B	**A**	2.2	ICSV93046
B	**A**	2.1	FAUANL-35A X FAUANL-39
B	**A**	2.1	KINGOLD
B	**A**	2.1	ICSA324 x SSV74
B	**A**	2.0	19S35SG0619
B	**A**	2.0	AVRT2057
B	**A**	2.0	SP081045-1
B	**A**	2.0	AVRT 2018-2
B	**A**	2.0	AVRT2061-1
B	**A**	2.0	AVRT1042-3
B	**A**	2.0	AVRT2061-2
B	**A**	1.9	OPV003
B	**A**	1.9	OPV008
B	**A**	1.9	CSH22SS
B	**A**	1.9	SP4487-3
B	**A**	1.9	OPV017
B	**A**	1.7	E36-1
B	**A**	1.7	FAUANL-34A X KELLER
B		1.6	KELLER
B		1.6	FAUANL-34A X FAUANL-39
B		1.5	AVRT1057-2
B		1.3	FORTUNA

There were two groups; the highest numerical value was for FAUANL-3 with 2.7 m and it was statistical equal to another 21 genotypes. In this particular group the lowest value was for FAUANL-34A x Keller with 1.7 m.

Keller (1.6 m) and Fortuna (1.3 m) were the extreme genotypes in the group with statistically lowest values. It is important to notice that the values shown in this table are lower compared with other cropping cycles, which is important to consider the effect of the environment on the height expression. According with Table 1, Fresh weight showed statistical differences only for genotypes, but Stem weight showed differences also for water treatments; this may be due to the differences of sources of the genotypes, since were tested genotypes from India, South Africa and México the variation was expected to be high.
The mean comparison for genotypes is show in Table 4.

Table 4: Mean comparison for genotype of fresh weight (Kg) and stem weight (Kg).

Freshweight			Genotype	Stemweight	
		Kg		Kg	
A		3.03	ICSV93046	0.98	A B
B	A	2.08	ICSA89002 X SPV422	1.55	A
B	A	2.08	AVRT2070-1	1.47	A B
B	A	2.00	KINGOLD	1.28	A B
B	A	1.93	19S35SG0619	1.46	A B
B	A	1.87	SP081045-1	1.35	A B
B	A	1.75	CSH22SS	1.15	A B
B	A	1.75	AVRT2057	1.28	A B
B	A	1.72	AVRT2061-1	1.17	A B
B	A	1.71	SP4487-3	1.24	A B
B	A	1.70	AVRT1057-2	1.05	A B
B	A	1.67	FORTUNA	0.88	A B
B	A	1.67	AVRT2015-3	1.17	A B
B	A	1.66	AVRT2061-2	1.27	A B
B	A	1.64	FAUANL-35A X FAUANL-39	1.17	A B
B	A	1.62	AVRT 2018-2	0.99	A B
B	A	1.58	FAUANL-3	1.15	A B
B	A	1.56	E36-1	1.07	A B
B	A	1.55	OPV003	1.11	A B
B	A	1.48	AVRT1042-3	1.09	A B
B		1.43	ICSA324 x SSV74	1.16	A B
B		1.43	OPV017	0.99	A B
B		1.30	OPV008	0.90	A B
B		1.14	KELLER	0.84	A B
B		0.99	FAUANL-34A X KELLER	0.72	B
B		0.98	FAUANL-34A X FAUANL-39	0.90	A B

For both variables there were two groups of genotypes, among the genotypes with the lowest values came from South Africa (OPV017and OPV008) for fresh weight and stem weight; however, FAUANL-34A x Keller and FAUANL-34A x FAUANL-39 showed the lowest values in both characteristics.

The highest values were for genotypes from India; ICSV93046 was not consistent, whereas ICSA89002 x SPV422 and AVRT2070-1 showed the highest values and they were consistent for both characteristics.

The genotypes showed high rank correlation between Fresh weight and Stem weight this is because stem represents more than 70 % of the fresh weight.

For the stem weight, the analysis of variance also showed statistical differences for the water treatments. The mean comparison is presented in Table 5.

Table 5: Mean comparison for water treatments for stem weight (kg).

	Kg	Watertreatment
A	1.21	FULL IRRIGATION
B A	1.15	POSTFLOWERING STRESS
B	1.02	PREFLOWERING STRESS

As it was expected, full irrigation showed the highest values for stem weight. Preflowering stress showed the lowest values as in height. The relation between them explains why this water treatment showed the lowest values. The stress preflowering affected the growth of the stem and by consequence the height and the stem weight.

Juice weight did not show statistical differences for water treatments nor for genotypes eventough there was a wide range of values obtained in this variable (0.59 Kg for Fortuna to 0.25 Kg for OPV008).

Table 6 shows the mean rank for juice weight for all genotypes tested in the experiment.

Table 6: Mean rank for juice weight in all genotypes tested in the experiment.

Rank		Kg	Genotype
1	A	0.59	FORTUNA
2	A	0.45	ICSA89002 X SPV422
3	A	0.44	ICSV93046
4	A	0.43	19S35SG0619
5	A	0.40	AVRT2057
6	A	0.40	FAUANL-35A X FAUANL-39
7	A	0.39	AVRT2070-1
8	A	0.38	AVRT2015-3
9	A	0.37	SP4487-3
10	A	0.36	AVRT2061-1
11	A	0.36	SP081045-1
12	A	0.34	E36-1
13	A	0.34	AVRT2061-2
14	A	0.33	AVRT 2018-2
15	A	0.32	CSH22SS
16	A	0.32	OPV003
17	A	0.32	KINGOLD
18	A	0.31	FAUANL-3
19	A	0.31	AVRT1042-3
20	A	0.30	ICSA324 x SSV74
21	A	0.30	AVRT1057-2
22	A	0.29	OPV017
23	A	0.27	KELLER
24	A	0.27	FAUANL-34A X FAUANL-39
25	A	0.25	FAUANL-34A X KELLER
26	A	0.25	OPV008

In spite of not found statistically differences, there was a tendency to show the highest values for those genotypes with high values in stem weight, height and fresh weight; this tendency id due to the fact that the juice is stored in the stem, consequently the higher the stem the higher capacity to store juice. It is important to conduct more experiments to show this relationship among the variables.

The analysis of variance in Table 1 shows statistically differences for ºBrix only among genotypes. Table 7 shows the genotype mean comparison for ºBrix.

Table 7. Genotypes mean comparison for ºBrix.

		ºBrix	Genotype
A		15.6	OPV003
B	A	13.9	E36-1
B	A	13.8	CSH22SS
B	A	13.6	KELLER
B	A	13.1	ICSA89002 X SPV422
B	A	12.8	OPV017
B	A	12.7	OPV008
B	A	12.5	FORTUNA
B	A	12.4	FAUANL-3
B	A	12.4	AVRT2070-1
B	A	12.4	KINGOLD
B	A	12.0	ICSA324 x SSV74
B	A	12.0	AVRT2057
B	A	11.9	AVRT1057-2
B	A	11.9	SP4487-3
B	A	11.5	SP081045-1
B	A	11.5	AVRT 2018-2
B	A	11.3	19S35SG0619
B	A	11.2	ICSV93046
B	A	11.2	FAUANL-34A X FAUANL-39
B	A	10.9	AVRT2061-2
B	A	10.4	AVRT2061-1
B		10.2	FAUANL-34A X KELLER
B		9.8	AVRT2015-3
B		9.8	FAUANL-35A X FAUANL-39
B		9.2	AVRT1042-3

The brix values showed in the Table 7 give the OPV003 from South Africa the highest value with 15.6 %. There were two groups and it was a light tendency to show the genotypes with the highest

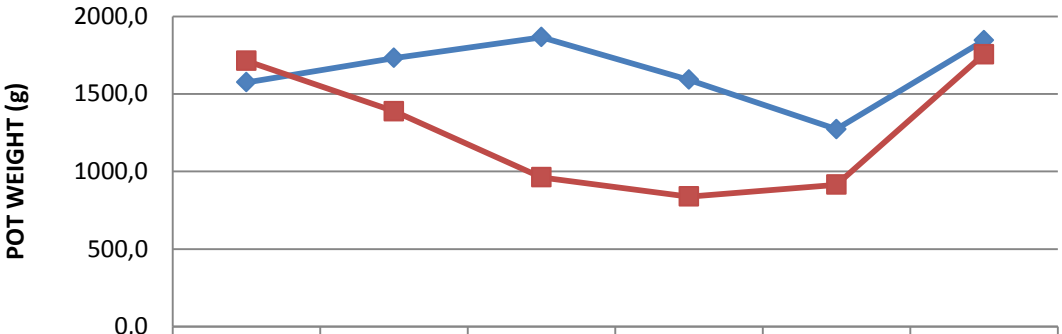
values for juice weight with low values of ºBrix such as FAUANL-35a x FAUANL-39 with 9.8 ºBrix and 0.40 Kg of juice weight; however, this relation may need to confirm with future experiments.
The ºBrix values shoed in all genotypes were not as high as was expected; this may be due to different reasons such as the growing cycle. In México, we have two growing cycles and the results are not the same, therefore it is important that before establish conclusions about sweet sorghum productivity.

2. Evaluate varietal tolerance to drought in terms of membrane integrity (electrolyte leakage);

Electrolyte leakage may be a good indicator to select genotypes under water and temperature stresses. To evaluate the response of eight genotypes to water stress and high temperature, it was established an experiment in 4 L pots. Three plants per experimental unit and three replications under a completely randomized design was established in Marín, México.

Materials and methods:
The genotypes were tested are: FAUANL-39, FAUANL-35A x FAUANL-39, KELLER, KANSAS COLLIER, FAUANL-3, FAUANL 33A X FAUANL-26 AND FAUANL-5. Three seed of each genotype was planted in each pot and after the seedling was emerging from the ground, one plant was left per pot. The moisture was kept uniform with not stress. 40 days after two water treatments were applied: Irrigation (not stress) and Drought (not water applied to the pots).

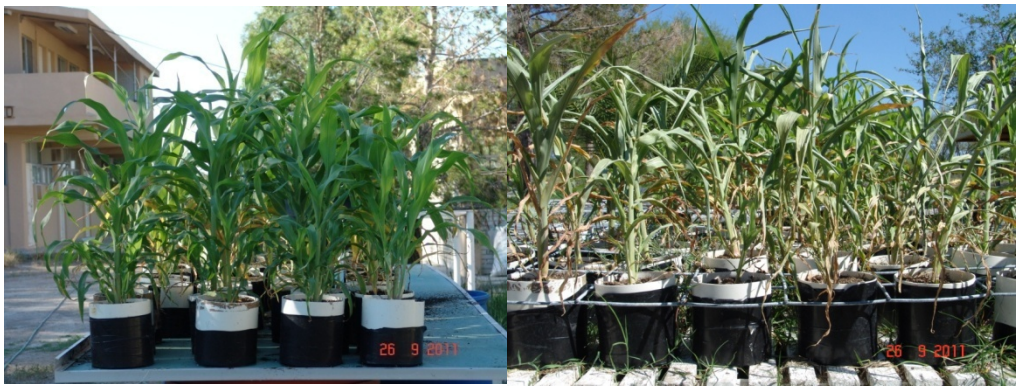
We left the plants under stress for 10 days. Figure 1 shows the weight average of the plots between the water treatments.



	19-sep-11	21-sep-11	23-sep-11	26-sep-11	29/SEP/11 B	29/SEP/11 A
IRRIGATION	1574,8	1731,0	1865,7	1591,2	1272,4	1844,4
DROUGHT	1713,3	1388,2	961,7	838,1	914,0	1754,6

Figure 1: Average weight of the pots between treatments for the period under stress.

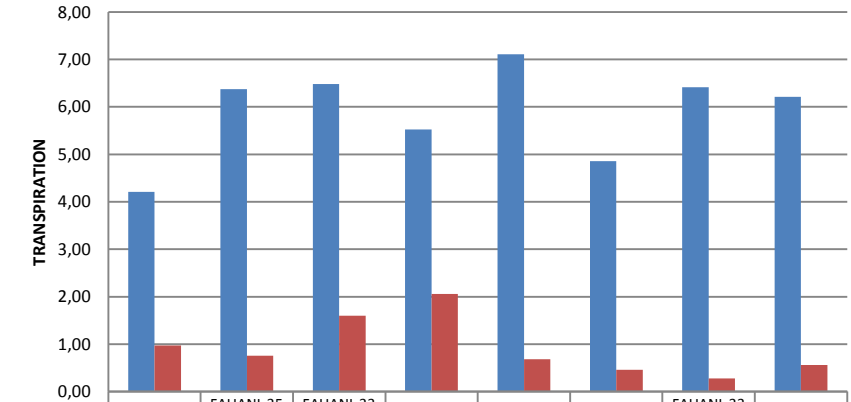
The Figure 2 shows the plants under the two water treatments.



Full Irrigation Drought

Results and Discussion:

After the stress we measured leaf transpiration using the porometer (LI-COR 1600). The values were quit low under stress due to the closure stomata and the lack of water in the soil. This showed that the effect of the water stress in the genotypes during the experiment. The transpiration values are shown in the Figure 3.



	FAUANL 39	FAUANL 35 A X FAUANL 39	FAUANL 33 A X FAUANL 5	KELLER	KANSAS COLLIER	FAUANL 3	FAUANL 33 A X FAUANL 26	FAUANL 5
IRRIGATION	4,21	6,37	6,49	5,53	7,11	4,86	6,42	6,21
DROUGHT	0,97	0,76	1,60	2,06	0,69	0,46	0,28	0,56

Figure 3: Leaf transpiration values in the genotypes under the water treatments in the experiment.

The transpiration was measured in the last completely expanded leaf in each plant in the two water treatments. The same leaf was used to take samples (5 discs for temperature treatment and

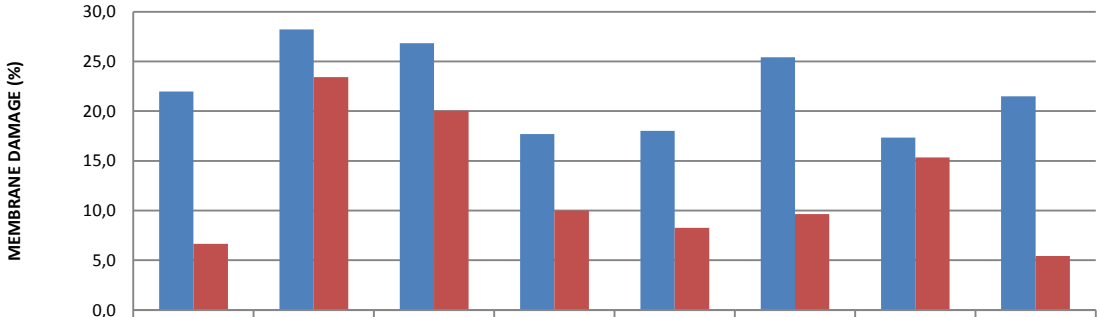
5 discs for the control temperature). The technique of Blum (1988) was adjusted for the experiment.

The formula used to calculate the electrolyte leakage is:

$$\% \text{ of damage} = 1 - \{ [1-(T1/T2)] / [1-(C1/C2)] \} * 100$$

Where: T = temperature treatment and C = control (no temperature treatment)

Figure 4 shows the % of damage of the cell membrane for all genotypes and the two water irrigation treatments.



	FAUANL 39	FAUANL 35 A X FAUANL 39	FAUANL 33 A X FAUANL 5	KELLER	KANSAS COLLIER	FAUANL 3	FAUANL 33 A X FAUANL 26	FAUANL 5
IRRIGATION	22,0	28,2	26,8	17,7	18,0	25,4	17,3	21,5
DROUGHT	6,6	23,4	20,0	10,0	8,3	9,6	15,3	5,4

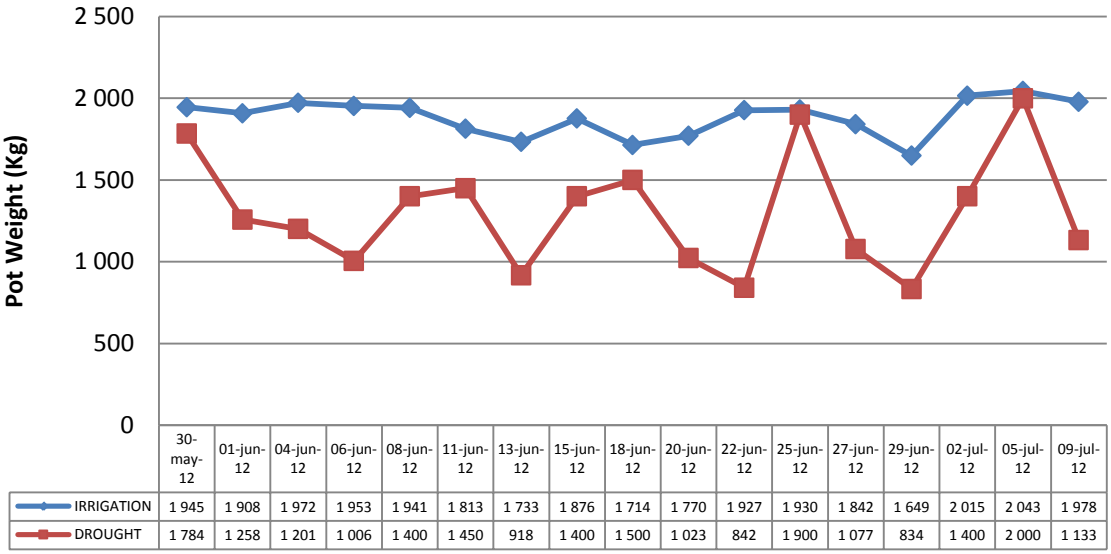
Figure 4: Percentage of the cell membrane damage in eight genotypes and two water regime.

The Figure 4 showed that the Drought water treatment showed the lowest values for the percentage of membrane damage in all genotypes. This may indicate that drought induces some resistance possible due to a better osmotic adjustment or organic substance such proline that normally accumulates under stress.

Based on the values of membrane damage Keller, Kansas Collier and FAUANL-5 and FAUANL-39 were less sensitive to the stress.

The same experiment was duplicated using the same methodology. The difference was the time of the application of the water stress.

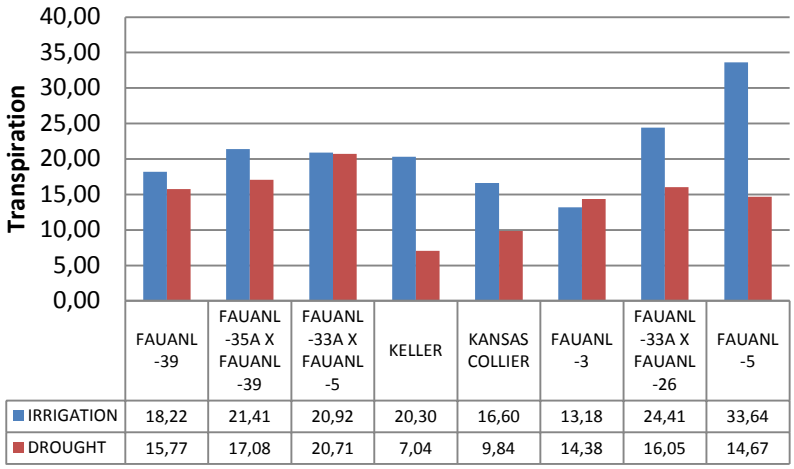
Figure 5 shows the pot weight to know the water stress used in this second experiment. The length of the application of the water treatment was longer that the previous one; however, we did applied water to avoid the death of the plants.



	30-may-12	01-jun-12	04-jun-12	06-jun-12	08-jun-12	11-jun-12	13-jun-12	15-jun-12	18-jun-12	20-jun-12	22-jun-12	25-jun-12	27-jun-12	29-jun-12	02-jul-12	05-jul-12	09-jul-12
IRRIGATION	1 945	1 908	1 972	1 953	1 941	1 813	1 733	1 876	1 714	1 770	1 927	1 930	1 842	1 649	2 015	2 043	1 978
DROUGHT	1 784	1 258	1 201	1 006	1 400	1 450	918	1 400	1 500	1 023	842	1 900	1 077	834	1 400	2 000	1 133

Figure 5: Water treatment in the second experiment aplied to the pots.

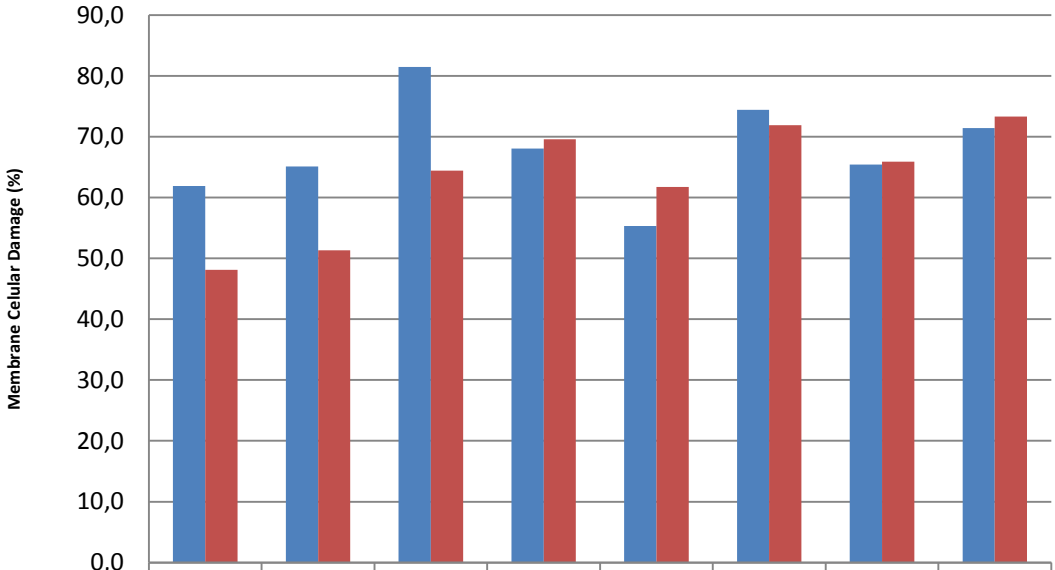
At the end of the stress period, we measured transpiration rates, which are shown in Figure 6.



	FAUANL-39	FAUANL-35A X FAUANL-39	FAUANL-33A X FAUANL-5	KELLER	KANSAS COLLIER	FAUANL-3	FAUANL-33A X FAUANL-26	FAUANL-5
IRRIGATION	18,22	21,41	20,92	20,30	16,60	13,18	24,41	33,64
DROUGHT	15,77	17,08	20,71	7,04	9,84	14,38	16,05	14,67

Figure 6: Transpiration rates in eight genotypes after water stress.

As it was in the previous experiment, the transpiration rates were lower in the drought treatment than the irrigation treatment. This is due to the lack of water in the tissue and closure of the stomata on the leaf (Figure 6).



	FAUANL-39	FAUANL-35A X FAUANL-39	FAUANL-33A X FAUANL-5	KELLER	KANSAS COLLIER	FAUANL-3	FAUANL-33A X FAUANL-26	FAUANL-5
IRRIGATION	61,9	65,1	81,5	68,1	55,3	74,4	65,4	71,4
DROUGHT	48,1	51,3	64,4	69,6	61,7	71,9	65,9	73,3

Figure 6: Membrane damage of eight genotypes under different water levels.

Membrane damages (Figure 6) did not show the same behavior of the genotypes in the other experiment, with exception of FAUANL-39, which shows the lowest membrane damage.

It is important to keep identifying the best genotypes especially for stress environment to keep increasing the productivity in these prone environments.

3. Identify candidate genes/gene sequences for high sugar content from association mapping using SCAR markers.

SCAR is a technique to identify markers associated with sugar concentration. In sorghum previous work done by this technique have been focused to identify a RAPD marker closely linked to a gene for resistance to anthracnose *Colletotrichum graminicola* (Ces.) and resistance to blight *Exserohilum turcicum* leaf.

Materials and methods:

We use fourteen genotypes, five hybrids and their parents, which are shown in Table 8 below:

Table 8: Genotypes used during the evaluation of molecular markers (SCAR).

Genotype number	Genotype name
1	FAUANL-33A x KANSAS COLLIER
2	AN601 x FAUANL-39
3	FAUANL-35A x FAUANL-39
4	FAUANL-33A x FAUANL-5
5	FAUANL-37A x FAUANL-5
6	FAUANL-37A
7	FAUANL-33A
8	FAUANL-35A x FAUANL-39

These genotypes were previously analyzed doing a morphological characterization and the sugars were quantified by HPLC technique, the evaluation was done by samplings at different phenological stages (Figure 7).

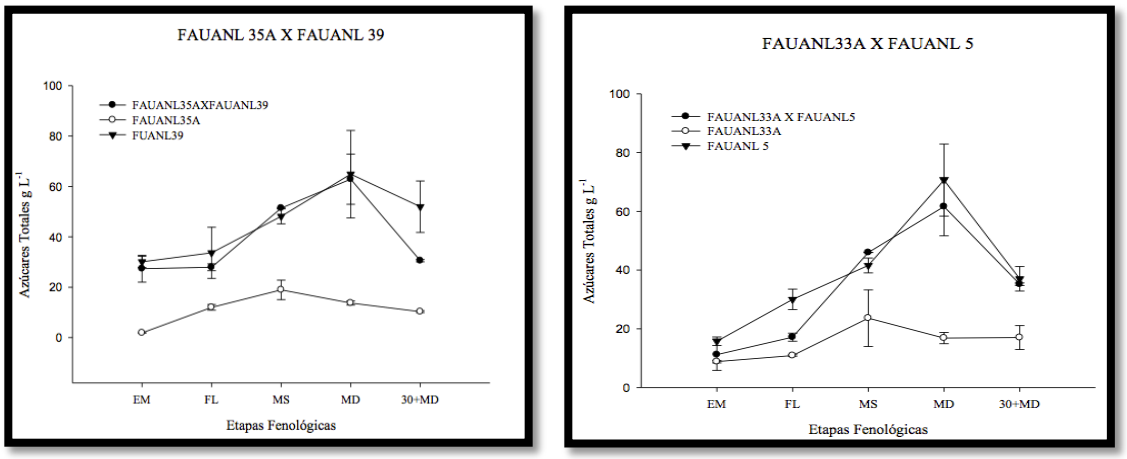


Figure 7: HPLC analysis of sugars in sweet sorghum genotypes at different growth stages (EM = boot stage; FL = Flowering; MS = Dough stage; MD = Physiological maturity; 30-MD = 30 day after physiological maturity).

DNA Extraction.Total DNA was extracted from sorghum leaves by method described by Chen *et al.* (2009), this DNA was used to screen RAPDs. For the RAPD-PCR analysis, 180 random decamer primer (Eurofins MWG Operon) were used for amplification by method described by Williams *et*

al., (1990) and Prakash *et al.,* (2006). The presence of polymorphics bands was confirmed by repeating PCR amplifications five times under the same conditions.

Results and Discussion:

Screening RAPD markers.The RAPD primers used amplified bands52 primers produced polymorphic and reproducible amplification of 256 bands (93.5% polymorphic; Figure 8).

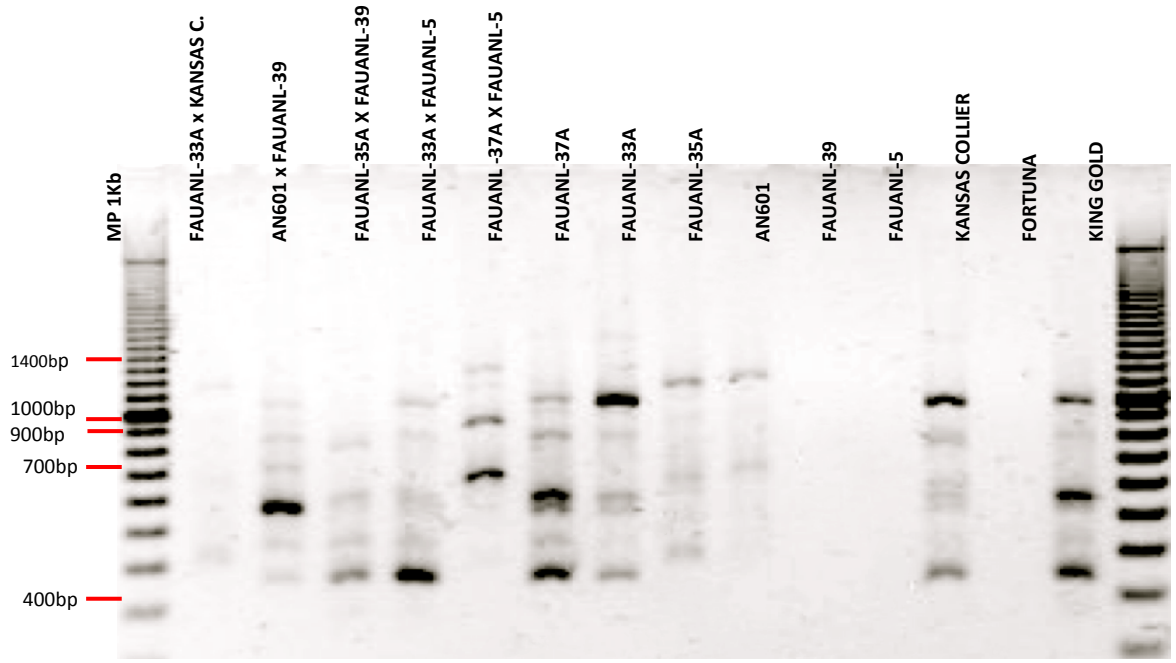


Figure 8: Bands produced by the RAPD analysis.

The cluster analysis with morphological and molecular data allowed the distinction of the lowest sugar genotypes from the highest; there were identified 52 RAPD markers that were present in the hybrids genotypes with the highest sugar content based on ºBrix: AN601 x FA-UANL 39; FA-UANL 35A x FA-UANL 39 and FA-UANL 33A x FA-UANL 5, while they were absent in the lowest genotypes sugar content: FA-UANL 37A, FA-UANL 33A and FA-UANL 35A (Figure 9).

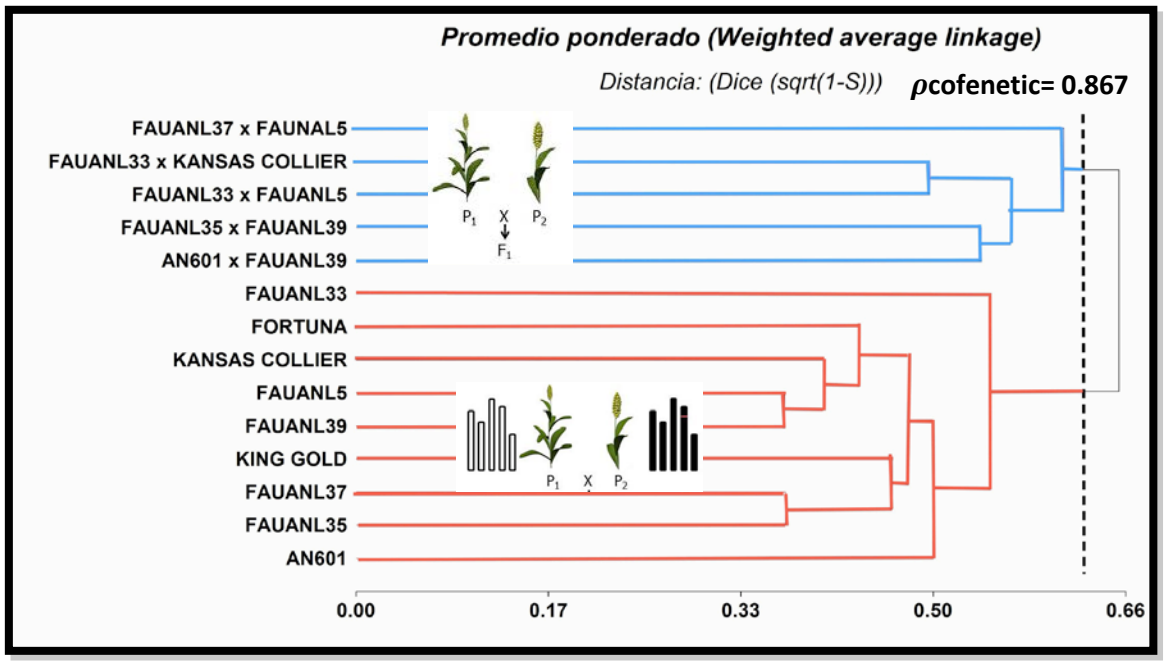


Figure 9: Dendogram of the cluster analysis for the genotypes being analyzed.

The 52 markers were identified according to the presence in the different genotypes according to the Table 9.

Table 9: Identification of the 52 markers in the different genotypes of sweet sorghum.

RAPD MARKER	FAUANL33 X KANSAS COLLIER	AN601 X FAUANL39	FAUANL35 X FAUANL39	FAUANL33 X FAUANL5	FAUANL37 X FAUANL5	FAUANL37	FAUANL33	FAUANL35	AN601	FAUANL39	FAUANL5	KANSAS COLLIER	FORTUNA	KING GOLD
A1-1600	0	0	1	0	0	0	0	0	0	0	0	0	0	0
A1-1500	0	0	0	1	0	0	0	0	0	0	1	0	0	0
A1-1300	0	0	0	1	0	0	0	0	0	0	0	0	0	0
A2-3500	0	0	0	1	0	0	0	0	1	1	1	0	0	0
A2-3285	1	1	1	1	0	0	0	0	0	1	0	0	0	0
A2-3072	0	0	1	0	0	0	0	0	1	0	0	0	0	0
A2-900	1	1	1	1	0	0	0	0	0	1	0	1	0	0
A2-850	1	0	0	1	0	0	0	0	0	0	0	0	0	0
A2-800	0	0	0	1	0	0	0	0	0	0	0	0	0	0
A2-550	0	0	1	0	0	0	0	0	0	0	0	1	0	0
A4-700	0	1	0	0	0	0	0	0	1	1	0	0	0	0
A10-500	0	0	0	1	0	0	0	0	0	0	1	0	0	0
A15-1400	0	0	1	1	0	0	0	0	0	0	0	0	0	0
B15-780	1	0	0	0	0	0	0	0	0	0	0	0	0	0
B17-1800	0	1	0	0	0	0	0	0	1	0	1	1	0	0
B17-980	0	1	0	0	0	0	0	0	1	0	0	0	0	0
B17-780	0	0	1	0	1	0	0	0	0	0	0	0	0	0
C1-1480	0	0	1	1	0	0	0	0	0	0	0	0	0	0
C1-800	0	0	1	1	1	0	0	0	0	0	0	0	0	0

Once the restriction fragments were identified, the next step was the extraction and purification od the DNA for sequencing. The sequences of markers were sent to be processed by Eurofins MWG Operon (Figure 10).

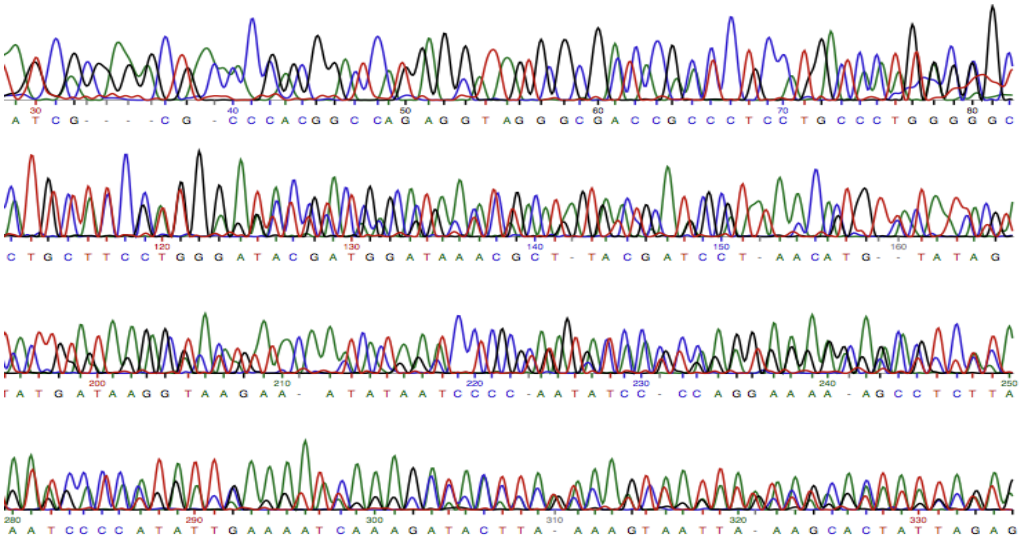


Figure 10: Sequence of the DNA fragments analyzed.

The sequencing process was not successful for all fragments; however, for fragments aligned with genomic sequences of *Sorghum bicolor* sp. were designed six SCAR primers (Table 10). There were made six replications more for each group to verify the sequence obtained using only the forward primer; however, is also important to test the reverse primers in all genotypes.

SCAR markers obtained provide further a biotechnology technique could be used to define activities for selection assisted by markers in varieties of UANL.

Specific primers designed (SCAR markers) need to be screened on F2 sorghum genotypes for early identification of appropriate genotypes for pre-breeding programs in sweet sorghum focus to sucrose production.

Development of SCAR markers will be allow make a early selection of individual possible candidates to breeding focused to high content of sugar.

Table 10: BLAST results and description of alignments with *Sorghum bicolor* sp. and *Zea mays*, sequences grouped by common primer. All GenBank sequences from *Sorghum bicolor* sp. contained at least 300 bp of 5´upstream sequences. Promoter sequence was defined as sequences upstream as indicated in the annotation for the GenBank entry. Design of primer forward and reverse derived from fragments aligned with *Sorghum bicolor* sp. sequences.

Group	Fragments	Accession with major identity	Expect Error	Identities	Definition	Reference		Sequence (5´-3´)	Length (pb)	Start	Stop	Tm ºC	GC%	Self-complementary
1	9 10 11 12 13 14	XM_002440182	3e-49	217/289 (75%)	*Sorghum bicolor* Hypothetic protein, mRNA	Paterson *et al.* 2009	**Pair primers 1**							
							Forward	GAGAGTCTTCTGTCCCGGTG	20	8	27	59.47	60	2
		NM_001156026	2e-13	149/222 (67%)	*Zea mays* Purine permease, mRNA	Alexandrov *et al.* 2009	**Reverse**	TGTCTGATCGGTTGGTCGAT	20	328	309	58.82	50	2
								Product size	321					
2	23 25 26	XM_002459992.1	3e-5	76/108 (70%)	*Sorghum bicolor* Hypothetic protein, mRNA	Paterson *et al.* 2009	**Pair primers 2**							
							Forward	ACCAAACCGCATTGTTCCAC	20	165	184	59.61	50	1
							Reverse	CTACCTACCTCCGGGCAGAT	20	692	673	60.18	60	3
								Product size	528					
3	92 93 94 95	XM_002458588.1	2e-28	116/144 (81%)	*Sorghum bicolor* Hypothetic protein, mRNA	Paterson *et al.* 2009	**Pair primers 3**							
							Forward	TGTCGTCCTTTCCCCGATCC	20	160	179	61.61	60	2
							Reverse	AAAGCAGTCACAGAGTGGAGT	21	385	365	59.23	48	1
								Product size	226					
4	46 47	XM_002488900.1	0.0	482/511 (94%)	*Sorghum bicolor* Hypothetic protein, mRNA	Paterson *et al.* 2009	**Pair primers 4**							
							Forward	GAGTGCTCCTACTCTTGCATCC	22	52	73	60.48	55	2
							Reverse	AGCTAATGTACATCAGCGGGT	21	392	372	60.00	48	2
								Product size	341					
5	48	AC196852.2	0.0	570/684 (83%)	*Sorghum bicolor* clone SB_BBc0169M22	Stanford Human Genome Center	**Pair primers 5**							
							Forward	ATCCAATCTAACTAAGCCAA	20	8	27	51.48	35	0
							Reverse	CGAGTTTTGGTGTTTGTCTAT	21	698	678	54.44	38	2
								Product size	691					
6	87 88	Sb01g035510	1e-12	90/119 (76%)	Nuclear cap-binding proteinCBP80	Paterson *et al.* 2009	**Pair primers 6**							
							Forward	TCCTTTGTCCCTACAGTTTTTCA	23	5	27	57.88	39	1
							Reverse	TCGAGTCGCCTTATCCTACCT	21	458	438	59.86	52	0
								Product size	454					