From: Bill Rooney
To: "Steven Thomas"
Subject: another letter
Date: Monday, June 22, 2009 6:27:00 AM

Steve:

Can you also provide a letter with

If that is a problem let me know. I'd like to to have options just in case my budget people balk.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From:	Bill Rooney
To:	"Steven Thomas"
Cc:	"Schmitt, Brian C."
Subject:	cbpr proposal
Date:	Friday, June 19, 2009 5:22:00 PM
Attachments:	6-19-2009 CPBR Letter Thomas.pdf CPBR Preproposal - Rooney.pdf

---

Steve:

It seems I have to do this correctly. So please see attached and respond if interested (you already have).

regards,
bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

AgriLIFE RESEARCH & EXTENSION
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COLLEGE OF AGRICULTURE
AND LIFE SC ENCES

Department of Soil and Crop Sciences

6/19/2009

Sincerely,

William L. Rooney

Sorghum Breeding and Genetics
Department of Soil & Crop Sciences
2474 TAMU
Texas A&M University
College Station, TX 77843-2474

Tel. 979.845.2151
Fax. 979.862.1931
wlr@tamu.edu

From:	Bill Rooney
To:	"Steven Thomas"
Cc:	"Walter Nelson"; "Karen Prihoda"
Subject:	confirmation letter for
Date:	Wednesday, June 24, 2009 11:40:00 AM

Steve:

I haven't yet received a confirmation letter of support for the . Any chance it got sent to the wrong address or fax?

Fax number is 979 862 1931. E-mail to me and copy karen prihoda (address above).

regaards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Walter Nelson"; "Clint Johnson"
Cc: "Collins, Stephen D"; "dustin borden"
Subject:
Date: Friday, July 03, 2009 7:01:00 PM

Walter and Clint:

After working out the details, the that you sent down this way appears to be about 30% female. It took awhile to figure this out, but it is in fact and the percentage of flowering plants (all seed parent height but variable due to the variation in a single cross seed parent) is about 30% - I've confirmed it in larger strip tests we planted and in replicated tests that we have included it in.

I don't know if you are seeing the same thing, but it is consistent in the plots that we have planted here in CS.

It is not as noticeable in the irrigated trials as the PS material is masking the presence of the flowering types, but in the dryland, that hybrid didn't growout as fast and quite frankly it does not look good at all. Hopefully, we'll get some rain and minimize the problem.

Ironically, I've seen very little to no contamination in the from Puerto Vallarta. Go figure.

I know you sent the same seed to Jurg's group, but I have not checked with them to determine the situation. I just wanted to make you aware of this in case you start seeing it elsewhere.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Walter Nelson"
Subject: lagf
Date: Wednesday, June 17, 2009 6:36:00 AM

Walter:

Have you contacted LAGF about the possibility of a Tuesday visit?

It's looking like I could do a one day (there and back) trip, if you still would like to do so

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Walter Nelson"
Subject: letter of collaboration on the SunGrant proposal
Date: Thursday, April 02, 2009 12:57:00 PM

Walter:

I should have asked about this earlier in the week - I forgot - and I'm writing this on the plane so it won't be sent until later. So, if you send the letter today, please ignore this reminder.

Have you (or whomever is designated) written a letter of collaboration for our South Central SunGrant? I need it by noon tomorrow so that I can upload the letter onto the website managing the proposals.

If you can't get it done, please let me know and I'll revise the document (I have to remove the references to Ceres involvement).

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From:	Bill Rooney
To:	"Edgar Haro"
Subject:	RE: anthracnose nursery
Date:	Tuesday, April 21, 2009 8:23:00 AM
Attachments:	Ceres 09 RIL 623xSC748 list.xls

Edgar:

Here is the file. The randomization in each location was the same, so you can use the same file for both locations.

We'll see you Thursday.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Edgar Haro [mailto:eharo@ceres-inc.com]
> **Sent:** Tuesday, April 21, 2009 7:53 AM
> **To:** Bill Rooney
> **Subject:** RE: anthracnose nursery
>
> Bill,
>
> Can you share a electronic copy of the materials in the anthracnose nursery.
> That may make life easier entering into the system.
>
> Thanks,
> Edgar

From:	Bill Rooney
To:	"Walter Nelson"
Cc:	"Steven Thomas"
Subject:	RE: confirmation letter
Date:	Thursday, June 25, 2009 11:01:00 AM

Yep, got it, and all is well.

Sorry, didn't copy you - I gotta get better at that.....

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, June 25, 2009 11:00 AM
To: Bill Rooney
Subject: RE: confirmation letter

I called Steve about this. Have you herd from him or gotten what you need?

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

-----Original Message-----
From: Bill Rooney <wlr@tamu.edu>
Sent: Wednesday, June 24, 2009 9:41 AM
To: 'Steven Thomas' <sthomas@ceres-inc.com>
Cc: 'Walter Nelson' <wnelson@ceres.net>; 'Karen Prihoda' <kprihoda@yahoo.com>
Subject: confirmation letter

Steve:

I haven't yet received a confirmation letter of support for the . Any chance it got sent to the wrong address or fax?

Fax number is 979 862 1931. E-mail to me and copy karen prihoda (address above).

regaards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Couple of questions
Date: Tuesday, April 07, 2009 1:58:00 PM

Walter:

We just finished up and I'm sitting in the airport waiting to go home. However, this e-mail may not go out until tomorrow morning (because I'm too cheap to pay for the service).

We have seed of the sudans and I have no problem with providing small quantities for evaluation and increase. I need to get the go ahead from Peter - I have a meeting with him Monday.

I found out last week that Jorge is going back to Brazil to work for Syngenta. His last day at Weslaco is April 15, so he is gone after this week. The wide hybridization will continue and I don't expect that this changes all that much (at least when it involves sorghum). So you're response is appropriate.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Tuesday, April 07, 2009 12:43 PM
To: Bill Rooney
Subject: Couple of questions

Hi Bill,

Hope you're not working too hard and finding some time to enjoy yourself a bit in Puerto Rico.

Two items:

Clint is doing some bloom date experiments in Amarillo and asked if there were any new materials coming out of your program. I mentioned those sudans you have developed. I am still interested in them. The new amendment I just did allows us to evaluate program materials from the program for things like this. If you have some seed from those for Clint to start looking at, do send it his way. I was also going to ask if you would be able to increase them a bit this season for handoff for production next year if you feel it appropriate to start preparing for that. We can discuss more later.

Also, we heard that Jorge da Silva left for Syngenta. An email went around here asking if his departure would affect the wide crossing project/pre-proposal. My response was we were fine in that you are the driver of that project and Mike Gould could fill any gaps if needed. Let me know if that was the correct response.

Thanks,

Walter

From:	Bill Rooney
To:	"Walter Nelson"
Subject:	RE: Couple of questions
Date:	Thursday, April 09, 2009 11:37:00 AM

Right move - with temps like that it won't work. Best not to pursue it. We can do it next winter.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, April 09, 2009 11:05 AM
To: Bill Rooney
Subject: RE: Couple of questions

Sounds good Bill. I think Clint is doing two plantings of things for evaluation, so we have some time on the sudans.

Regarding Arica, Chile and a short-day nursery, we've had some discussions with the group there and internally and decided not to do it this year. Turns out it's pretty cold there despite the latitude...average high's and lows of 60s and 50s through the winter growing season. They have a tough time with corn if planted after April 15 due to the low temps, so we decided we would be asking for trouble with sorghum.

Lastly, do you know the name of and where to buy that microbicide/stabilizing solution you guys use for the sweet sorghum juice?

Thanks,

Walter

-----Original Message-----
From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Wednesday, April 08, 2009 4:55 AM
To: Walter Nelson
Subject: RE: Couple of questions

Walter:

We just finished up and I'm sitting in the airport waiting to go home. However, this e-mail may not go out until tomorrow morning (because I'm too cheap to pay for the service).

We have seed of the sudans and I have no problem with providing small quantities for evaluation and increase. I need to get the go ahead from Peter - I have a meeting with him Monday.

I found out last week that Jorge is going back to Brazil to work for Syngenta. His last day at Weslaco is April 15, so he is gone after this week. The wide hybridization will continue and I don't expect that this

changes all that much (at least when it involves sorghum). So you're response is appropriate.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Tuesday, April 07, 2009 12:43 PM
To: Bill Rooney
Subject: Couple of questions

Hi Bill,

Hope you're not working too hard and finding some time to enjoy yourself a bit in Puerto Rico.

Two items:

Clint is doing some bloom date experiments in Amarillo and asked if there were any new materials coming out of your program. I mentioned those sudans you have developed. I am still interested in them. The new amendment I just did allows us to evaluate program materials from the program for things like this. If you have some seed from those for Clint to start looking at, do send it his way. I was also going to ask if you would be able to increase them a bit this season for handoff for production next year if you feel it appropriate to start preparing for that. We can discuss more later.

Also, we heard that Jorge da Silva left for Syngenta. An email went around here asking if his departure would affect the wide crossing project/pre-proposal. My response was we were fine in that you are the driver of that project and Mike Gould could fill any gaps if needed. Let me know if that was the correct response.

Thanks,

Walter

From:	Bill Rooney
To:	"Walter Nelson"
Subject:	RE:
Date:	Monday, June 15, 2009 4:51:00 PM

Thanks. Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Monday, June 15, 2009 4:51 PM
To: Bill Rooney
Subject: FW:

FYI...

Looks like Steve Thomas will handle the letter for you. I told them you needed by the end of June. Looks like Steve will drop you a line sometime to discuss.

W

-----Original Message-----
From: Steven Thomas
Sent: Monday, June 15, 2009 12:41 PM
To: Walter Nelson
Cc: Steven Bobzin
Subject: RE:

Thanks, Walter. I'll give Bill a call. Steve

****
Steven R. Thomas, Ph.D.
Director of Bioproducts
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320

ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net
****

-----Original Message-----
From: Walter Nelson
Sent: Monday, June 15, 2009 12:26 PM
To: Steven Bobzin; Steven Thomas
Subject:

He said before the end of June....

W

-----Original Message-----
From: Steven Bobzin
Sent: Monday, June 15, 2009 11:59 AM
To: Walter Nelson; Steven Thomas
Subject: RE: Sorghum flowering

Walter, Steve T and I talked and he will provide a letter for Bill. Does he have a deadline for this?

Sincerely,

Steve

Steven C. Bobzin, Ph.D.
Director
Technology Planning, Protection, and Acquisition
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
Phone: 805-376-6515 FAX: 805-498-1002
sbobzin@ceres-inc.com
www.ceres.net

-----Original Message-----
From: Walter Nelson
Sent: Wednesday, June 10, 2009 1:27 PM
To: Steven Thomas; Steven Bobzin
Subject: FW: Sorghum flowering

Steve and Steve,

Bill Rooney mentioned that he needs a . Are you guys familiar with what he is asking for and can you arrange it or would you like me to investigate and take care of?

Thanks,

W

-----Original Message-----
From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Thursday, June 04, 2009 1:08 PM
To: Walter Nelson
Subject: RE: Sorghum flowering

Walter:

John has more experience than I in the greenhouse, but we've treated these in the field with a trashcan for about two weeks and that was enough to get them to flower. I assume similar in a GC, but it might be less.

I'm working on the
. I assume that comes from Steve Thomas, correct?

Regards,
Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, June 04, 2009 2:30 PM
To: John Mullet; William Rooney
Subject: Sorghum flowering

John or Bill,

I have a couple of growing in our long-day greenhouse in Thousand Oaks. In theory they won't flower while in there as I understand it.

If I wanted to trigger flowering in them by moving them into a short-day growth chamber, how long would I have to keep the plant in the short-day lighting before I could move it bck to the greenhouse to finish flowering?

Thanks!

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

From: Bill Rooney
To: "Steven Thomas"
Subject: RE: CPBR letter
Date: Monday, June 22, 2009 6:04:00 AM

Steve:

That is correct. I'll need the letter by tomorrow.

I'm open to using either or both for analysis.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Steven Thomas [mailto:sthomas@ceres-inc.com]
> **Sent:** Tuesday, June 16, 2009 4:52 PM
> **To:** Bill Rooney
> **Cc:** Walter Nelson
> **Subject:** RE: CPBR letter
>
> Thanks for sending this, Bill. So, it looks like I should write a letter committing to a
>
> One thought right now: Are you planning on relying on a Ceres NIR method for sugar concentration and composition, or your own method?
>
> Good ta king with you, Steve
>
> **********
> Steven R. Thomas, Ph.D.
> Director of Bioproducts
> Ceres, Inc.
> 1535 Rancho Conejo Blvd.
> Thousand Oaks, CA 91320
>
> ph: (805) 376-6514
> cell: (805) 807-6412
> email: sthomas@ceres-inc.com
> web: http://www.ceres.net
> **********
>
> > -----Original Message-----
> > **From:** Bill Rooney [mailto:wlr@tamu.edu]
> > **Sent:** Tuesday, June 16, 2009 2:05 PM
> > **To:** Steven Thomas
> > **Subject:** RE: CPBR letter
> >
> > Here is a first draft, obviously subject to change...
> >
> > bill
> >
> > Dr. William L. Rooney
> > Professor, Sorghum Breeding and Genetics
> > Chair, Plant Release Committee
> > Texas A&M University
> > College Station, Texas 77843-2474

979 845 2151

-----Original Message-----
From: Steven Thomas [mailto:sthomas@ceres-inc.com]
Sent: Tuesday, June 16, 2009 1:08 PM
To: wlr@tamu.edu
Cc: Walter Nelson
Subject: CPBR letter

Hi Bill,

Walter tells me that you have requested a letter of

If so, I'd like to take advantage of that and include it in the letter. Also, when do you need the letter?

If you think we should talk through this, let me know when you are available and I will call you.

Thanks and best regards, Steve

****
Steven R. Thomas, Ph.D.
Director of Bioproducts
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320

ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net
****

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: discussion
Date: Friday, May 08, 2009 7:32:00 PM

Jeff:

No, I'm on the road to Iowa. You can call me tomorrow if it urgent, but if not, I'll be back in the office on Monday.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
> **Sent:** Friday, May 08, 2009 10:33 AM
> **To:** Bill Rooney
> **Subject:** discussion
> **Importance:** High
>
> Are you around this afternoon and have time for a brief chat re several items?
>
> J. Jefferson Gwyn, Ph.D.
> Director of Breeding
> Ceres, Inc.
> 3199 County Road 269 East
> Somerville, TX 77879
> off 979.272.2265
> fax 979.272.2269
> cell 805.490.0070
> www.ceres.net

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Embrapa contact and female increases
Date: Thursday, July 23, 2009 2:20:00 PM

Walter:

The e-mail contact that I have for Geraldo de Franca is

Not happy to hear that you've got fertiles. Hopefully it is not a large problem, but I agree, we need to continue to push harder on the females to reduce the chances of this ever happening.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Thursday, July 23, 2009 9:04 AM
> **To:** Bill Rooney
> **Subject:** Embrapa contact and female increases
>
> Bill,
>
> Can you send me Geraldo's contact information? Spencer and I are gearing up for another trip to Brazil and we wanted to follow up with some contacts there at Embrapa.
>
> Also, wanted to let you know that Clint called yesterday and said we appear to be having some problems with the . He mentioned a high frequency of shedders as well as some segregating head types. Will be probably another week or two before we know exactly what's going on, but wanted to give you a heads up that there may be an issue. Mike didn't seem to be too concerned about it and was just planning to have them rogue everything a lot harder, but this will probably increase the urgency of getting some alternate/more advanced females into production.
>
> W

> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Tuesday, May 26, 2009 1:00 PM
> **To:** Walter Nelson
> **Subject:** RE: Possible Meeting
>
> Walter:
>
> Dr. Geraldo De Franca is (or was last time I asked) quite high in the EMBRAPA administration. I don't know if he will be there but he is the contact with whom I have know since the late 1980s'. I haven't spoken with Geraldo about sweet sorghum in nine months or so - th emost recent discussion have been about his son coming to College Station for a internship with our program next year.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Tuesday, May 26, 2009 11:25 AM
> **To:** Bill Rooney
> **Subject:** RE: Possible Meeting
>
> Thanks Bill...will send Omar a note.
>
> Quick question. Who is the senior guy at Embrapa that you said used to be at A&M and is a key player for sweet sorghum interest there? I am going to a big conference in Sao Paulo next week and was going to look for him and also need to talk to McCutchin about what he has in mind for this "joint" program that he brought up at the last quarterly.
>
> Thanks!
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Tuesday, May 26, 2009 6:13 AM
> **To:** 'Omar A Diaz'
> **Cc:** Jorgebdelatorre@gmail.com; 'Jorge de la Torre Valdes'; Walter Nelson
> **Subject:** RE: Possible Meeting
>
> Omar:
>
> Thanks for your interest in testing sweet sorghum in the DR. I don't provide adequate quantities of seed to do large scale testing and I don't commercialize the hybrids. Ceres, Inc. is an Energy Crop Breeding Company that is testing and may eventually develop a commercial version of some of our sweet sorghum hybrids. Mr. Walter Nelson is their sorghum product manager and he would be a primary contact for larger scale testing when they have seed available.
>
> regards,
>
> bill
>
> Dr. William L. Rooney
> Professor, Sorghum Breeding and Genetics
> Chair, Plant Release Committee
> Texas A&M University
> College Station, Texas 77843-2474
> 979 845 2151

-----Original Message-----
From: Omar A Diaz [mailto:omar.diaz@transseed.com]
Sent: Monday, May 25, 2009 11:59 AM
To: Bill Rooney
Cc: Jorgebdelatorre@gmail.com; Jorge de la Torre Valdes
Subject: RE: Possible Meeting
Importance: High

Hi Bill

It has been a while, but wanted to take the opportunity to say hi and wish you a good memorial day. Jorge and I are contemplating testing your best sorghum hybrids in the Dominican Republic and analyze behaviour. I believe this is an opportunity to either have you visit us yourself or allow us to test the varieties. We could arrange for a small shipment to DR or your visit, considering your schedule I'd dare say it'd be best to arrange a sample. Please let me know costs associated with either option. This should potentially be a major crop in the country and your expertise along the process among other things will be highly appreciated.

Look forward to hearing from you.

Warm Regards

Omar A. Diaz
USA: 214-914-6664
Fax: 517-947-6664
Mob: 972-904-2296

> -------- Original Message --------
> Subject: RE: Possible Meeting
> From: "Bill Rooney" <wlr@tamu.edu>
> Date: Fri, May 30, 2008 10:49 am
> To: "'Omar A Diaz'" <omar.diaz@transseed.com>
>
> Thanks, Omar
>
> Bill
>
> Dr. William L. Rooney
> Professor, Sorghum Breeding and Genetics
> Chair, Plant Release Committee
> Texas A&M University
> College Station, Texas 77843-2474
> 979 845 2151
>
> > -----Original Message-----
> > **From:** Omar A Diaz [mailto:omar.diaz@transseed.com]

Sent: Friday, May 30, 2008 10:47 AM
To: Bill Rooney
Cc: Jorge de la Torre Valdes
Subject: RE: Possible Meeting
Importance: High

Bill

I'd like to thank you again for your time and the outstanding attention you paid to us during our visit last Wednesday. We are in the planning stages and are considering your consulting services and maybe the research part we talked about. We will be in touch soon, or if we have any questions.

Have a great weekend.

Omar A. Diaz
Exec VP Business Development
TransSeed Biofuels International.
www.transseed.com
1330 Post Oak Blvd., Suite 1600
Houston, Texas 77056 USA
USA: 214-914-6664
Fax: 214-576-2794
Mob: 972-904-2296
DR: 829-948-3565

-------- Original Message --------
Subject: RE: Possible Meeting
From: "Bill Rooney" <wlr@tamu.edu>
Date: Wed, May 28, 2008 7:27 am
To: "'Omar A Diaz'" <omar.diaz@transseed.com>

Omar:

When you get near please call me at the office 979 845 2151 or my cell 979 220 1951, and I'll get you directions.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474

979 845 2151

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Flowering time data:
Date: Thursday, May 14, 2009 8:06:00 AM

I talked with Clint yesterday, We are also checking in data to find more, but I think I relay all I could to Clint off the top of my head.

I looked at our Ceres supplied seed trials yesterday. They look good - stands, emergence and growth to date fine.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Monday, May 11, 2009 10:58 AM
> **To:** Bill Rooney
> **Cc:** Clint Johnson
> **Subject:** Flowering time data:
>
> Bill,
>
> Clint is planning to set up some of our planned hybrid productions in the panhandle this summer. We are planning to use with the respective females.
>
> We may have asked this before, but could you give us any information about the bloom dates for this lines in College Station, Halfway and Amarillo? Any information you would have would be halpful as most of ours is currently for short day or end of season stuff where photoperiod and temperature differences may have been having some influence.
>
> Thanks!
>
> Walter

Walter E Nelson
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
voice: (805)376-6548
www.ceres.net

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: lagf
Date: Wednesday, June 17, 2009 1:45:00 PM

Walter

I'll call Juan Pablo on Friday - that'll give me time to confirm my availability.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Wednesday, June 17, 2009 8:01 AM
To: Bill Rooney
Subject: RE: lagf

I had not yet as I figured I'd check with you first. Would you like to drop them a line or would you like me to? I would have to go through Spencer or Bud as I have not met them yet.

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

-----Original Message-----
From: Bill Rooney <wlr@tamu.edu>
Sent: Wednesday, June 17, 2009 4:37 AM
To: 'Walter Nelson' <wnelson@ceres.net>
Subject: lagf

Walter:

Have you contacted LAGF about the possibility of a Tuesday visit?

It's looking like I could do a one day (there and back) trip, if you still would like to do so

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From:	Bill Rooney
To:	"Walter Nelson"
Cc:	"dustin borden"
Subject:	RE:
Date:	Friday, May 08, 2009 7:42:00 PM

Yes! I'll replace the mixed hybrids in late College Station Plantings and in Halfway. About 1/2 pound total of each will be acceptable.

Dustin - we need to wait for these hybrids before we make our May plantings.

thanks,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Friday, May 08, 2009 11:18 AM
> **To:** Bill Rooney
> **Subject:**
>
> Hi Bill,
>
> The M81 hybrids have been harvested in Hawaii and should be in Texas any day. It appears to have gone well and I think we have 30-50 lbs for 5 females. Do you want seed for them and, if so, how much and by when?
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Wednesday, April 29, 2009 5:30 AM
> **To:** Walter Nelson; Bud Wylie
> **Subject:** RE: Umbrella hybrids
>
> Walter and Bud:

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Tuesday, April 28, 2009 7:06 PM
To: Bill Rooney
Subject: Umbrella hybrids

Hi Bill,

I need to re-code the umbrella hybrids you gave Bud for our trials. Can you send me the codes and pedigrees for the hybrids you gave him?

Thanks!

Walter

From: Bud Wylie
Sent: Tuesday, April 28, 2009 3:26 PM
To: Walter Nelson
Subject: Umbrella

Walter,

What are the codes for the Umbrella hybrids?

Bud Wylie
Manager, Commercial Trials
Ceres, Inc
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
210-882-7257
bwylie@ceres-inc.com

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: meeting on June 2nd
Date: Friday, May 15, 2009 1:21:00 PM

That'lll be fine.

See you there.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
> **Sent:** Friday, May 15, 2009 11:33 AM
> **To:** Bill Rooney
> **Cc:** Nickolai Alexandrov
> **Subject:** meeting on June 2nd
> **Importance:** High
>
> For the meeting with Nick and I on June 2, I suggest you coming out to our station around 9 am, we can meet a few hours, go back to town, eat lunch around noon, Nick wants a quick tour of your shop.
>
> Would that work?
>
> J. Jefferson Gwyn, Ph.D.
> Director of Breeding
> Ceres, Inc.
> 3199 County Road 269 East
> Somerville, TX 77879
> off 979.272.2265
> fax 979.272.2269
> cell 805.490.0070
> www.ceres.net

From: Bill Rooney
To: "Edgar Haro"
Subject: RE: Pedigree information
Date: Thursday, April 23, 2009 11:44:00 AM

Edgar:

As I mentioned this morning during our visit

Hope that helps.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Edgar Haro [mailto:eharo@ceres-inc.com]
> **Sent:** Wednesday, April 22, 2009 11:48 AM
> **To:** wlr@tamu.edu
> **Subject:** Pedigree information
>
> Bill,
> I will appreciate the information on the selection/pedigree of the following seed sources we got from you last year.
> Thanks
> Edgar

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Possible Meeting
Date: Tuesday, May 26, 2009 3:00:00 PM

Walter:

Dr. Geraldo De Franca is (or was last time I asked) quite high in the EMBRAPA administration. I don't know if he will be there but he is the contact with whom I have know since the late 1980s'. I haven't spoken with Geraldo about sweet sorghum in nine months or so - th emost recent discussion have been about his son coming to College Station for a internship with our program next year.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Tuesday, May 26, 2009 11:25 AM
> **To:** Bill Rooney
> **Subject:** RE: Possible Meeting
>
> Thanks Bill...will send Omar a note.
>
> Quick question. Who is the senior guy at Embrapa that you said used to be at A&M and is a key player for sweet sorghum interest there? I am going to a big conference in Sao Paulo next week and was going to look for him and also need to talk to McCutchin about what he has in mind for this "joint" program that he brought up at the last quarterly.
>
> Thanks!
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Tuesday, May 26, 2009 6:13 AM
> **To:** 'Omar A Diaz'
> **Cc:** Jorgebdelatorre@gmail.com; 'Jorge de la Torre Valdes'; Walter Nelson
> **Subject:** RE: Possible Meeting
>
> Omar:
>
> Thanks for your interest in testing sweet sorghum in the DR. I don't provide adequate quantities of seed to do large scale testing and I don't commercialize the hybrids. Ceres, Inc. is an Energy Crop Breeding Company that is testing and may eventually develop a commercial version of some of our sweet sorghum hybrids. Mr. Walter Nelson is their sorghum product manager and he would be a primary contact for larger scale testing when they have seed available.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Omar A Diaz [mailto:omar.diaz@transseed.com]
Sent: Monday, May 25, 2009 11:59 AM
To: Bill Rooney
Cc: Jorgebdelatorre@gmail.com; Jorge de la Torre Valdes
Subject: RE: Possible Meeting
Importance: High

Hi Bill

It has been a while, but wanted to take the opportunity to say hi and wish you a good memorial day. Jorge and I are contemplating testing your best sorghum hybrids in the Dominican Republic and analyze behaviour. I believe this is an opportunity to either have you visit us yourself or allow us to test the varieties. We could arrange for a small shipment to DR or your visit, considering your schedule I'd dare say it'd be best to arrange a sample. Please let me know costs associated with either option. This should potentially be a major crop in the country and your expertise along the process among other things will be highly appreciated.

Look forward to hearing from you.

Warm Regards

Omar A. Diaz
USA: 214-914-6664
Fax: 517-947-6664
Mob: 972-904-2296

> -------- Original Message --------
> Subject: RE: Possible Meeting
> From: "Bill Rooney" <wlr@tamu.edu>
> Date: Fri, May 30, 2008 10:49 am
> To: "'Omar A Diaz'" <omar.diaz@transseed.com>
>
> Thanks, Omar
>
> Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Omar A Diaz [mailto:omar.diaz@transseed.com]
Sent: Friday, May 30, 2008 10:47 AM
To: Bill Rooney
Cc: Jorge de la Torre Valdes
Subject: RE: Possible Meeting
Importance: High

Bill

I'd like to thank you again for your time and the outstanding attention you paid to us during our visit last Wednesday. We are in the planning stages and are considering your consulting services and maybe the research part we talked about. We will be in touch soon, or if we have any questions.

Have a great weekend.

Omar A. Diaz
Exec VP Business Development
TransSeed Biofuels International.
www.transseed.com
1330 Post Oak Blvd., Suite 1600
Houston, Texas 77056 USA
USA: 214-914-6664
Fax: 214-576-2794
Mob: 972-904-2296
DR: 829-948-3565

> -------- Original Message --------
> Subject: RE: Possible Meeting
> From: "Bill Rooney" <wlr@tamu.edu>
> Date: Wed, May 28, 2008 7:27 am
> To: "'Omar A Diaz'" <omar.diaz@transseed.com>
>
> Omar:
>
> When you get near please call me at the office 979 845 2151
> or my cell 979 220 1951, and I'll get you directions.
>
> regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: question
Date: Thursday, April 30, 2009 3:07:00 PM

Jeff:

is an old sweet sorghum variety developed in the Rio Grande Valley. It does very well in South Texas but it doesn't do as well in the Southeast. It is photoperiod sensitive like but it is quite different than

Sure we've crossed a lot with . In fact much of our initial sweet sorghum germplasm is based on

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
Sent: Thursday, April 30, 2009 9:27 AM
To: Bill Rooney
Subject: question
Importance: High

Where does come from? Can we get some? I here it's an like thing. Have you crossed with it?

J. Jefferson Gwyn, Ph.D.
Director of Breeding
Ceres, Inc.
3199 County Road 269 East
Somerville, TX 77879
off 979.272.2265
fax 979.272.2269
cell 805.490.0070
www.ceres.net

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: question
Date: Wednesday, May 20, 2009 12:08:00 PM

Jeff:

We treat everything with Concep and then apply Bicep (Dual/Atrazine) as a preemerge.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
> **Sent:** Wednesday, May 20, 2009 11:42 AM
> **To:** Bill Rooney
> **Subject:** question
>
> What herbicide regime do you use on your nursery (inbreds) and yield trials? Do you treat everything?
>
> We are not treating with Concep so evaluating our choices. So far have only come up with atrazine or propazine at a reduced rate for inbreds.
>
> Please advise.
>
> J. Jefferson Gwyn, Ph.D.
> Director of Breeding
> Ceres, Inc.
> 3199 County Road 269 East
> Somerville, TX 77879
> off 979.272.2265
> fax 979.272.2269
> cell 805.490.0070
> www.ceres.net

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: questions
Date: Monday, June 22, 2009 11:08:00 AM

Walter:

I'm planning to be in my office all of this afternoon. We just finished crossing - the crew can handle bagging.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Monday, June 22, 2009 7:13 AM
> **To:** Bill Rooney
> **Subject:** RE: questions
>
> Thanks Bill.
>
> Should we plan to leave by 5am or so?
>
> Also, will you be at your office this afternoon at all?
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Sunday, June 21, 2009 7:01 PM
> **To:** 'Juan Pablo Rebolledo R.'
> **Cc:** 'Mauricio Santacoloma'; Walter Nelson
> **Subject:** RE: questions
>
> Juan Pablo:
>
> We (Walter Nelson and I) will plan on being in Lacassine between 9:30 and 10 am. We'll leave no later than 2:00 pm.
>
> As for the proposal, I have not yet heard anything. I expect to hear something by July 1.
>
> See you on Tuesday.
>
> Regards,
>
> bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Juan Pablo Rebolledo R. [mailto:juan.re@lagreenfuels.com]
Sent: Friday, June 19, 2009 6:44 PM
To: Bill Rooney
Cc: Mauricio Santacoloma
Subject: RE: questions

Hello Bill

You are very welcome here any time. To answer your questions:

1. Tuesday 23 June is good for me in the morning. Something around 8 or 9 to 12 pm. If you want after we review our program and go to the experimental trials, we can have lunch together.

2. We are willing to receive the Vice minister of agriculture from Ecuador to show him our project based on fermentable sugars from sweet sorghum. So, go forward and disclose our contact information to the TAMU people that is coordinating the visit.

Bill, is there any new about the Sun Grant that we applied for sweet sorghum research?

I will look to see you soon,

Best Regards

Louisiana Green Fuels LLC
Juan Pablo Rebolledo R.
Agricultural Manager
juan.re@lagreenfuels.com
Phone: (337) 5884944 /5/6 Ext. 281
Mobil: (337) 4999897
Fax: (337) 5884493
14342 Walker Kimbrough
Lacassine, LA 70650, USA

From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Friday, June 19, 2009 12:34 PM
To: Juan Pablo Rebolledo R.
Subject: questions

Juan Pablo:

I've got two questions for you:

1. Mr. Walter Nelson, Sorghum Product Manager of Ceres, will be visiting me next Monday. He was interested in visiting LAGF on Tuesday if you were available. It wouldn't hurt me to see what you are up to and I could come along. However, I want to make sure that you are available for a mid day visit on Tuesday, June 23.

2. The vice-minister of agriculture of Ecuador is scheduled to make a visit to Texas A&M. They have specifically asked to visit with people who are working with sweet sorghum for ethanol production. I've agreed to visit with him in College STation on the development and logistics of sweet sorghum for energy. It might be interesting for him to visit your facility in Lacassine - IF YOU ARE WILLING FOR SUCH A VISIT. I have not disclosed your contact information to anyone regarding this visit. I wanted to make sure you would be interested before doing so. If you are interested, just let me know and I'll forward your contact information to the folks at TAMU who are developing the schedule. The date is not finalize as of yet.

regards,
bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From:	Bill Rooney
To:	"Walter Nelson"
Subject:	RE: Sorghum flowering
Date:	Thursday, June 04, 2009 3:07:00 PM

Walter:

John has more experience than I in the greenhouse, but we've treated these in the field with a trashcan for about two weeks and that was enough to get them to flower. I assume similar in a GC, but it might be less.

I'm working on the , I'll need a letter of confirmation that we can use year 2 and 3 of our supplemental grant as matching for my proposal. I assume that comes from Steve Thomas, correct?

Regards,
Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, June 04, 2009 2:30 PM
To: John Mullet; William Rooney
Subject: Sorghum flowering

John or Bill,

I have a couple of growing in our long-day greenhouse in Thousand Oaks. In theory they won't flower while in there as I understand it.

If I wanted to trigger flowering in them by moving them into a short-day growth chamber, how long would I have to keep the plant in the short-day lighting before I could move it bck to the greenhouse to finish flowering?

Thanks!

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Sorghum flowering
Date: Thursday, June 11, 2009 6:15:00 AM

This is due in late June so it would great to have it by late next week.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Wednesday, June 10, 2009 3:25 PM
To: Bill Rooney
Subject: RE: Sorghum flowering

Thanks Bill. Sorry for the delay in reply. When do you need the letter by? I sent the request to Steve T.

W

-----Original Message-----
From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Thursday, June 04, 2009 1:08 PM
To: Walter Nelson
Subject: RE: Sorghum flowering

Walter:

John has more experience than I in the greenhouse, but we've treated these in the field with a trashcan for about two weeks and that was enough to get them to flower. I assume similar in a GC, but it might be less.

I'm working on the CPBR grant, I'll need a letter of confirmation that we can use year 2 and 3 of our supplemental grant as matching for my proposal. I assume that comes from Steve Thomas, correct?

Regards,
Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

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Thanks!

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Sorghum flowering
Date: Thursday, June 11, 2009 6:40:00 AM

Things look good here, the sweets are well into the log phase of growth and will be flowering in the next few weeks. The material is little later, but it is also taking off. We haven't seen anything flowering that shouldn't be.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, June 11, 2009 6:37 AM
To: Bill Rooney
Subject: RE: Sorghum flowering

Thanks. I will pass that along to Steve.

How is the sorghum looking? Clint reported a few boots in the that he's growing out in Arizona....

W

-----Original Message-----
From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Thursday, June 11, 2009 4:16 AM
To: Walter Nelson
Subject: RE: Sorghum flowering

This is due in late June so it would great to have it by late next week.

Regards,

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Wednesday, June 10, 2009 3:25 PM
To: Bill Rooney
Subject: RE: Sorghum flowering

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W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Sorghum flowering
Date: Thursday, June 11, 2009 9:51:00 AM

Anytime for the remainder of the morning. The afternoon is full.

Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]
Sent: Thursday, June 11, 2009 9:37 AM
To: Bill Rooney
Subject: RE: Sorghum flowering

Just realized there are a few other things that I meant to ask you about. There a good time to call you today?

W

-----Original Message-----
From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Thursday, June 11, 2009 4:40 AM
To: Walter Nelson
Subject: RE: Sorghum flowering

Things look good here, the sweets are well into the log phase of growth and will be flowering in the next few weeks. The PS material is little later, but it is also taking off. We haven't seen anything flowering that shouldn't be.

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W

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To: Walter Nelson
Subject: RE: Sorghum flowering

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Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

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Sent: Wednesday, June 10, 2009 3:25 PM
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Subject: RE: Sorghum flowering

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W

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Sent: Thursday, June 04, 2009 1:08 PM
To: Walter Nelson
Subject: RE: Sorghum flowering

Walter:

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I'm working on the CPBR grant, I'll need a letter of confirmation that we can use year 2 and 3 of our supplemental grant as matching for my proposal. I assume that comes from Steve Thomas, correct?

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Bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics Chair, Plant Release Committee Texas A&M University
College Station, Texas 77843-2474 979 845 2151

-----Original Message-----
From: Walter Nelson [mailto:wnelson@ceres.net]

Sent: Thursday, June 04, 2009 2:30 PM
To: John Mullet; William Rooney
Subject: Sorghum flowering

John or Bill,

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If I wanted to trigger flowering in them by moving them into a short-day growth chamber, how long would I have to keep the plant in the short-day lighting before I could move it bck to the greenhouse to finish flowering?

Thanks!

W

Walter E Nelson
Ceres, Inc.
mobile: (805)410-0503

sent from treo

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: sorghum for energy
Date: Thursday, April 09, 2009 11:14:00 AM

I doubt it very much.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Thursday, April 09, 2009 11:08 AM
> **To:** Bill Rooney
> **Subject:** RE: sorghum for energy
>
> Thanks Bill, this guy contacted me a few days ago and I hadn't gotten back to him yet. I'm sending a small bulk of seed to Germany and will partition out to the various European requestors as appropriate.
>
> How do you think the high biomass sorghums will do in Belgium? Worthwhile? I'm a bit skeptical....
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Thursday, April 09, 2009 8:55 AM
> **To:** 'Ghekiere Greet'
> **Cc:** Walter Nelson
> **Subject:** RE: sorghum for energy
>
> Greet:
>
> Thanks for your interest in trying some of the photoperiod sensitive hybrids.
>
> As you know those hybrids are being commercially developed through our partnership with Ceres, and they have produced experimental quantities of seed for evaluation. I don't produce large quantities (2kg) of seed, so I've copied Mr. Walter Nelson, who is sorghum product manager for Ceres and he can inform you of Cere's interest in providing seed.
>
> regards,
>
> bill
>
> Dr. William L. Rooney
> Professor, Sorghum Breeding and Genetics
> Chair, Plant Release Committee
> Texas A&M University

College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Ghekiere Greet [mailto:Greet.Ghekiere@west-vlaanderen.be]
Sent: Thursday, April 09, 2009 3:14 AM
To: wlr@tamu.edu
Subject: sorghum for energy
Importance: High

Dear professor Rooney,

As member of a technical research station for agriculture in Belgium, Europe, I follow the results on sorghum of TAMU since several years, as we have in our own trial site since 2005 trials on sorghum for anaerobic digestion.
Although it is quite visible that the varieties of sorghum on the market today, are not designed for our growing circumstances (could in spring), we can also see the potential of this crop concerning biomass accumulation in short time.
The best varieties in our trials, of which are and , reached yields of 20 tons of dry matter per hectare in trial.

I've read with interest on the new developments at TAMU concerning the energy-sorghum in cooperation with . I really would like to do a small test with one or two of these varieties in our growing conditions, because in fact, also for anaerobic digestion it is not really the sugar but the celluloses that is important.
I'm not sure that it will work out. I've understood that the varieties are photosensitive, and in 2007 we had the PS-variety 1990 (Sorghum partners) in trial and that didn't work out al all. But I think it is worth the try.

I don't know if it would be possible to receive samples (± 2 kg) from your institute or Ceres?
It is no problem for us to pay the costs for the seeds and the shipment.

Sincerely yours,

Greet Ghekiere

POVLT - afdeling Innovatie, Verbreding & Advies
Ieperseweg 87
8800 Rumbeke (Beitem)
Tel 051/273384
Fax 051/240020
www.povlt.be

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: sorry
Date: Thursday, May 07, 2009 8:51:00 AM

That is a GREAT ONE! I haven't gotten one quite like that yet. Quite frankly, I think that one will be hard to beat.....

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Thursday, May 07, 2009 8:46 AM
> **To:** Bill Rooney
> **Subject:** RE: sorry
>
> No worries...we get lots of such things.
>
> My current favorite is the guy who bought a couple of bags of switchgrass and then, when I was speaking to him later, told me he thought he just needed to press/crush the switchgrass through rollers to squeeze the fuel out.
>
> I warned Cory he was going to have some customer complaints about the performance of his product at the end of the season....
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Thursday, May 07, 2009 6:16 AM
> **To:** Walter Nelson
> **Subject:** sorry
>
> Walter:
>
> FYI, I just gave a fellow your name as a potential contact for sweet sorghum and energy sorghum seed (I told him you would not give out sweet sorghum seed, but I wasn't sure about energy sorghum).
>
> He's from Maryland, claimed he hasn't talked to you, but he may have. He's one that has all the answers and big plans, but nobody to fund it.
>
> Sorry about this, but thought deserved a heads up before you get the phone call.
>
> regards,
>
> bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From:	Bill Rooney
To:	"Edgar Haro"
Cc:	"Walter Nelson"
Subject:	RE: Sweet bulked inbred list
Date:	Tuesday, May 19, 2009 6:54:00 AM

Edgar - see below

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Edgar Haro [mailto:eharo@ceres-inc.com]
> **Sent:** Monday, May 18, 2009 5:33 PM
> **To:** wlr@tamu.edu
> **Cc:** Walter Nelson
> **Subject:** Sweet bulked inbred list
>
> Hi Bill,
> Could you please corroborate the pedigrees of sweet females you provided for hybrid and inbred production.
>
> The Plant selection number out of each sweet female.
>
> Thanks.
> Edgar
>
> Edgar Haro PhD.
> Senior Manager Sorghum Research
> Ceres Inc.
> Cel. (979) 324-8046

From: Bill Rooney
To: "Edgar Haro"
Subject: RE: Sweet bulked inbred list
Date: Thursday, May 21, 2009 6:02:00 AM

---

friday is better.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Edgar Haro [mailto:eharo@ceres-inc.com]
> **Sent:** Wednesday, May 20, 2009 4:55 PM
> **To:** Bill Rooney
> **Subject:** RE: Sweet bulked inbred list
>
> Bill,
> I would like to stop by your office for a couple of quick questions just to understand your selection recording process.
> Thanks,
> Edgar
>
> > -----Original Message-----
> > **From:** Bill Rooney [mailto:wlr@tamu.edu]
> > **Sent:** Tuesday, May 19, 2009 6:55 AM
> > **To:** Edgar Haro
> > **Cc:** Walter Nelson
> > **Subject:** RE: Sweet bulked inbred list
> >
> > Edgar - see below
> >
> > Dr. William L. Rooney
> > Professor, Sorghum Breeding and Genetics
> > Chair, Plant Release Committee
> > Texas A&M University
> > College Station, Texas 77843-2474
> > 979 845 2151
> >
> > > -----Original Message-----
> > > **From:** Edgar Haro [mailto:eharo@ceres-inc.com]
> > > **Sent:** Monday, May 18, 2009 5:33 PM
> > > **To:** wlr@tamu.edu
> > > **Cc:** Walter Nelson
> > > **Subject:** Sweet bulked inbred list
> > >
> > > Hi Bill,
> > > Could you please corroborate the pedigrees of sweet females you provided for hybrid and inbred production.

Thanks.
Edgar

Edgar Haro PhD.
Senior Manager Sorghum Research
Ceres Inc.
Cel. (979) 324-8046

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: sweet sorghum association mapping
Date: Monday, April 27, 2009 10:50:00 AM
Attachments: 2008 Murray et al. II Crop Science.pdf
2008 Murray et al. I Crop Science.pdf
Murray et al., 2009.pdf

Jeff:

In the presentation on Thursday, I mentioned that we had a mapping population in the field (in cooperation with Veremis at UFL). I also mentioned that this was followup work to research done by Seth Murray (while he was a Ph.D. student at Cornell). From that work, Seth has published three papers (this was the final publication from that work. I've attached the first two (in case you don't have them).

The fieldwork for this paper was completed prior to the Ceres agreement. We have a version of this sweet sorghum panel in the field, mainly for reference and crossing work. We don't have plans to collect specific data on it this summer. If there is an interest from Ceres, you would be able to utilize it as long as it doesn't interfere with any crosses or seed production.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
Sent: Monday, April 27, 2009 10:35 AM
To: Bill Rooney
Cc: Nickolai Alexandrov
Subject: FW: sweet sorghum association mapping

What do we know about this? I don't remember any discussion on sweets and genes and phenology.

Please advise.

From: Nickolai Alexandrov
Sent: Saturday, April 25, 2009 10:40 PM
To: Jeff Gwyn
Subject: RE: sweet sorghum association mapping

Interestingly, we have briefly discussed this paper on our MAB journal club last Wednesday. Jeff, do you think we should collect this kind of data for our internal breeding program?
Nick

From: Steven Thomas
Sent: Sat 4/25/2009 11:25 AM
To: Jeff Gwyn; Edgar Haro; Walter Nelson; Nickolai Alexandrov; John Bouck
Cc: Bonnie Hames; Tanya Kruse; Joon-Hyun Park; Roger Pennell; Richard Flavell; Spencer Swayze
Subject: sweet sorghum association mapping

See attachment for more detail (from The Plant Genome). st

Making Sweet Sorghum Sweeter

Submitted by James Giese on Fri, 04/17/2009 - 14:48

- Feature

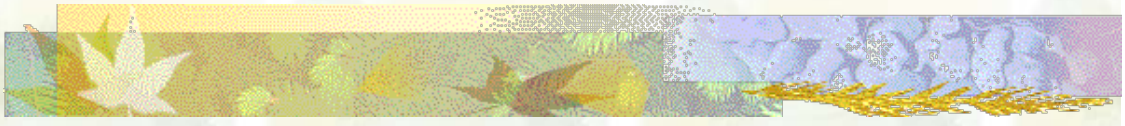
Sweet sorghum, like its close relative, sugarcane, has been bred to accumulate high levels of edible sugars in the stem. Sweet sorghums are tall and produce high biomass in addition to sugar. However, there is little documentation about the genetic relationships and diversity within sweet sorghums and how sweet sorghums relate to grain sorghum racial types.

Researchers from Cornell and Texas A&M genotyped with simple sequence repeats and single nucleotide polymorphisms a diverse panel of 125 (mostly sweet) sorghums. Using both distance-based and model-based methods, the researchers identified three main genetic groupings of sweet sorghums. Based on observed phenotypes and known origins, these were classified as historical and modern syrup, modern sugar/energy, and amber types.

Three significant associations for height were detected. Two of these, on chromosomes 9 and 6, support published studies. One significant association for brix, on chromosome 1, was detected.

****
Steven R. Thomas, Ph.D.
Director of Bioproducts
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320

ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net
****



Genetic Improvement of Sorghum as a Biofuel Feedstock: I. QTL for Stem Sugar and Grain Nonstructural Carbohydrates

Seth C. Murray, Arun Sharma, William L. Rooney, Patricia E. Klein, John E. Mullet, Sharon E. Mitchell, and Stephen Kresovich*

ABSTRACT

Genetic improvement of sorghum [*Sorghum bicolor* (L.) Moench] has traditionally focused on a single nonstructural carbohydrate, either grain starch or stem sugar. Sorghum starch and sugar may both be used as feedstocks for biofuel production. To investigate genetic tradeoffs between grain and stem sugar, a population derived from sweet sorghum cultivar Rio and grain sorghum 'BTx623' was evaluated for 27 traits related to grain and stem sugar yield and composition. Across three environments, a total of 129 quantitative trait loci (QTL) were identified. Tradeoffs identified between grain and stem sugar yield QTL colocalized with height and flowering time QTL. Most importantly, QTL were identified that increased yield and altered the composition of stem sugar and grain without pleiotropic effects. For example, a QTL on chromosome 3 that explained 25% of the genetic variance for stem sugar concentration did not colocalize with any grain QTL. These results suggest that total nonstructural carbohydrate yield could be increased by selecting for major QTL from both grain and sweet sorghum types. We conclude that altering grain and stem sugar genetic potential for yield traits should lead to greater feedstock improvement than altering composition traits.

S.C. Murray, S.E. Mitchell, and S. Kresovich, Institute for Genomic Diversity and Dep. of Plant Breeding and Genetics, Cornell Univ., Ithaca, NY 14853; A. Sharma, P.E. Klein, and J.E. Mullet, Institute for Plant Genomics and Biotechnology, Texas A&M Univ., College Station, TX 77843; W.L. Rooney, Dep. of Soil and Crop Sciences, Texas A&M Univ., College Station, TX 77843. Received 8 Jan. 2008. *Corresponding author (sk20@cornell.edu).

Abbreviations: ADF, acid detergent fiber; AFLP, amplified fragment length polymorphism; CIM, composite interval mapping; HPLC, high performance liquid chromatography; IM, interval mapping; LOD, likelihood of odds; NIRS, near infrared spectroscopy; QTL, quantitative trait locus (loci); RIL, recombinant inbred line; SSR, simple sequence repeat; WINQTL, Windows QTL Cartographer.

There is renewed interest in using sugars derived from agricultural crops as feedstocks for biofuel production (Farrell et al., 2006; USDOE, 2006; Somerville, 2007), large-scale manufacture of more complex molecules (Lichtenthaler and Peters, 2004), and in planta syntheses of harvestable biomolecules (i.e., nutraceuticals) (Mazur et al., 1999; Mohanty et al., 2002). Improving a species for use as biofuel feedstock requires a change in perspective: crops must be regarded as living systems for capturing and storing energy rather than simply as a sole source of food, feed, or fiber products. This changes the basic biological question from "how much of a crop's energy can be converted into food?" to "how can we maximize the total useable energy that can be produced and stored throughout the growing season?". Furthermore, characterization and quantification of environmental and postharvest energy degradation in addition to genetics will be crucial for developing economically feasible biofuel feedstocks.

Published in Crop Sci. 48:2165–2179 (2008).
doi: 10.2135/cropsci2008.01.0016

677 S. Segoe Rd., Madison, WI 53711 USA

Sorghum [*Sorghum bicolor* (L.) Moench], a hardy drought-tolerant and nutrient-efficient C_4 grass, is widely adapted throughout the world. Sorghum is also closely related to other potential biofuel crops such as sugarcane (*Saccharum officinarum* L.), the principal sugar feedstock, and maize (*Zea mays* L.), the most important starch feedstock. Sweet sorghums accumulate up to 25% sugar, 1.4 to 2.7 times more whole-plant nonstructural carbohydrates than grain sorghums, in the parenchyma of juicy stalks (Vietor and Miller, 1990; Ming et al., 2001). The physiological mechanism of sugar accumulation, however, appears to differ between sorghum and sugarcane (Tarpley and Vietor 2007). Additionally, many of the enzymes associated with sugar accumulation in sugarcane (i.e., sucrose phosphate synthase and invertase) do not appear to play major roles for sugar accumulation in sorghum (Lingle, 1987; Tarpley et al., 1994). Sweet sorghums are more water and nutrient efficient than sugarcane and maize, and can be grown over a wide area of the United States (Jackson et al., 1980; Hallam et al., 2001). To date, fewer than 50 U.S. elite inbred sweet sorghum cultivars have been released. Among these cultivars are syrup types, lines selected for high quality and quantity of stem juice sugar to be boiled into syrup, and a few sugar types, lines selected for high sucrose yield only (Jackson et al., 1980). Because both sweet sorghum types were selected specifically for extractable stem sugar, these lines generally produce small amounts of grain with undesirable characteristics such as small seed and high tannin content. It is unclear whether there is a genuine physiological tradeoff between high stem sugar production and reduced grain yield or if the relationship is simply because sweet sorghum cultivars have never been improved for grain traits. In sorghum, the mode of inheritance of increased stem sugar depends on the cross and has been shown to be either additive or dominant (Schluhuber, 1945; Clark, 1981). Genetic mapping experiments for sorghum stem sugar have identified one to a few loci (Natoli et al., 2002; Bian et al., 2006; Ritter 2007) but the small variance explained suggests that additional loci with complex interactions may also be involved.

In the United States, most ethanol is produced from maize grain starch, which is enzymatically converted to glucose and then fermented. The same process is used for grain sorghum; in fact, sorghum is the second most commonly used grain in ethanol production in the United States (Renewable Fuels Association, 2007). In Brazil, ethanol is produced from sucrose extracted from sugarcane. This process is simpler, as it eliminates the need for enzymatic degradation of starch and requires less processing. Sweet sorghum juice could certainly be used in a similar system as sugarcane. In addition, harvesting grain from sweet sorghum provides another important source of fermentable carbohydrates for conversion to ethanol (Jackson et al., 1980; Kresovich and Henderlong, 1984). The "dual-purpose" nature of sorghum raises the possibility that energy production could be maximized by concurrent improvement of both grain and stem sugar yields. Because elite sorghum cultivars have traditionally been bred for a single use (i.e., grain for human or animal consumption, stem sugar for syrup production, or forage–silage for animal feed), little is known about the physiological tradeoffs of simultaneously improving both grain and sugar traits.

In this study, we investigated the potential of developing high-starch grain sorghums with increased stem sugar for the ultimate goal of improving sorghum as a dedicated feedstock crop. To accomplish this, we identified and mapped quantitative trait loci (QTL) controlling yield and composition of sugar in the stem as well yield and composition of starch, fat, protein, fiber, and phosphorus in grain. Specifically, we were interested in determining (i) the genetic tradeoffs between grain and stem sugar yield, (ii) the genetic tradeoffs between grain composition and yield of stem sugar or grain, and (iii) if there were significant effects of harvest date and postharvest handling on the production of fermentable carbohydrates.

MATERIALS AND METHODS

Plant Material and Plot Design

A mapping population consisting of 176 $F_{4:5}$ recombinant inbred lines (RILs) from a cross between cultivar Rio, a high-biomass sweet sorghum (Broadhead, 1972), and 'BTx623', an elite inbred grain sorghum (Frederiksen and Miller, 1972), was phenotyped for grain yield, grain composition, and stem sugar traits. The RILs and the parental lines were planted during the summer growing season in 2005 at Weslaco, TX (WE05), and in 2005 at College Station, TX (CS05). In 2006, 165 $F_{5:6}$ RILs were planted in College Station (CS06) from self-pollinated seed produced at CS05. In each location, two replicates of 3.05-m rows were planted in a randomized complete block design. Seeds were planted at a rate of 160,000 plants ha^{-1} with either 76-cm (CS05, CS06) or 102-cm row spacings (WE05). The WE05 site had 5 cm of pre–plant irrigation and received <2 cm of rainfall for the remainder of the growing season. The CS05 emerged based on available soil moisture; 430 mm of rain fell during the growing season primarily during flowering. In CS06, the total rainfall of 360 mm was distributed evenly throughout the growing season.

Phenotypic Measurement of Field Traits

In total, we measured 28 traits of agronomic and quality importance (Table 1). Plant height was measured either in the field (WE05) or at harvest, due to high lodging (CS05, CS06). Stand density and tillering were each estimated on a scale from 1 to 10 in the harvested area of each row. Flowering time was measured as 50% plot anthesis (WE05 and CS05).

Harvests were staggered over 16 d (WE05), 14 d (CS05), and 11 d (CS06) because of the volume of work and logistics of labor and equipment. Harvest date was used as one of the cofactors in subsequent statistical analyses to control for experimental

Table 1. Trait values for Rio × BTx623 parental and recombinant inbred lines (RILs) at three locations in Texas.

Trait	Weslaco, 2005				College Station, 2005				College Station, 2006			
	Rio	BTx623	RILs mean (SD)†	RILs range	Rio	BTx623	RILs mean (SD)†	RILs range	Rio	BTx623	RILs mean (SD)†	RILs range
Brix, °brix	19.4	14.4	17.2 (1.7)	12.3–22.5	19.7	9.7	15.5 (1.9)	9.8–20	21.7	14.5	16.3 (2.4)	7.9–21.5
Juice sugars, g L^{-1}	195	98	148 (25)	54–288	178	60	142 (23)	75–209	199	87	135 (30)	22–202
Juice glucose, g L^{-1}	17	12	11 (3)	0–25	15	13	13 (6)	5–40	12	19	12 (5)	4–43
Juice fructose, g L^{-1}	14	23	14 (10)	4–121	13	12	11 (6)	3–47	7	5	8 (4)	0–34
Juice sucrose, g L^{-1}	164	63	123 (28)	8–193	151	36	117 (25)	43–182	180	64	114 (31)	9–172
Total sugar yield, t ha^{-1}‡	2.9	0.8	2.2 (.8)	0.5–6.5	7.3	0.6	3.6 (1.4)	0.6–9.4	6.6	1.1	3.1 (1.2)	0.3–7.9
Sugar in dry stem, g kg^{-1}§	560	310	500 (80)	230–790	460	260	530 (100)	310–1640	530	330	500 (100)	120–730
Juice yield first press, t ha^{-1}	7.9	3.5	7.8 (3.5)	0.5–24	9.7	1.6	5.7 (2.3)	1–17.7	19.8	5.7	13.1 (4.5)	2.6–29.8
Stem fresh yield, t ha^{-1}	23.3	10.3	21.9 (6)	6.2–48.1	65.5	13.2	36 (12)	6.5–79.3	53	16.8	32.5 (10)	8.5–69.3
Stem juiciness, g kg^{-1}¶	740	790	770 (20)	680–830	720	810	790 (20)	720–920	730	790	790 (20)	710–850
Panicle fresh yield, t ha^{-1}	4.2	7.3	5.5 (1.1)	2.6–9.8	3.6	4.3	3.8 (1.7)	0.4–11.4	2.3	6.5	4.3 (1.7)	0.9–11.3
Grain dry yield, t ha^{-1}#	2.6	4.6	3.5 (0.8)	0–6.2	1.1	2	1.4 (1.2)	0–6.3	0	3.8	2.3 (1.2)	0–6.7
Thousand seed weight, g	18.5	23.3	22 (2.9)	15.3–30.8	14.9	20.9	16.9 (3.2)	7.9–28.8	14.6	27.4	24.6 (3.3)	11.8–32.9
Thousand seed density, g mL^{-1}	0.73	0.75	0.76 (0.03)	0.63–1.00	0.64	0.65	0.64 (0.05)	0.45– 0.75	0.77	0.87	0.85 (0.04)	0.64–0.92
Corneous endosperm	7.2	7.3	6.7 (1.8)	1.5–9.6	7.3	2.8	4.8 (2.1)	0–9.2	na††	na††	na††	na††
Grain starch, g kg^{-1}	630	670	650 (10)	600–690	600	650	610 (30)	450–660	570	680	640 (20)	550–680
Grain fat, g kg^{-1}	40	40	40 (0)	30–50	30	30	30 (0)	10–40	30	30	40 (0)	30–50
Grain crude protein, g kg^{-1}	140	110	130 (10)	90–170	160	130	150 (20)	120–190	180	120	140 (10)	110–190
Grain moisture, g kg^{-1}	88	87	87 (1)	70–82	88	87	86 (20)	80–90	84	84	86 (30)	79–94
Grain phosphorus, g kg^{-1}	2.1	1.1	16 (0.4)	0.5–2.7	2.9	1.2	2 (0.6)	0.5–3.5	3.0	1.0	2 (0.5)	0.6–3.4
Grain ADF, g kg^{-1}‡‡	130	90	100 (10)	80–140	140	90	110 (20)	80–170	140	90	110 (10)	90–170
Glumes retained after threshing, %	2	10	9 (6)	0–30	6	13	14 (11)	0–75	1	1	1 (3)	0–40
Stand density	7.5	6.5	7.7 (0.6)	4–9	6	4.5	5.3 (1.4)	1–8	7	7	6.6 (1.3)	2–9
Tillering	6.5	2.5	6.1 (1.5)	2–9	4.5	1.5	4.5 (1.7)	0–8	8	4	5.5 (1.7)	1–9
Mean stem thickness	2	6	3.4 (0.9)	1.5–7	5.8	5.8	4.3 (1)	2–7	4	5	3.5 (1)	1.5–7
Plant height, cm	210	130	200 (26)	130–274	273	119	227 (29)	119–297	227	123	204 (26)	109–259
Flowering time, d	116	109	111 (5)	104–123	180	161	168 (5)	157–185	na††	na††	na††	na††

†Standard deviation in parentheses.

‡Total sugar yield = {juice sugars × [pressed juice + (pressed stem weight wet – pressed stem weight dry]}

§Sugar concentration in dry stem = total sugar yield/{dry stem biomass yield – [(pressed stem weight wet – pressed stem weight dry) × juice sugars]}.

¶Percent water of fresh stem by weight = total stem water weight/(total stem water weight + dry stem weight + total sugar yield).

#Grain dry matter yield = panicle fresh yield × (dry grain subsample/fresh panicle subsample) × (NIRS grain dry matter content). NIRS, near infrared spectroscopy.

††Not assayed.

‡‡ADF, acid detergent fiber.

error. From each row, a random meter of plant material was harvested in the morning from a central stand by cutting the plants within 3 cm of the ground. Each cut row was then bundled in clear plastic sheeting and taken to a shaded central processing facility within 2 h of harvest. The bundled row was stripped into panicles and stems, and each was weighed (panicle fresh weight, stem fresh weight traits, respectively). Strip date was also recorded because only about half the plants could be processed on the day they were harvested. At CS05 and CS06, replications were harvested simultaneously; at WE05 harvest was sequential, meaning harvest date and replicate sources of error would be nested for statistical analysis.

Fresh stem tissue was crushed in a three-roller sugar mill (WE05, CS06) or a potato starch drier (CS05) to extract the juice. At this time, juice volumes and weights were recorded, brix was measured using a handheld refractometer (Atago U.S.A. Inc., Bellevue, WA), and aliquots of juice (15 mL $sample^{-1}$) were frozen for high performance liquid chromatography (HPLC) analysis of sugars. Brix, a measure of the mass ratio of soluble solids to water, is a widely used approximation for sugar content and is reported as a trait in the rest of the text.

For each experimental unit, random subsamples of grain panicles and pressed stems were collected. Subsamples were then weighed and dried in a greenhouse (WE05) or in a forced-air drier at 38°C (CS05, CS06). Dry stem and panicle subsamples were weighed, and panicles were threshed; stem dry weight, panicle dry weight, and grain dry yield were calculated from these measurements. All dried material and frozen juice samples were then shipped to Ithaca, NY, for further analysis.

Measurement of Sugar Traits

To determine sugar composition and quality, frozen juice was evaluated by HPLC based on the instrument manufacturer's instructions (Dionex, 2006). Juice samples were thawed, lactose

was added as an internal standard, samples were diluted 250× in water and filtered through a 0.45-μm filter (PALL acroprepTM96; Pall Life Sciences, Ann Arbor, MI). Samples were analyzed on a Dionex HPLC (Dionex, Sunnyvale, CA) with EP50 gradient pump, AS40 autosampler, ED40 HPAE-PAD detector, and CarboPac PA1 analytical and guard columns. Results were evaluated using the software package PeakNet (Dionex). Stem juice samples were run for 10 min with a flow rate of 1 mL min^{-1} of 150 mM sodium hydroxide buffer. A standard curve for sucrose, glucose, fructose, and lactose was developed each time the buffer was replenished. For each sample, sugar values were corrected based on the ratio of lactose detected/lactose expected. Sucrose, glucose, and fructose weights were converted to grams per liter of juice (juice sucrose, juice glucose, and juice fructose traits) and these values were summed for total sugar concentration (juice sugars).

Measurement of Grain Quality Traits

One thousand seeds were counted with a seed counter and weighed to obtain thousand seed weight. Thousand seed volume was then measured in a graduated cylinder. Thousand seed density was calculated as thousand seed weight divided by thousand seed volume. The ratio of corneous to floury grain endosperm (corneous endosperm) was the mean of 10 seeds that were halved and scored visually on a scale from 1 to 10 (not measured for CS06). The percentage of seeds retaining glumes after threshing was also estimated by visual inspection.

Approximately 60 g of seed were ground in a cyclone mill (UDY Corporation, Fort Collins, CO) with a 1-mm screen with a stainless steel grinding ring and an aluminum impeller. Ground grain samples were then stored at 4°C for approximately 4 wk. Before assaying, samples were acclimated for 3 wk in a room housing the analytical instrument, a FOSS Model 5000 Feed and Forage Analyzer (FOSS NIRS Systems, Silver Spring, MD), and analyzed with WinISI II software (Infrasoft International, State College, PA). Near infrared spectroscopy (NIRS), a technique for rapid measurement of most organic and some inorganic compounds in tissue is an accurate, reliable, and repeatable method for analyzing components of grains, including sorghum (Williams and Sobering, 1993; Hicks et al., 2002; de Alencar Figueiredo et al., 2006; Hooks et al., 2006). A total of 1006 samples from this population were analyzed by NIRS.

NIRS calibration

To obtain accurate data from NIRS, the system must be calibrated based on values obtained from chemical analyses of a subset of samples. For developing calibration equations, 111 of the most informative grain samples were chosen with the WinISI software (76 from the Rio × BTx623 RILs and 35 from diverse sweet and grain sorghums grown at same time in the same locations as the RILs). Grain samples were then analyzed for starch (YSI Incorporated, 2000), fat (Padmore, 1990), crude protein (Miller et al., 1998), and moisture content by Ward Laboratories (Kearney, NE).

Near infrared spectroscopy equations for each grain trait were developed with WinISI. Trait values from a randomly selected group comprising 74 of the 111 samples were used to produce the calibration equations while values from a second group, the remaining one-third of the samples, were used to evaluate the derived equations. In all, 28 equations (each with different wavelengths and math treatments, and the inclusion of the repeatability file) were tested for each trait. The equations that maximized the prediction of trait values (based on low standard errors of prediction and high R^2) were retained. This process was repeated three times with different subsets of random samples for deriving and validating equations. The best calibration equations were then evaluated using the full subset of 111 samples, and the best equation from each repetition (a total of three equations) was used to predict composition values from the NIRS spectra of all grain samples (n = 1006).

To investigate the effect of calibration sample size on NIRS calibration, we repeated the above procedure for all traits except grain moisture (data were not available) using an expanded data set that included raw data from additional samples (Hooks et al., 2006). Sample sizes for each trait are reported in Supplementary Table S1. We therefore evaluated predicted values from a total of six different calibration equations, the best three from our samples only and the best three equations from the larger sample set that included both our samples and those from Hooks et al. (2006). From these, one equation was selected for each trait based on low standard error of calibration, low standard error of cross-validation, high R^2, high heritability, and repeatable detection of QTL in the RIL population (see below).

The best calibration equation for each trait is shown in Supplementary Table S1. Starch, fat, and acid detergent fiber (ADF) trait prediction improved with the inclusion of additional sample data but, overall, little difference between most equations was observed. Acid detergent fiber and phosphorus calibration equations were based solely from the calibration samples of Hooks et al. (2006).

Statistical Analyses

Identifying sources of experimental variation and trait heritability

For the statistical analyses, WE05, CS05, and CS06 were treated as different environments. Models were evaluated separately for each trait with SAS PROC MIXED (SAS Institute, 2007) software. Trait variance σ^2_{Trait} was estimated as:

$$\sigma^2_{\text{Trait}} = \sigma^2_{\text{G}} + \sigma^2_{\text{E}} + \sigma^2_{\text{G×E}} + \sum(\sigma^2_{\text{X}})_{ij} + \sigma^2_{\text{error}} \qquad [1]$$

where σ^2_{G} is variance due to genotype, σ^2_{E} is the variance due to environment (i.e., location), $\sigma^2_{\text{G×E}}$ is variance due to interaction of genotype and environment, $\sum(\sigma^2_{\text{X}})_{ij}$ is the sum of variances due to a number of predicting effects, X, ranging from i to j, and σ^2_{error} is the variance due to error. Here, the predicting effects (X) constitute nongenetic sources of experimental error, such as harvest date (see Table 2 column headings for a complete list of predicting effects). Most of these effects were nested in environment. For testing effects, all were treated as random except genotype. Genotype was treated as fixed to allow inferences on specific RILs in later data correction and analyses. Only effects deemed significant (P = 0.05) by Type III sums of squares and the main effects, in the case of significant interactions, were retained in the reduced model (Table 2). Type III sums of squares were also used to estimate variance components from the reduced model with all effects, including genotype, as random. Variance components for genotype (G), environment

Table 2. Trait heritability and variance component percentage attributable to genetic (G), environmental (E), genetic × environment interaction (G × E), and other effects.

Trait	Heritability	Variance component†														
		G	E	G × E	(E)‡ Rep.	(E)‡ Har. date	(E)‡ Har. date × strip date	(E)‡ North border	(E)‡ East border	(E)‡ South border	(E)‡ West border	(E)‡ Storage box	HPLC dilution date	Grain grind date	NIRS date	Residual error
Brix, °brix	0.65	0.21***	0.17***	0.15***		0.07***										0.40
Juice sugars, g L^{-1}	0.56	0.16***	0.05	0.15***		0.03**							0.14***			0.47
Juice glucose, g L^{-1}	0.55	0.09***	0	0			0.34***						0.11***			0.45
Juice fructose, g L^{-1}	0.15	0.01	0.14	0		0.27*	0.15***						0.02*			0.4
Juice sucrose, g L^{-1}	0.58	0.17***	0	0.1***									0.09***			0.53
Total sugar yield, t ha^{-1}	0.62	0.15***	0.38***	0.11***								0.02**	0.02**			0.32
Sugar in dry stem, g kg^{-1}	0.43	0.09***	0	0.08**		0.11***							0.11***			0.59
Juice yield first press, t ha^{-1}	0.69	0.1***	0.63***	0.02*		0.02***										0.22
Stem fresh yield, t ha^{-1}	0.71	0.2***	0.39***	0.07***												0.34
Stem juiciness, g kg^{-1}	0.49	0.11***	0.13	0.09***	0.01*	0.08***						0.06*			0.05*	0.47
Panicle fresh yield, t ha^{-1}	0.65	0.18***	0.2	0.07***					0.11***							0.44
Grain dry yield, t ha^{-1}	0.63	0.08***	0.5*	0.07***					0.05**	0.05**	0.04**	0.01**		0.03***		0.17
Thousand seed weight, g	0.79	0.16***	0.64***	0.07***												0.13
Thousand seed density, g mL^{-1}	0.53	0.03***	0.83***	0.03***		0.02***						0.01**				0.08
Corneous endosperm	0.72	0.25***	0.19*	0.09**	0.02**							0.07***				0.38
Grain starch, g kg^{-1}	0.70	0.16***	0.47***	0.08***		0.02***									0.03***	0.25
Grain fat, g kg^{-1}	0.80	0.26***	0.37***	0.09***								0.06***			0.03***	0.19
Grain crude protein, g kg^{-1}	0.80	0.14***	0.67***	0.05***		0.01**								0.01***	0.01**	0.12
Grain moisture NIRS, g kg^{-1}	0.70	0.13***	0.11	0		0.02*						0.37***		0.03**		0.34
Grain phosphorus, g kg^{-1}	0.78	0.27***	0.27***	0.12***								0.04***		0.04***	0.05***	0.22
Grain ADF, g kg^{-1}	0.89	0.54***	0.10***	0.07***		0.01*									0.03***	0.25
Glumes retained after threshing, %	0.43	0.04***	0.60***	0.05***			0.02**	0.03**				0.04***				0.18
Stand density	0.43	0.06***	0.47*	0.09***						0.04**	0.06***					0.27
Tillering	0.54	0.14***	0.17	0.11*	0.05***											0.52
Mean stem thickness	0.59	0.17***	0.16*	0.09***	0.02***	0.03**										0.52
Plant height, cm	0.83	0.36***	0.07	0.08***		0.04***				0.15***						0.29
Flowering time, days	0.68	0.21***	0.53***	0.12***												0.14

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†Variance component of each significant effect divided by total variance components. Genetic (G), environment (E), genetic × environment (G × E), and main effects were retained regardless of significance. May not sum to 1 due to rounding. Rep., replication; Har. date, harvest date; HPLC, high performance liquid chromatography; NIRS, near infrared spectroscopy.

‡Effect is nested in environment (E).

(E) (i.e., location), and genetics × environment interaction (G × E) were used to calculate broad-sense heritability:

$$H^2 = \sigma_G^2 \Big/ \sigma_G^2 + \frac{\sigma_{G\times E}^2}{E} + \frac{\sigma_{error}^2}{ER} \quad [2]$$

where E is the number of environments and R is the number of replicates.

Data Correction Model, QTL, and Trait Correlation Analyses

Input for QTL and trait correlation analyses was obtained from residual values with a mixed model that corrected for the sources of nongenetic experimental error identified above (Table 2; i.e., harvest date).

$$\text{Trait}^{\star}_{\text{Line,RL}} = \text{Trait}_{\text{Line,RL}} - \overline{x_E} - \sum(\overline{x})_{ij} \quad [3]$$

where $\text{Trait}^{\star}_{\text{Line,RL}}$ is a residual trait value of an RIL replicate, $\text{Trait}_{\text{Line,RL}}$ is the observed trait value in an RIL replicate, $\overline{x_E}$ is the mean effect in each environment, and $\sum(\overline{x})_{ij}$ is the sum of all the other predicting effects (X) identified from the reduced model of Eq. [1] except the G and G × E interactions. The estimates of residuals ($\text{Trait}^{\star}_{\text{Line,RL}}$) from this model therefore contained only the G, G × E, and unexplained error variances. These residuals appeared to be near normally distributed in all traits. This approach was similar to statistical correction methodology used in microarray experiments (Wolfinger et al., 2001).

The corrected residuals ($\text{Trait}^{\star}_{\text{Line,RL}}$) were used as input for QTL analysis, after averaging across replicates within a location, and similarly for fitting a second model that partitioned variance explained by genetic effects, G and G × E, from the unexplained error variance.

$$\text{Trait}^{\star}_{\text{Line,RL}} = \text{error}_{\text{Line,RL}} + (\overline{x_G} + \overline{x_{GE}}) \quad [4]$$

To identify trait correlations across environments, the predicted, replicate trait values containing only G and G × E were used as input for SAS PROC CORR (SAS Institute, 2007) software. This was similar to using the corrected residuals replicate mean.

Single marker analysis and QTL interval and composite interval mapping were performed with Windows QTL Cartographer version 2.5 (WINQTL) (Wang et al., 2007). We used WINQTL settings RI1 for the cross type and 2 cM for the walk speed. For composite interval mapping, markers were selected as cofactors using a stepwise multiple regression with a 0.01 in/out probability and with a window size of 10 cM. A conservative permutation threshold at the 0.01 significance level was obtained for each trait using 1000 permutations. The QTL Fig. 1 was created using R (R Development Core Team, 2005). We should note that the difference in degrees of freedom after fitting the Eq. [3] model was trivial but may result in minor overestimation of QTL and correlation effects.

Genetic Map Construction

Leaf tissue was collected from all 176 RILs and the parents at the CS05 location. DNA was extracted from pooled tissue from four or more plants line^{-1} using a standard cetyltrimethyl ammonium bromide extraction protocol (Doyle and Doyle, 1987). A total of 300 markers was scored in this population, including 68 simple sequence repeat (SSR) and 222 amplified fragment length polymorphism (AFLP) markers (AFLP is a registered trademark of Keygene N.V., Wageningen, the Netherlands). Genotyping was performed according to Menz et al. (2002). Missing, nonparental, and heterozygous alleles were treated as missing data for map construction and QTL mapping. Mapmaker/Exp version 3.0b (Whitehead Institute, Cambridge,

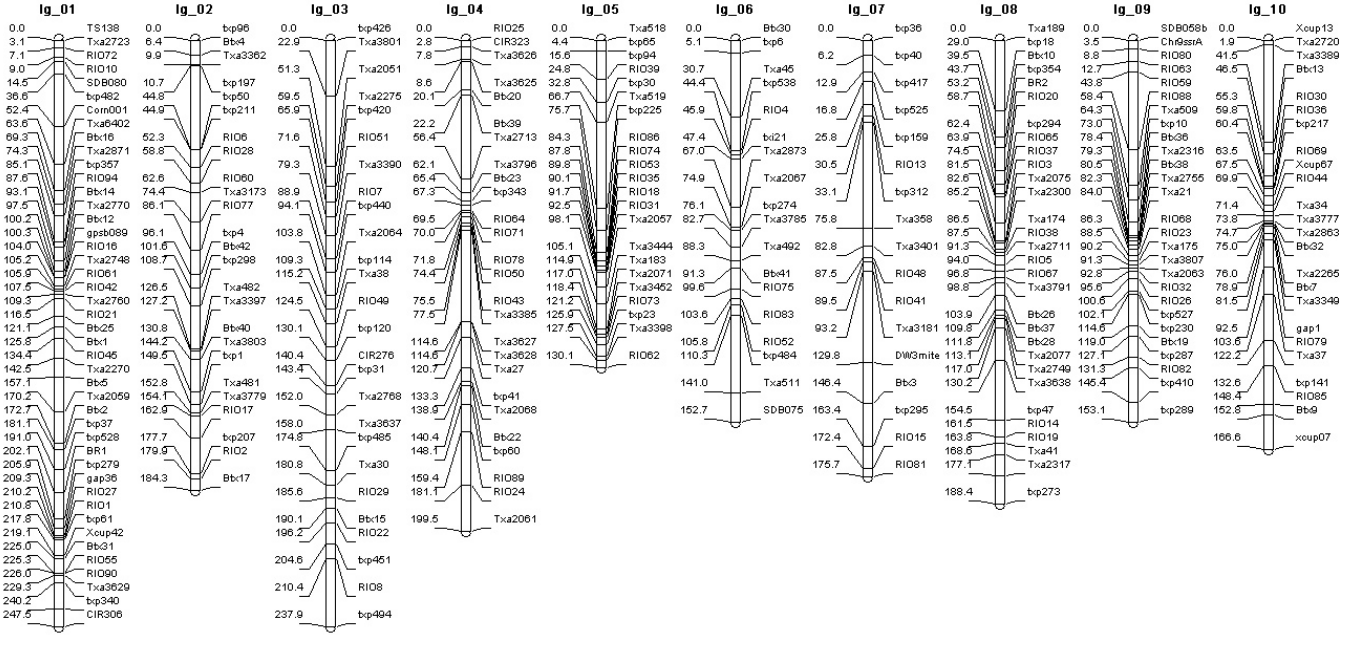


Figure 1. Genetic map derived from the Rio × BTx623 recombinant inbred line (RIL) population. The 10 sorghum chromosomes are named by convention of Kim et al. (2005) and displayed in the orientation of Menz et al. (2002). Marker names beginning with *txp*, *CIR*, *gap*, and *xcup* denote simple sequence repeats. The *Txa* markers are amplified fragment length polymorphisms (AFLPs) that were previously mapped in RIL population (BTx623 × IS3620c). Marker names beginning with *BTx* and *Rio* denote AFLPs that are the unique to our RIL population (Rio × BTx623). lg, linkage group.

MA), Kosambi centiMorgan function, was used to create the genetic map, and JoinMap 4 (Van Ooijen and Voorrips., 2001) was used to place previously unmapped AFLP markers. Linkage groups were assigned to chromosomes using the designations of Kim et al. (2005).

RESULTS

Phenotypic Data

Phenotypes of the RIL parents were as expected based on their selection history. Rio, the sweet sorghum parent, was a tall, late-flowering, high sugar content, and high juice producer that exhibited more secondary growth (increased tillering) than BTx623 (Table 1). BTx623, the grain sorghum parent, was shorter, earlier, and produced larger panicles with more seed than Rio. Overall, trait values for the RILs tended to be intermediate but transgressive segregation was observed for all traits (Table 1).

Sugar and grain yields varied between locations due to both experimental and biotic factors. For example, experimental variation in extraction efficiency of different cane presses (on average, 46% of the stem juice was extracted in WE05, 20% in CS05, and 51% in CS06) resulted in variation in first-press juice yield across locations (Table 1). Grain yield and its components were affected primarily by environment, which was optimal in WE05, and poor in CS05 and CS06 (i.e., damage from rain in CS05, and sorghum midge [*Stenodiplosis sorghicola* (Coquillett)] in both CS05 and CS06). Finally, latitudinal differences (Weslaco = 26.2° N lat, College Station = 30.6° N lat) led to longer flowering times in CS05 and CS06 for the slightly photoperiod-sensitive Rio parent (Table 1).

Variance Components and Error Sources

The relative contributions of genetic, environmental, and experimental causes of variation from calculated variance components are presented in Table 2. Evaluation in distinctly different environments resulted in environmental effects (E) accounting for the largest proportion of total phenotypic variance for many traits. Therefore, genetic effects (G) often explained a smaller, yet highly significant, proportion of the phenotypic variation. Genetic × environmental effects (G × E) were also highly significant for most traits including sugar yield, grain yield, and grain composition. Environmental effects on sugar yield were primarily due to locational differences in fresh stem biomass and sugar concentration. Environmental effects on grain yield and composition, on the other hand, were caused by variation in weather and midge damage between locations. Notably, E and G × E effects were markedly reduced for ADF, suggesting that this was the only grain composition trait unaffected by midge or weather. For some traits (e.g., stem juiciness or the sugar in dry stem; Table 2), location was not significant although it accounted for a large amount of variation. This result was due to other significant experimental effects in the model nested within location, which if removed, made the location effect highly significant but did not much alter the percentage of variation explained.

Of the other significant effects, within-location harvest date influenced almost half of the traits including both sugar and grain composition (Table 2). Strip date, or the number of days that elapsed between harvesting and stripping plants and subsequent processing, nested within harvest date and location had a significant effect on sugar composition because of increased degradation of sucrose to fructose and glucose over time. More importantly, total sugar yield, brix, sugar concentration, and juice yield were not affected by strip date. Within-location storage effects (subsamples were stored in the same box as they were removed from the drying oven) influenced many traits because of differences in sample residual moisture resulting from variation between oven-drying cycles. As might be expected, sample processing dates had significant impacts on experimental values of both sugar (HPLC dilution dates) and grain (grain grind and NIRS dates) composition traits. Although grain samples were assayed over a 1-wk period, the NIRS date was highly significant for composition traits, moisture, starch, fat, protein, ADF, and phosphorus. This variation is likely the result of fluctuations in room temperature and humidity affecting the instrument rather than physiological changes in the sample. Over all traits, the within-location border effect was fairly minimal. Accounting for significant experimental error effects reduced the error variance for all traits, and correcting the raw data for significant error sources modestly increased trait normality, heritability, and QTL detection (see below).

Trait Heritability

In general, the broad-sense heritability of measured traits was fairly high (Table 2). As has been reported by other authors, height, flowering time, and thousand seed weight had very high heritability values (Brown et al., 2006; Ritter, 2007). For many of the calculated traits (e.g., stem juiciness, sugar concentration in dry stems, and total sugar), multiplicative error led to a lower heritability than for traits that were measured directly. Also, lower heritability was observed for HPLC-measured sugar composition traits in general (juice glucose, fructose, and sucrose) than for brix, although HPLC results were highly repeatable. This apparent inconsistency was due to degradation of the HPLC samples before analysis. Brix measurements were collected from juice samples soon after pressing. Samples were then placed on ice and later frozen at –20°C for transport to the HPLC laboratory. Equal heritabilities of sugars measured by HPLC and brix would have been expected had juice samples been frozen in liquid nitrogen immediately after pressing (Ritter 2007).

Trait Correlations

Overall, correlations among sugar composition and yield traits were highly significant and appropriate with respect to sign (Table 3). For example, brix was correlated with increased juice sucrose, total juice sugars, and sugar yield traits (correlation coefficients were positive). Total sugar yield was only moderately correlated with brix but had very high positive correlations with stem water weight and stem sugar yield. Therefore, juice yield had a larger influence than sugar concentration in determining total sugar yield. Sugar traits, in general, exhibited low to moderate negative correlations with grain yield and grain starch content.

Grain production measurements are presented both as panicle fresh yield (harvest weight of panicles and associated stems) and dry grain yield (weight of grain after panicles were dried and threshed corrected for moisture content estimated by NIRS). Our results showed that the minor grain moisture variation was correlated with both grain composition and yield (Table 3), suggesting that the chemical composition of grain affects moisture retention. Grain composition was measured as percent grain starch, protein, fat, ADF, and phosphorus. These combined traits explained nearly 100% of grain sample weight, with a small remainder consisting of ash and/or error. Starch, the most important feedstock for ethanol, was the major component of grain weight. Starch had a low positive correlation with thousand seed weight and density and a strong positive correlation with grain yield. Because grain composition measurements are reported on a percentage basis, it was not surprising that starch had negative correlations with all other composition traits (Table 3).

The ratio of corneous to floury endosperm and percentage of glumes retained after threshing were investigated for relationships with composition. Corneous endosperm was moderately negatively correlated with ADF and positively correlated with the amount of protein. Glumes, being mostly fiber, were expected to be positively correlated with ADF but our results showed a small negative correlation, likely because glumes constitute a very small component of the seed sample weight. In general, increased grain starch composition for biofuel had small negative correlations with improved stem sugar concentration and yield. Grain yield had low negative correlations with stem sugar yield and moderately negative correlations with stem sugar concentration. Therefore, improved grain and stem sugar yield and composition appear to have only small physiological tradeoffs.

Table 3. Pearson correlation coefficients of genetic and genetic × environment effects.

Trait	Juice sugars	Juice glucose	Juice fructose	Juice sucrose	Total sugar yield	Sugar in dry stem	Juice yield first press	Stem fresh yield	Stem juiciness	Panicle fresh yield	Grain dry yield	Thousand seed weight	Thousand seed density
Brix	0.91***	−0.12***	−0.14***	0.88***	0.58***	0.59***	0.27***	0.34***	−0.57***	−0.37***	−0.38***	−0.2***	−0.05
Juice sugars	–	−0.05	−0.1**	0.95***	0.65***	0.65***	0.33***	0.38***	−0.57***	−0.36***	−0.37***	−0.2***	−0.05
Juice glucose		–	0.53***	−0.25***	0.04	−0.07*	0.04	0.07*	0.0–	−0.29***	−0.3***	−0.21***	−0.15***
Juice fructose			–	−0.32***	−0.06	−0.03	−0.0–	−0.04	0.07*	−0.08*	−0.11***	−0.13***	0
Juice sucrose				–	0.61***	0.62***	0.31***	0.36***	−0.55***	−0.29***	−0.29***	−0.15***	−0.03
Total sugar yield					–	0.51***	0.83***	0.92***	−0.38***	−0.21***	−0.28***	−0.12***	−0.08*
Sugar in dry stem						–	0.37***	0.3***	0.15***	−0.16***	−0.24***	−0.1**	−0.04
Juice yield first press							–	0.93***	−0.13***	−0.05	−0.14***	−0.02	−0.07*
Stem fresh yield								–	−0.27***	−0.11***	−0.19***	−0.08**	−0.07*
Stem juiciness									–	0.33***	0.25***	0.15***	0.02
Panicle fresh yield										–	0.93***	0.34***	0.1**
Grain dry yield											–	0.39***	0.1**
Thousand seed weight												–	0.04
Thousand seed density													–

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†NIRS, near infrared spectroscopy.

‡ADF, acid detergent fiber.

Table 3. Continued.

Trait	Corneous endosperm	Grain starch	Grain fat	Grain crude protein	Grain moisture NIRS†	Grain phosphorus	Grain ADF‡	Glumes retained after threshing	Stand density	Tillering	Mean stem thickness	Plant height	Flowering time
Brix	–0.04	–0.25***	–0.04	0.13***	0.0	0.11***	0.29***	–0.21***	0.0–	0.04	–0.03	0.37***	0.39***
Juice sugars	–0.05	–0.24***	–0.06*	0.12***	0.0	0.09**	0.26***	–0.17***	0.0–	0.05	–0.04	0.43***	0.4***
Juice glucose	–0.03	–0.25***	0.16***	0.25***	–0.18***	0.21***	0.07*	–0.08*	0.09**	0.07*	0.04	–0.02	0.16***
Juice fructose	0.14***	–0.0–	0.0–	0.05	–0.12***	–0.02	–0.17***	0.07*	–0.03	0	0.05	–0.06*	0.0–
Juice sucrose	–0.07*	–0.19***	–0.08*	0.06*	0.05	0.06	0.26***	–0.15***	0.02	0.05	–0.06*	0.42***	0.36***
Total sugar yield	0	–0.32***	–0.03	0.2***	–0.05	0.15***	0.24***	–0.02	0.12***	0.16***	0.04	0.75***	0.43***
Sugar in dry stem	0.04	–0.12***	–0.03	0.04	–0.05	–0.09**	0.04	–0.07*	–0.12***	–0.09**	0.11***	0.25***	0.34***
Juice yield first press	0	–0.24***	0.0–	0.17***	–0.04	0.11***	0.15***	0.05	0.13***	0.18***	0.05	0.77***	0.34***
Stem fresh yield	0	–0.3***	0	0.21***	–0.03	0.19***	0.22***	0.04	0.19***	0.23***	0.02	0.8***	0.35***
Stem juiciness	0.11***	0.2***	0.03	–0.11***	–0.05	–0.19***	–0.3***	0.1***	–0.13***	–0.18***	0.19***	–0.39***	–0.14***
Panicle fresh yield	–0.11***	0.57***	–0.05	–0.6***	0.36***	–0.5***	–0.19***	0.14***	0.12***	0.12***	–0.16***	–0.2***	–0.41***
Grain dry yield	–0.13***	0.67***	0	–0.66***	0.36***	–0.54***	–0.23***	0.1**	0.11***	0.11***	–0.21***	–0.2***	–0.46***
Thousand seed weight	–0.05	0.19***	0.09**	–0.12***	–0.02	–0.24***	–0.14***	0.17***	–0.14***	–0.22***	0.16***	–0.1**	–0.28***
Thousand seed density	0.11***	0.04	0.07*	–0.06*	0.07*	0.06	0.02	0.02	0.23***	0.15***	–0.2***	–0.15***	–0.15***
Corneous endosperm	–	0.0–	0.13***	0.34***	–0.41***	0.32***	–0.49***	0.15***	–0.26***	–0.29***	0.25***	0.03	0.14***
Grain starch		–	–0.07*	–0.79***	0.35***	–0.69***	–0.47***	–0.1***	0	0.05	–0.16***	–0.14***	–0.27***
Grain fat			–	0.26***	–0.38***	0.34***	–0.05	0.05	0.11***	0.15***	–0.18***	–0.07*	–0.05
Grain crude protein				–	–0.65***	0.84***	0.08**	0.0–	–0.13***	–0.18***	0.24***	0.07*	0.35***
Grain moisture NIRS					–	–0.35***	0.45***	–0.14***	0.22***	0.24***	–0.21***	0.04	–0.27***
Grain phosphorus						–	0.31***	–0.08**	0.05	0	0.06	0.05	0.25***
Grain ADF							–	–0.2***	0.36***	0.35***	–0.21***	0.13***	0.04
Glumes retained after threshing								–	–0.0–	0.0–	–0.09**	0.1**	–0.32***
Stand density									–	0.72***	–0.58***	0.05	–0.09**
Tillering										–	–0.75***	0.13***	–0.15***
Mean stem thickness											–	–0.02	0.33***
Plant height												–	0.29***
Flowering time													–

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†NIRS, near infrared spectroscopy.

‡ADF, acid detergent fiber.

Height and flowering time were highly correlated, as taller plants tended to flower later (Table 3). The effects of these two traits, most quantifiably height, were significantly correlated with most of the traits of interest for biofuel production. Taller plants had more stem biomass, more juice, higher stem sugar concentrations, and ultimately more sugar yield per hectare. Conversely, later-flowering, and therefore often taller plants had moderate correlations with lower grain yield, lower grain starch, higher protein, and phosphorus, though these grain traits must be carefully evaluated for bias due to midge grain damage in some locations. Flowering time, in particular, played an important role in reduced grain yield in midge-infested plots (CS05 and CS06) where establishment of sorghum midge on early-flowering lines led to large populations that were then able to overwhelm the lines that flowered later.

Increasing mean stem thickness, which was not correlated with height, had negative effects on grain yield and composition similar to height, but without concurrent improvements in sugar yield and concentration. Mean stem thickness also had very low correlations with stem juiciness and no correlation with first-press juice yield. The lack of correlation suggests larger stems do not hold more moisture and are not easier to press. Because stand density was tightly correlated with tillering, we could not adequately separate the two traits. In general, the effect of increasing stand density–tillering was minor but significant for improving sugar yield, grain yield, and overall grain composition for biofuel.

Genetic Mapping

The genetic map derived for our RIL population contained a total of 259 SSR and AFLP markers that were assembled into 10 linkage groups with good colinearity with a previously published map (BTx623 × IS3620C; Menz et al., 2002) (Fig. 1). The total genetic distance represented on the map was 1836 cM. In total, eight markers were not included in calculating the map distances, one marker was unlinked, and 32 AFLPs could be placed on to their respective chromosomes but the exact positions of these markers could not be determined.

Low residual heterozygosity was observed in the RILs and heterozygous alleles were treated as missing data for both genetic map construction and QTL mapping. There was very little marker segregation distortion (BTx623 parent average = 48.3%, min. = 32%, max. = 64%), the only exception being a single marker tightly linked with a major height QTL, dw_3 (Multani et al., 2003; Patrick J. Brown, personal communication, 2007) on chromosome 7 where only 14% of lines had the dwarf BTx623 allele.

QTL Mapping

Both interval mapping (IM) and composite interval mapping (CIM) consistently identified and mapped QTL at the same positions. Single marker analysis supported many of these QTL and also identified additional QTL that were not significant under the stringent permutation thresholds used for IM and CIM (Supplementary Table S1). Compared with IM, CIM detected more significant QTL effects with smaller genetic distance (1 and 2 likelihood of odds [LOD] intervals). Approximate QTL map positions (CIM, 2-LOD intervals) are presented for all locations in Fig. 2 and exact positions are shown in Supplementary Table S1. The majority of favorable alleles for QTL were derived from the expected parent. For example, BTx623 had positive QTL for grain yield while Rio had positive QTL for sugar traits. However, for most traits at least one positive QTL across locations was contributed by the unexpected parent. For example, on chromosome 1 (WE05), Rio had a positive allele for thousand seed weight and BTx623 had a positive allele for sugar yield. For many traits, QTL colocalized either within or between locations, especially for the major height and/or flowering time genes. Such colocalization of QTL across traits suggested a single gene pleiotropic effect, especially when the traits have obvious biological relationships. Genetic linkage of multiple genes, however, cannot be ruled out.

Quantitative trait loci colocalization clusters were observed on chromosomes 4, 6, 7, and 9. These corresponded to height, flowering time, or stand density–tillering QTL. The height and flowering time QTL at the proximal end of chromosome 9 colocalized with low grain yield and high stem sugar yield (WE05). In CS05, there was a much larger effect flowering time QTL on chromosome 6 that also colocalized with QTL having highly opposing effects between grain yield and stem sugar.

The QTL for brix and stem sugar concentration (total juice sugars) mapped to near identical locations on chromosome 3. Quantitative trait loci peaks on chromosome 3 were also observed for stem sugar in CS05 and CS06 and brix in CS06 but were not significant ($P > 0.01$). As would be expected based on heritability estimates (Table 2), the brix QTL had higher significance than total juice sugars and explained 25% of the variance for WE05 and 12% for CS05. Additional brix QTL were present in CS05 and the strongest of these, accounting for 14% of the variance, colocalized with the chromosome 7 height QTL, likely dw_3. Subsequent analysis for the brix QTL on chromosome 3 and dw_3 showed the highest significance for the interaction term, suggesting an epistatic interaction (data not shown). However, because only 14% of the population had the dw_3 allele, we lack power for a rigorous test of epistasis at this locus.

For grain yield in the midge-free environment, WE05, we observed multiple positive QTL from both parents. In the stressed environments, CS05 and CS06, only two QTL for grain yield were identified. Here, the major QTL allele on chromosome 6 for increased grain

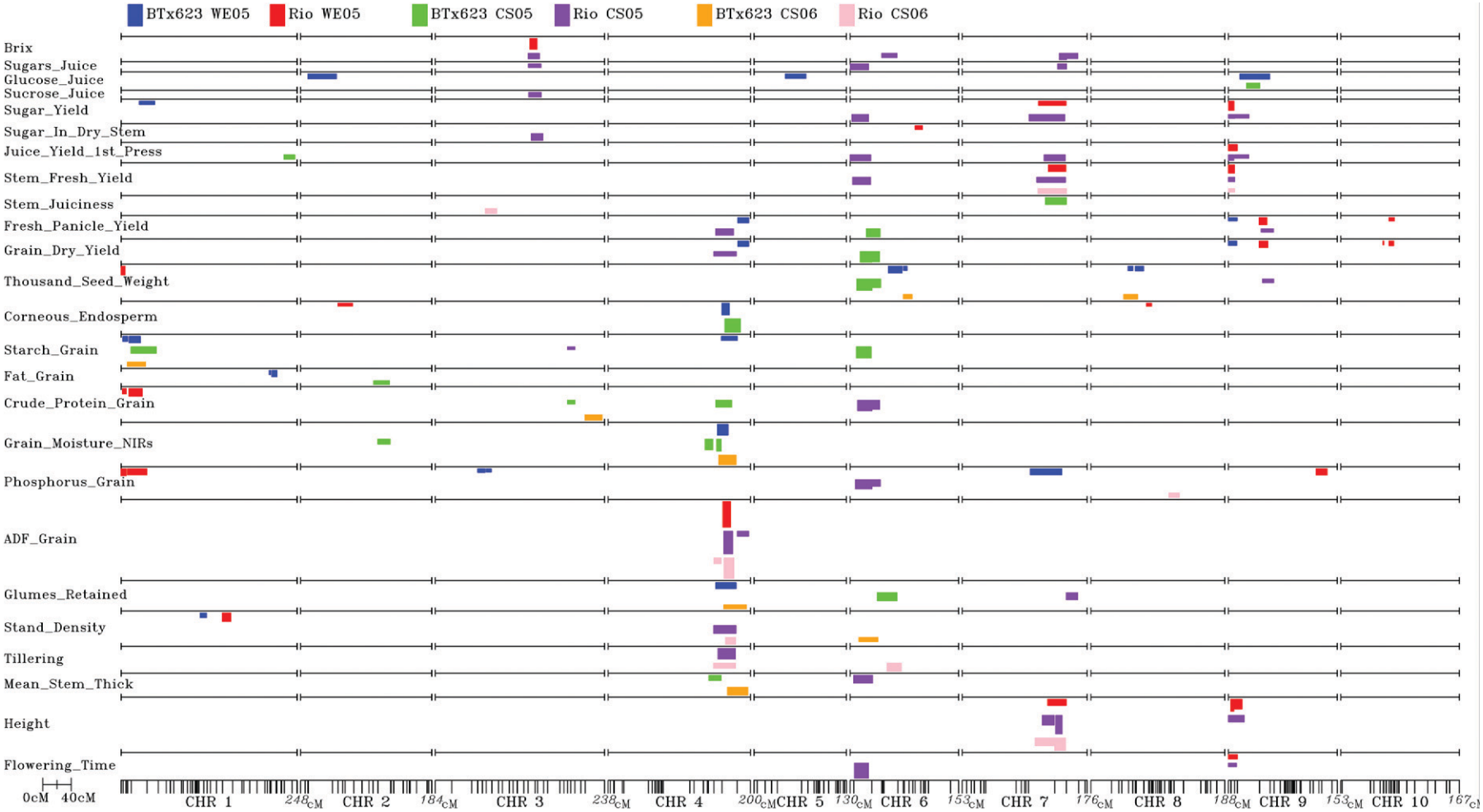


Figure 2. Map positions and strength of QTL effects from data collected at three locations in Texas: Weslaco, 2005 (WE05); College Station, 2005 (CS05); and College Station, 2006 (CS06). Colored bars represent QTL detected by composite interval mapping. Alleles that increased trait values inherited from BTx623 (grain parent) are shown in blue (WE05), green (CS05), and orange (CS06). Alleles that increased trait values inherited from Rio (sweet parent) are shown in red (WE05), purple (CS05), and pink (CS06). Trait names are shown on the left side of the figure. Marker positions are shown as vertical lines on the bottom above chromosome (CHR) numbers. The length of each bar represents the 2-likelihood of odds QTL interval, and height represents the variance (R^2) explained by the QTL. No QTL were detected for juice fructose or thousand seed weight, and QTL for other traits were not always detected in all locations. Exact QTL map positions are presented in Supplementary Table S1.

yield under stress originated from BTx623 (the grain sorghum parent) and colocalized with both increased grain starch and decreased flowering-time QTL. A minor QTL for increased grain yield under stress on chromosome 4 originated from Rio (the sweet sorghum parent) and colocalized with increased stem density–tillering. Another QTL on chromosome 1 for increased grain starch from BTx623 did not colocalize with grain yield but, oddly, did colocalize with a positive sugar yield QTL, also from the grain parent. This QTL on chromosome 1, in addition to the QTL on chromosome 3 for sugar concentration, would be good breeding targets for improved energy content without physiological tradeoffs.

DISCUSSION

Breeding Sorghum for Increased Sugar Yield

For BTx623, the "grain" parent of our RIL population, grain starch was the primary sink for nonstructural carbohydrates. Phenotypes of grain-related traits generally had higher heritability than those related to stem sugar composition or yield. Variation of total energy per hectare in grain was primarily associated with grain yield. Composition, primarily from increased starch content in grain, slightly increases ethanol yield and, importantly, increases ethanol fermentation efficiency (Wu et al., 2007). Thus, breeding to increase ethanol yields from grain should focus primarily on increasing grain yield, with increasing the proportion of starch in grain as an important secondary goal.

Total stem sugar yield per hectare is dependent on two traits, sugar concentration in the stem and stem juice yield per hectare. Increasing sugar concentration would be very valuable to increase energy density and reduce processing and transportation costs. However, sugar yield from sugarcane has increased almost exclusively by increasing crop biomass and stem juice yield rather than sugar concentration, perhaps because the concentration has been maximized at 62% of dry weight, or 25% of fresh weight (Moore and Maretzki, 1996; Jackson, 2005). Juice sugar concentrations in many elite sweet sorghum cultivars already reaches 20 to 25 brix, or 66 to 70% of stem dry weight (Murray, unpublished data, 2006). Furthermore, sorghum stem sugar concentration appears to be a primarily additive trait across genetic backgrounds, with no noticeable increases in hybrids (Clark, 1981; Rooney, unpublished data, 2005). Therefore, stem sugar concentration in sweet sorghum is unlikely to be significantly increased by breeding practices. However, stem sugar concentration in grain sorghum may be increased by introducing QTL alleles from sweet sorghum.

In our population, stem juice yield accounted for almost twice as much variation in stem sugar yield than sugar concentration and, therefore, may be a better initial target for improvement. This supposition is also supported by the fact that sugar yield QTL colocalized with juice yield and stem fresh weight but not with sugar concentration. Juice yield is a function of both stem juiciness (total stem water content/stem fresh weight) and stem fresh weight. Stem juiciness in our population differed little between the parents and had little genetic variance but a major QTL for low stem juiciness, or "dry stalk" (Bennetzen et al., 2001), suggests that sorghum harbors additional genetic variation that could be exploited. The other component of juice yield, stem fresh weight, was highly correlated with height and slightly correlated with stand density–tillering. Stem fresh yield has high genetic variation and heterosis potential in sorghum (Rooney, unpublished data, 2005). Therefore, stem sugar yield per hectare may be best improved in sweet sorghums by increasing stem fresh weight while maintaining maximum sugar concentration and stem juiciness. For grain sorghums, increasing stem fresh weight by increasing height may be undesirable. Because grain sorghums have not been selected for sugar traits, however, stem sugar concentration could be easily improved.

Tradeoffs between Grain and Stem Sugar Yield

In the environment that experienced no major biotic stress (WE05), only the proximal QTL on chromosome 9 exhibited a genetic basis for tradeoffs between stem sugar yield (~50% of the variation identified by all QTL; see Supplementary Table S2) and grain yield (~16% of the QTL variation). This tradeoff was offset by a closely linked locus contributed by the sweet sorghum parent that increased grain yield (representing ~23% of QTL variation). Under midge and rain stress (CS05), only the QTL on chromosome 6 exhibited a genetic tradeoff between stem sugar and grain yield QTL. These results suggest that stress created negative relationships between grain and stem sugar yields under standard, nonlimited agronomic practices. Consequently, breeders should be able to improve grain starch and stem sugar simultaneously in both grain and sweet sorghum types, but tradeoffs will increase with stress. The feasibility of concurrent improvement of grain and sugar yields is supported by other studies. Lingle (1987) found sink (in this case, energy stored in grain starch) but not source (photosynthetic) limitations in crops grown in nonlimited environments. This study concluded that the developing grain is not a significant sink for whole-plant carbohydrates. Other studies (Wu and Birch, 2007) have found that transforming sugarcane to produce a second sugar (an additional sink), isomaltulose, in addition to sucrose, nearly doubled the total sugar concentrations in harvested juice. Results from both studies imply that sinks operate independently and it is possible to increase a plant's ability to store photosynthates. In the past, cheap energy and a lack of infrastructure allowed growers to focus on

harvesting a single product (i.e., grain, stem sugar, or forage). Plants may be most efficient at producing energy, however, if there are a number of different sinks for storing nonstructural carbohydrates throughout the growing season, especially under ideal agronomic conditions.

Colocalization with QTL from Other Studies

In QTL analyses, only loci with alleles that differ between the parents of the study population can be identified and mapped. Here, we evaluated a population derived from a low sugar-accumulating grain sorghum (~12.6 brix and low juice volume) crossed to a very high sugar-producing sweet sorghum (~20 brix and high juice volume). Therefore, we were likely to find major QTL for high sugar accumulation. Three other studies have identified QTL for sugar concentration in sorghum. Natoli et al. (2002) used an F_2 population derived from two sweet sorghum parents (brix of 15.4 and 15.9) to map QTL that differed between high sugar types. Ritter (2007) used a RIL population derived from a very low sugar grain sorghum parent (~6.6 brix) crossed to a low-sugar (~12.1 brix) photoperiod-insensitive dwarf grain variety of Rio (not selected for high sugar) to identify QTL for stem sugar in grain sorghum. Finally, Bian et al. (2006) identified brix QTL in an F_3 population derived from a sweet sorghum and a grain inbred line but did not report sugar values.

Given differences in populations, locations, and measurements, we did not expect high QTL colocalization between these studies but found a number of strong similarities. As in our study, Natoli et al. (2002) identified and mapped a major QTL for brix to the middle of chromosome 3. In Ritter (2007) it is likely that an unassigned linkage group containing the largest height, sugar, and flowering time QTL corresponds to QTL near the telomere of the long arm of chromosome 9 in our study. No brix QTL were shared with Bian et al. (2006). Both Natoli et al. (2002) and Ritter (2007) detected height and sugar yield QTL on chromosome 5 that were not identified in this study, possibly because BTx623 carried the same alleles as Rio in this region.

To date there have been few published molecular genetic studies of grain yield or composition in sorghum. Rami et al. (1998) evaluated two sorghum mapping populations for grain yield and quality traits, and found little colocalization between QTL except for a few major height genes which affected many traits, findings similar to our results. There were also several QTL identified by Rami et al. (1998) that colocalized with our study, specifically a grain protein QTL on chromosome 1, a fat and thousand seed weight QTL on chromosome 1, and a corneous grain starch QTL on chromosome 2. There may also be a common grain yield QTL on chromosome 10 with Ritter (2007), but in our study the allele from the Rio parent had an opposite effect. The QTL on chromosome 10 reported by Ritter (2007) also colocalized with increased dry matter and stem sugar (though it slightly decreased sugar concentration). Thus, Ritter (2007) found no QTL tradeoffs between grain yield and stem sugar yield, indicating that differences in height caused tradeoffs under nonstress conditions.

The height QTL identified on chromosome 7 of this study has been detected in other studies (Rami et al., 1998; Brown et al., 2006) and is likely dw_3 (Pereira and Lee, 1995; Multani et al., 2003). The height and flowering time QTL identified on chromosome 9 was also detected by Pereira and Lee (1995) and by Lin et al. (1995). The QTL effects identified on chromosome 1 are consistent with flowering time QTL from Crasta et al. (1999), Ritter (2007), and Natoli et al. (2002). The major flowering time QTL on chromosome 6 in CS05 was reported by Lin et al. (1995) as ma_1, and was also detected by Rami et al. (1998) and Brown et al. (2006). Ma_1 is known to be regulated by photoperiod and the fact that we do not detect this large QTL in WE05 can be explained by latitudinal differences between locations (Quinby and Karper, 1945; Lin et al., 1995).

Limits of QTL Studies

Population size, trait heritability, and recombination all affect the ability to accurately detect QTL (Beavis et al., 1994; Kearsey and Farquhar, 1998; Collard et al., 2005). Given the large amount of sample processing and the cost of phenotyping, the population size evaluated for this experiment was as large as feasible. There are two other major limitations to identifying QTL in biparental RIL populations. First, only two alleles, at most, can be evaluated. We assume the parental lines adequately represent respective grain and stem sugar sorghum types and, judging from the results of Natoli et al. (2002) and Ritter (2007), this assumption seems reasonable. However, more sweet and grain sorghums need to be investigated. Second, elite varieties in the developed world are usually grown in hybrid combinations that rely on significant dominance effects. Dominance effects cannot be evaluated in a study of homozygous RILs, but additive effects can be identified and are of more universal value for crop improvement. Although stem sugar concentration (as indicated by brix and total juice sugar) appears to be additive, the dominance heterosis in hybrids for biomass, juice volume, and grain yield can be up to 150%, raising total sugar yields and grain yields significantly (Rooney, unpublished data, 2005). It is possible that heterosis could affect the relationships between nonstructural carbohydrates identified in this study.

CONCLUSIONS

To our knowledge, this study is the first to investigate the genetics of tradeoffs between grain starch and stem sugar production in sorghum. By measuring many traits concurrently, we indentified QTL clusters where colocalization

of sugar and grain QTL are likely due to changes in plant architecture (height, flowering time, stand density–tillering). Results suggested that increases in plant sinks may increase total energy production potential, especially in nonstressed growing environments. This work represents only a first step in understanding the genetics of carbohydrate accumulation and partitioning in sorghum. Future studies should (i) determine whether these findings are common in sorghum by surveying a larger number of parental alleles from other grain and sweet sorghum cultivars, (ii) investigate trait heterosis by evaluating populations as hybrids useful to growers, and (iii) use phenotypic assays that are less time consuming and costly by evaluating whole plants with NIRS calibrated for stem sugar and grain starch. These studies will ultimately allow genetic improvement of sorghum to maximize energy capture and storage for sustainable biofuel production.

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RESEARCH

Genetic Improvement of Sorghum as a Biofuel Feedstock: II. QTL for Stem and Leaf Structural Carbohydrates

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ABSTRACT

Digestion and fermentation of lignocellulosic biomass (i.e., structural carbohydrates) are predicted to deliver higher yields of energy per hectare than sugar and starch (nonstructural carbohydrates), yet little research on genetic variation in crop feedstock biomass traits has been conducted. We investigated the genetic basis of leaf and stem biomass yield and composition in a population derived from a high-biomass sweet sorghum, 'Rio', and a grain sorghum inbred line, 'BTx623', and compared these results with those from analyses of grain and stem sugar traits that we reported previously. Thirty-one traits were evaluated and a total of 110 quantitative trait loci (QTL) were identified across three locations. Many QTL for structural and nonstructural carbohydrate yields colocalized with loci for height, flowering time, and stand density–tillering. Quantitative trait loci for composition had little colocalization across tissues and environments. Separate genetic control for leaf and stem structural carbohydrate composition was identified, as well as separate genetic control of protein accumulation in leaf, stem, and grain. To maximize energy yields from grain and dedicated biomass sorghums, results suggest yield traits should be targeted for improvement before composition traits.

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Abbreviations: ADF, acid detergent fiber; CIM, composite interval mapping; IM, interval mapping; LOD, likelihood of odds; NDF, neutral detergent fiber; NIRS, near infrared spectroscopy; QTL, quantitative trait locus (loci); RIL, recombinant inbred line; SSR, simple sequence repeat; WINQTL, Windows QTL Cartographer.

Production of biofuels from plant structural carbohydrates (the cellulose, hemicellulose, and lignin-containing portion of stem, leaf, and root tissue) is predicted to yield five times more net energy per unit land area than using grain starch and sugar while producing only a quarter of the greenhouse gases (Farrell et al., 2006; USDOE, 2006; Somerville, 2007). These predictions focus on the C_4 grasses such as maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], sugarcane (*Saccharum officinarum* L.), miscanthus (*Miscanthus* spp.), and switchgrass (*Panicum virgatum* L.) that can efficiently produce high yields of structural carbohydrates in biomass. Structural carbohydrates, specifically cellulose, may derive either from crop residue, the byproduct of crops bred and harvested primarily for grain or stem sugar, or from dedicated biomass crops bred primarily for production of structural carbohydrates. Competition with food streams can therefore be minimized if crop residue is used as an ethanol feedstock or if dedicated biomass crops are grown on marginal land. These approaches may

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still, however, conflict with goals for wisely managed soil and wildlife conservation, and the need for cautious development of sustainable technologies for dedicated biomass production remains (Lal, 2005; Bies, 2006).

Currently, progress in improving crops for structural carbohydrate production lags behind advancement in biofuel production technologies. Thus, the first lignocellulose processing plants will likely use both crop residues and feedstocks that have not been improved for biomass yield or composition traits. To attain maximal efficiency, however, the system will eventually require feedstocks that have been selected for various compositional characteristics (i.e., high cellulose and low lignin content). Competing feedstock conversion technologies such as acid hydrolysis, enzymatic hydrolosis, thermochemical methods (syngas), and direct combustion will require both different feedstock traits and economic considerations for optimal system performance (Hamelinck et al., 2005). For example, the degree of feedstock cellulose polymerization, crystallinity, surface area, lignin content, and protein content will affect end sugar yield, although these effects have yet to be quantified (Hamelinck et al., 2005; USDOE, 2006; Somerville, 2007). Genetic variation for improving these traits likely exists, but the tremendous expense of directly measuring cellulose quality and quantity within a crop species makes assessment economically impractical.

As a starting point for advancing biomass quality, we should first consider forage and silage crops for which structural carbohydrates have already been the focus of improvement and economical analysis methods are currently available. Goals shared between forage and biomass feedstock improvement include yield, resistance to lodging, regrowth potential (perennial habit), high cellulose content, and nutrient use efficiency. An important difference, however, is that in forage crops, high protein and mineral content is necessary for animal feed but undesirable in a biofuel feedstock (Jenkins et al., 1998; Casler and Vogel, 1999; Wu et al., 2007). Proteins reduce cellulose digestibility and fermentation efficiency during ethanol production and create air pollution in systems that use direct combustion. Minerals, on the other hand, can foul processing equipment (Jenkins et al., 1998).

Because of its drought tolerance, nutrient use efficiency, and ability to adapt to a variety of environments, the C_4 grass sorghum is a promising crop for biofuel production (Rooney et al., 2007). In the only published common garden studies for dry biomass yield, sweet and forage sorghums out-yielded maize, switchgrass, reed canarygrass (*Phalaris arundinacea* L.), big bluestem (*Andropogon gerardii* var. *gerardii* Vitman), and alfalfa (*Medicago sativa* L.) for dry biomass, especially under low-input regimes (Anderson et al., 1995; Hallam et al., 2001). Grain, sweet, and forage type sorghums are all compatible with current agricultural and ethanol production systems, allowing sorghum to function as an improved feedstock crop residue or as a dedicated biomass crop.

We have previously identified quantitative trait loci (QTL) for grain and stem sugar composition and yield, and results indicated that overall energy yields could be increased by concurrent improvement for both sorghum grain and sugar traits (Murray et al., 2008). In this study, we identified lignocellulosic leaf and stem structural biomass yield, composition, and regrowth QTL that could be used to improve sorghum as a biomass feedstock. We were also interested in exploring the relationships between (i) whole-plant nonstructural and structural carbohydrate yields; (ii) leaf and stem structural carbohydrate composition; and (iii) protein levels in stems, leaves, and grain. Finally, we address the question of whether genetic improvement of sorghum should be focused on residue or on dedicated structural biomass production.

MATERIALS AND METHODS

Plant Material

A recombinant inbred line (RIL) population consisting of 176 $F_{4:5}$ lines was developed from a cross between 'BTx623' (a grain sorghum inbred line; Frederiksen and Miller, 1972) and 'Rio' (a high-biomass inbred sweet sorghum cultivar; Broadhead, 1972). Two replicates of RILs were planted during the normal summer growing season in 2005 in Weslaco, TX (WE05), and College Station, TX (CS05). In 2006, two replicates of 167 $F_{5:6}$ RILs were planted in College Station (CS06) from seed harvested in CS05. Two replicates of 3.05-m rows were planted in a randomized complete block design in each location. Environmental conditions of photoperiod, wind, and moisture between Weslaco and College Station locations were very different (Murray et al., 2008). Through the growing season, rainfall was 2 cm in WE05, 43 cm in CS05 primarily through flowering, and 36 cm in CS06.

Field Measurements

Plant height was measured either in the field (WE05) or, due to lodging, at the time of stripping (i.e., division of plants into panicles, stem, and leaf components) (CS05, CS06). Stand density and tillering were visually assessed using a scale that ranged from 0 (no plants or tillers) to 10 (very dense main stalks, very dense tillering) in the harvested area of each row. Average stem thickness was estimated by visual assessment of the base node using a scale from 0 (thin) to 10 (very thick). Flowering time was measured as time from planting to 50% anthesis (WE05, CS05).

Biomass Measurements

Plant harvest was staggered across 16 d (WE05), 14 d (CS05), and 11 d (CS06) due to the volume of work and the logistics of labor and equipment. Harvest date, therefore, was recorded and used as a cofactor in later statistical analyses. For each row, plants were harvested from a randomly selected area (1 m in length) by cutting within 3 cm above the soil. Plants were bundled in clear plastic sheeting and taken to a central processing facility within

2 h of harvest. The bundled plants were stripped into panicles, stems, and leaves, and were weighed (panicle fresh yield, stem fresh yield, and leaf fresh yield, respectively). Although most plants were stripped on the same day, some were not processed for up to 3 d after harvest. Strip date, therefore, was also noted and used as a cofactor in subsequent analyses. Wet stem tissue was pressed to remove the juice on press date, either the same or next day as stripping. Brix, a measure of soluble solids that in sweet sorghums is composed mostly of sucrose, was measured with two different handheld refractometers (Atago U.S.A. Inc., Bellevue, WA) and averaged.

For each plot, random subsamples of panicles, leaves, and pressed stems were weighed. These were then dried for a few days in a greenhouse (WE05) or a grain drier set at 38°C (CS05, CS06). Dry stem and leaf subsamples were reweighed, and stem dry yield and leaf dry yield were calculated by dividing these values by wet subsample weight and multiplying by full wet sample weight. Stem dry harvest index was then calculated by dividing the dry stem yield by the sum of dry stem sugar, stem, leaf, and panicle yields. Leaf, panicle, and grain harvest indices were calculated in the same manner as above, substituting the appropriate tissues.

Regrowth Measurements

After harvest in CS06, full plots were subjected to uniform mowing and allowed to regrow to maturity. A second harvest of both replications was conducted in a single day. One meter of plants from each row was harvested, weighed (total biomass including grain), and pressed in a sorghum press to extract juice. Measurements on juice volume, brix, height, and maturity stage at harvest were recorded, in addition to wet biomass. This material was not analyzed further.

Stem and Leaf NIRS

For each sample, at least 40 g of dry stem tissue was cut and ground in a no. 8 Christy mill (Christy and Norris Ltd., Chelmsford, United Kingdom)with 2-mm screen. Approximately 15 g of dry leaf tissue, as well as the previously ground stem tissue, were processed separately in a UDY cyclone mill (UDY Corporation, Fort Collins, CO) using a 1-mm screen with a stainless steel grinding ring and an aluminum impeller. The ground tissue was stored in a redline zipper storage bag for 4 to 12 wk, then moved to the near infrared spectroscopy (NIRS) laboratory and acclimated for 3 wk before analysis. Scanning was done on a FOSS Model 5000 Feed and Forage Analyzer with 1/4-cup cells (NIRS Systems, Silver Spring, MD) and analyzed with WinISI II software (Infrasoft International, State College, PA). A total of 1051 leaf samples and 1050 stem samples from this population were analyzed by NIRS.

To obtain accurate data from NIRS, the system must be calibrated based on values obtained from chemical analyses in a subset of samples. Therefore, leaf and stem samples from each location were selected for chemical analysis to maximize the information content using the WinISI software. In all, 107 leaf samples (72 from the RIL population and 35 from diverse sweet and grain sorghum accessions grown in the same environment as the RILs) and 168 stem samples (82 RILs and 86 diverse sorghums) were analyzed. Amounts of acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, crude protein, and dry matter in stems and leaves were measured by Dairy One (Ithaca, NY) with the ANKOM A200 Filter Bag Technique (ANKOM Technology, Macedon, NY) (Vogel et al., 1999). Stems and leaves were dried in a 135°C oven for 2 h, and amounts of crude protein (Association of Official Analytical Chemists, 1990b) and dry matter (Association of Official Analytical Chemists, 1990a) in each tissue were also measured by Dairy One. Neutral detergent fiber is a measure of three structural carbohydrates: cellulose, hemicellulose, and lignin. Acid detergent fiber is a measure of cellulose and lignin only (Theander and Westerlund, 1993). Thus, cellulose was calculated as ADF minus lignin, and hemicellulose was calculated as NDF minus ADF.

For each trait, the selected samples (107 leaf or 168 stem) were randomly assigned to one of two groups. The calibration set comprised two-thirds of the samples, and experimental values from these were used to establish the best calibration equations. The second group (validation set) consisted of the remaining one-third of the samples. Equations developed from the calibration set data were evaluated for their ability to correctly predict trait values in the validation set.

Twenty-eight different equation treatments, each testing different wavelengths, math treatments, and the use of the repeatability file, were applied to data from the calibration set. For each trait, the treatment that maximized the prediction of appropriate values in the validation set, as evidenced by low standard error and high R^2, was retained. This process was repeated a total of three times, each with different randomized calibration and validation sets. The best three equations (one from each repetition) were then evaluated in the full sample subset (107 leaf or 168 stem samples) and the best of these equations was used to predict trait values of all samples (1051 leaf or 1050 stem samples).

The best calibration equations are reported for each trait in Supplementary Table S1. Criteria for selecting the best equation included low calibration standard error, low cross-validation standard error, high R^2, high heritability, and adequate ability to predict QTL (see below). The equations derived from each repetition were quite similar.

Statistical Analyses

For each trait, we identified significant experimental effects (error) and their variance components, corrected the data for any confounding nongenetic effects, and performed correlation and QTL analyses (Murray et al., 2008). We first analyzed the impact of genotype, environment, genotype × environment interaction, and nongenetic effects such as harvest date and others (see Table 2 for a complete list of nongenetic effects) on trait data in a mixed model. WE05, CS05, and CS06 were considered as different environments and all variables were treated as random except genotype. Genotype was fixed so that inferences on effects would be appropriate for later data correction and analyses in which values were obtained for individual RILs. SAS PROC MIXED (SAS Institute, 2007) software was used to test all experimental effects in the column headings of Table 2 for significance. Only effects deemed significant ($P = 0.05$) by Type III sums of squares were retained in the reduced model (main effects of significant interactions were also retained irrespective of significance). From the reduced model, variance

components were obtained using Type III sums of squares, with genotype considered a random effect for appropriate population inferences. Variance components were then used to calculate broad-sense heritability (Murray et al., 2008). We used Type III sums of squares so that the order of effects would not influence the results.

Next, we corrected the trait data for nongenetic sources of experimental error. To accomplish this, the reduced model, determined above, was further reduced by removing the variable terms for genetic and genetic × environment interaction from the model. The residuals from this new model (containing only genetic, genetic × environment, and unexplainable error) appeared to be fairly normally distributed for all traits (data not shown). These residuals were then used as input for correlation and QTL analysis. This statistical approach was similar to the "two-step model" correction methodology used in microarray experiments (Wolfinger et al., 2001).

For correlation analysis, the data (residuals from above) were used to fit a simple model with genetic and genetic × environment terms only. The predicted trait values separated genetic effects (including genetic × environment interactions) from error and were used as input for SAS PROC CORR (SAS Institute, 2007) software. These correlations should be interpreted as genetic correlations within environments averaged over all environments. These measurements were determined to be most useful for comparing genetic tradeoffs across locations (environments) with results from the QTL analysis.

Single marker analysis and QTL interval mapping (IM) and composite interval mapping (CIM) were performed with Windows QTL Cartographer version 2.5 (WINQTL) (Wang et al., 2007). Genetic data and the genetic map were identical to those presented in Murray et al. (2008). We used WINQTL settings RI1 for the cross type and 2 cM for the walk speed. For QTL identification, forward and backward regression with 0.01 in/out probability and a 10-cM window size was used. A conservative permutation threshold at the 0.01 significance level was obtained for each trait using 1000 permutations. Resulting QTL maps were created using R (R Development Core Team, 2005).

RESULTS

Phenotypic Data

A total of 31 structural carbohydrate and related traits were measured or calculated (Table 1 and Supplementary Table S1). Across all locations, Rio was tall, late flowering, had high leaf–stem biomass, tillered and produced more secondary growth than BTx623 (Table 1). BTx623 was dwarf, early flowering, had much higher panicle and grain harvest indices, and slightly higher leaf harvest indices. Transgressive segregation was observed in the RILs for all traits (Table 1). Correcting raw values of all traits for significant nongenetic sources of error modestly increased observed trait normality and heritability.

Biomass Yield

High dry matter content at harvest increases energy density which, in turn, results in decreased harvest, transport, and drying costs. Total biomass dry matter content averaged 62% of fresh yield in WE05, 68% in CS05, and 66% in CS06 (data not shown). Considering the arid conditions at WE05 and the abundance of rain in the CS05 and CS06 locations, the similarity of dry matter content between the Weslaco and College Station locations was surprising. In all locations, dry matter content was lowest in the juicy stem for all lines. Heritability values for fresh stem, fresh panicle, and total fresh biomass yields were higher than the corresponding values for dry material (Table 2). This result was likely due to multiplicative error associated with calculating dry component heritabilities and differences in sample residual moisture. Heritabilities for stem, leaf, and panicle harvest indices were even higher than the dry yields and roughly equivalent to the grain harvest index heritability. This result suggests that the harvest indices of structural components may be as useful as grain harvest index for targeted selection.

Dry structural stem yields (not including stem sugar) were higher than leaf yield by an average of 10% in WE05, 12% in CS05, and 17% in CS06 RILs (Table 1). Stem composition, therefore, contributed more to total plant biomass than did leaf composition, and this effect was more pronounced in taller plants having much greater stem harvest indices. Stem yield also had a higher proportion of genetic variance than leaf yield (fresh stem 20% vs. fresh leaf yield 8%; dry stem 21% vs. dry leaf yield 12%; 19% stem harvest index vs. 15% leaf harvest index).

Stem and Leaf Composition

Near infrared spectroscopy calibration equations generally performed well (Supplementary Table S1) and resulted in high heritabilities across stem–leaf composition traits in this population (Table 2). Protein had the best fitting NIRS prediction equations while moisture and, as might be expected, lignin (a complex heterogeneous biopolymer) had the lowest. Poor performance of NIRS equations for moisture was unexpected, though this outcome could have been at least partially due to low moisture variation between samples. It also seems plausible that the high temperature used to evaluate dried sample residual moisture (135°C) may have affected molecules other than water (i.e., residual sugars). Because levels of moisture and moisture variation were low, the effect of poor calibration did not substantially impact other NIRs measurements performed in the study.

Transgressive segregation of progeny (Table 1) and genetic variance (Table 2) were lower for biomass composition traits than for yield. In leaf tissue, BTx623 had higher levels of lignin, cellulose, and protein, while Rio had much higher amounts of hemicellulose. Combined, the measurements consistently explained ~70% of the total leaf composition across all lines and locations; the remaining 30% was expected to comprise nonstructural carbohydrates (starch

Table 1. Trait values for Rio × BTx623 parental and recombinant inbred lines (RILs) at three locations in Texas: Weslaco, 2005 (WE05); College Station, 2005 (CS05); and College Station, 2006 (CS06).

Trait	WE05				CS05				CS06			
			RILs				RILs				RILs	
	Rio	BTx623	Mean (SD)†	Min. –Max.	Rio	BTx623	Mean (SD)†	Min. –Max.	Rio	BTx623	Mean (SD)†	Min. –Max.
Fresh biomass yield												
Fresh total biomass yield, t ha^{-1}	36.8	28	37.2 (7.5)	16.4–70.9	90.2	23.5	55.8 (16)	18.7–103	70.7	33.7	48.5 (13.3)	10.9–96.9
Fresh stem yield, t ha^{-1}	23.3	10.3	21.9 (6)	6.2–48.1	65.5	13.2	36 (12)	6.5–79.3	53.1	16.8	32.5 (10)	8.5–69.3
Fresh leaf yield, t ha^{-1}	9.3	10.4	9.8 (2.2)	4.1–18.6	21.1	6	15.9 (4.9)	4–32.5	12.8	9.8	10.6 (3.1)	1.6–20.1
Fresh panicle yield, t ha^{-1}	4.2	7.3	5.5 (1.1)	2.6–9.8	3.6	4.3	3.8 (1.7)	0.4–11.4	2.3	6.5	4.3 (1.7)	0.9–11.3
Dry biomass yield												
Dry total biomass yield, t ha^{-1}	14.1	13.4	14.1 (2.5)	7–24.2	31.4	6.5	17.6 (4.9)	5.5–31.9	24.2	12.4	16.3 (4.4)	3.9–31.4
Dry stem structural yield, t ha^{-1}	5.1	1.9	4.5 (1.3)	1.2–9.4	15.7	2.3	6.9 (2.4)	1.3–14.7	12.4	3.1	6.3 (2.1)	1.6–13.9
Dry leaf yield, t ha^{-1}	2.7	2.6	2.9 (0.7)	1.3–5.2	5.6	1.5	4.5 (1.4)	1.1–9.6	4.6	2.7	3.4 (1)	0.7–6.4
Dry panicle yield, t ha^{-1}	3.4	6	4.5 (1)	0–7.9	2.9	2.5	2.6 (1.5)	0–7.9	0.5	5.4	3.4 (1.4)	0–8.8
Dry harvest indices‡												
Stem dry harvest index	36	18	31 (5)	15–47	49	34	38 (5)	12–51	51	25	38 (5)	24–61
Leaf dry harvest index	19	24	21 (3)	12 35	18	19	26 (5)	13–46	19	22	21 (4)	14–46
Panicle dry harvest index	24	52	32 (8)	0–51	9	39	15 (9)	0–43	2	44	21 (8)	0–51
Grain dry harvest index	18	40	25 (6)	0–41	5	19	9 (7)	0–30	0	31	14 (7)	0–38
Stem composition												
Stem NDF, g kg^{-1}§	67.3	68.7	69.6 (3.8)	58.3–80.1	55	67.6	60.4 (3.4)	50.8–69.7	62.7	63.8	65.4 (3.6)	55.7–77.3
Stem cellulose, g kg^{-1}¶	36.9	39.3	38.7 (2.3)	31–45.7	29.1	37.9	32.4 (2.1)	26.8–38.5	34.3	35.7	36.1 (2.2)	30.5–43.3
Stem hemicellulose, g kg^{-1}¶	22.6	23.5	23.5 (1.4)	19.5–28	20.8	23.5	22.3 (1.3)	16.5–26.8	23.1	22.8	23.5 (1.1)	20.2–27.1
Stem lignin, g kg^{-1}	6.1	5.8	6.5 (0.7)	4.7–8.7	4.8	6.1	5.2 (0.7)	3.5–7.5	6.4	5.6	6.3 (0.6)	4.9–9.4
Stem crude protein, g kg^{-1}	3.7	4.7	4.2 (0.5)	3–6.4	4.2	5.7	4.5 (0.7)	2.6–7.3	3.7	4.4	3.6 (0.6)	2.4–5.9
Leaf composition												
Leaf NDF, g kg^{-1}	56.4	57.9	58.9 (2)	52.6–64.9	58	57.8	59.8 (2.6)	51.7–67	59	57.8	60.9 (2.3)	55.1–69.6
Leaf cellulose, g kg^{-1}¶	33	36.1	34.3 (1.9)	29.9–41.9	31.9	32.8	32.7 (2.5)	26.4–38.5	28.8	28.5	31.9 (2)	27.5–37.7
Leaf hemicellulose, g kg^{-1}#	21.7	18.6	20.8 (1.9)	14.9–25.9	24.1	18.4	22.8 (1.9)	16.5–27.3	26.1	24	24.4 (1.4)	19.1–27.7
Leaf lignin, g kg^{-1}	3.1	3.9	3.7 (0.4)	2.4–4.8	3.2	4.4	3.7 (0.5)	2.3–5.4	3.7	4.3	4.2 (0.5)	2.9–5.3
Leaf crude protein, g kg^{-1}	11.2	12.7	12 (1)	8.9–16.3	12.2	12.9	12.6 (1.2)	9.9–17.3	10.6	12.1	11.1 (1.1)	7.8–14.6
Other traits												
Stand density	7.5	6.5	7.7 (0.6)	4–9	6	4.5	5.3 (1.4)	1–8	7	7	6.6 (1.3)	2–9
Tillering	6.5	2.5	6.1 (1.5)	2–9	4.5	1.5	4.5 (1.7)	0–8	8	4	5.5 (1.7)	1–9
Mean stem thickness	2	6	3.4 (0.9)	1.5–7	5.8	5.8	4.3 (1)	2 7	4	5	3.5 (1)	1.5–7
Plant height, cm	210	130	200 (26)	130–274	273	119	227 (29)	119–297	227	123	204 (26)	109–259
Flowering time, days	116	109	111 (5)	104–123	180	161	168 (5)	157–185	na††	na††	na††	na††
Lodging, %	20	10	25 (15)	10–80	73	50	83 (16)	0–100	35	0	60 (34)	0–100

†Standard deviation in parentheses.

‡Harvest index (stem, leaf, grain, or stem sugar) = yield of (stem, leaf, grain, or stem sugar)/total biomass yield.

§NDF, neutral detergent fiber.

¶Cellulose = acid detergent fiber (ADF) – lignin.

#Hemicellulose = neutral detergent fiber (NDF) – ADF.

††Not assayed.

and sugar) and ash. Stem composition had higher heritability than leaf composition. In absolute values, stems had more lignin and cellulose and much less protein than leaves. In the stem, BTx623 had composition levels of NDF structural components equal to or higher than Rio due to lower residual nonstructural carbohydrates (stem sugar). Differences in residual sugar complicated comparisons of structural carbohydrate composition.

As shown in Table 2, sources of experimental variance influenced biomass composition more than yield traits.

Table 2. Trait heritability and variance component percentage attributable to genetic (G), environmental (E), genetic × environment interaction (G × E), and other significant experimental effects.

Trait	Heritability	Variance component†														
		G	E	G × E	(E)‡ Rep.	(E)§ Har. date	(E) Har. date × strip date	(E) Har. date × press date	(E) North border	(E) East border	(E) South border	(E) West border	(E) Storage box	Sample grind date	NIRS date	Residual error
Fresh yield																
Fresh total biomass yield, t ha^{-1}	0.65	0.16***	0.36***	0.05*												0.43
Fresh stem yield, t ha^{-1}	0.71	0.2***	0.39***	0.07***												0.34
Fresh leaf yield, t ha^{-1}	0.48	0.08***	0.42***	0.08***		0.05***										0.38
Fresh panicle yield, t ha^{-1}	0.65	0.18***	0.2	0.07**						0.11***						0.44
Dry yield																
Dry total biomass yield, t ha^{-1}	0.52	0.14***	0.16*	0.08**	0.01*											0.61
Dry stem structural yield, t ha^{-1}	0.68	0.21***	0.3***	0.12***												0.37
Dry leaf yield, t ha^{-1}	0.53	0.12***	0.26***	0.1***		0.07***										0.44
Dry panicle yield, t ha^{-1}	0.60	0.12***	0.38***	0.08***									0.04***	0.07***	0.01*	0.3
Dry harvest indices																
Stem dry harvest index	0.72	0.19***	0.35*	0.11***		0		0.07***		0.05*			0.02*			0.22
Leaf dry harvest index	0.65	0.15***	0.34***	0.02		0	0.07***									0.44
Panicle dry harvest index	0.73	0.2***	0.43***	0.11***		0	0.03**									0.22
Grain dry harvest index	0.70	0.16***	0.52***	0.1***		0	0.03**									0.2
Stem composition																
Stem NDF, g kg^{-1}	0.61	0.06***	0.61***	0.03**		0.06*		0.03**					0.02**			0.18
Stem cellulose, g kg^{-1}	0.60	0.06***	0.66***	0.03***		0.06*		0.03***								0.17
Stem hemicellulose, g kg^{-1}	0.50	0.09***	0.2*	0.05*		0.14*		0.05*					0.05**			0.42
Stem lignin, g kg^{-1}	0.75	0.11***	0.61***	0.03**		0.02**							0.03***		0.05***	0.16
Stem crude protein, g kg^{-1}	0.59	0.12***	0.34***	0.11***		0		0.07***					0.06***			0.29
Leaf composition																
Leaf NDF, g kg^{-1}	0.55	0.11***	0.16	0.07**	0.04***	0.07	0.15***									0.41
Leaf cellulose, g kg^{-1}	0.65	0.13***	0.1	0.03		0.19*	0.16***				0.04*					0.35
Leaf hemicellulose, g kg^{-1}	0.45	0.08***	0.24*	0.06*	0.03***	0.12***									0.01*	0.47
Leaf lignin, g kg^{-1}	0.74	0.18***	0.41***	0.06***		0.05	0.04***									0.27
Leaf crude protein, g kg^{-1}	0.66	0.1***	0.39**	0.04**	0.03***	0.12*	0.07***									0.24*
Other traits																
Stand density	0.43	0.06***	0.47*	0.09***							0.04**	0.06***				0.27
Tillering	0.54	0.14***	0.17	0.11*	0.05***											0.52
Mean stem thickness	0.59	0.17***	0.16*	0.09***	0.02***	0.03**										0.52
Plant height, cm	0.83	0.36***	0.07	0.08***		0.04***					0.15***					0.29
Flowering time, d	0.68	0.21***	0.53***	0.12***												0.14
Lodging, %	0.56	0.05***	0.58	0.04***	0**						0.14***					0.18

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†Variance component of each significant effect divided by total variance components. Genetic (G), environment (E), genetic × environment (G × E), and main effects were retained regardless of significance. May not sum to 1 due to rounding. Rep., replication; Har. date, harvest date; NIRS, near infrared spectroscopy.

‡Effect was nested within environment (E).

§Retained when nonsignificant due to higher-level interaction term(s).

For example, harvest date affected leaf and stem composition to a greater extent than biomass yield. This was due to the fact that whole plants were harvested within a 3-wk window, which contained the separate optimum harvest times for leaf and stem biomass, stem sugar, and grain. During this harvesting period, plant biomass reached and passed maturity. As plants matured, older leaves and stems began to show the effects of physiological age (senescence), weathering, and disease while grain matured and new tillers and axillary branches were produced. The date the plant was harvested and stripped also accounted for as much or more variation than genetics for leaf ADF, NDF, cellulose, and crude protein. The date NIRS was performed was less important for leaf and stem composition than for grain composition traits (Murray et al., 2008).

We tested for genetic correlations between composition traits in leaf and stem (Table 3). Correlations were low to moderate for cellulose and lignin and there was no correlation for either hemicellulose or protein, suggesting separate genetic controls for leaf and stem composition. Leaf and stem protein were both significantly negatively correlated with fresh and dry biomass production. However, leaf protein was positively correlated with measures of grain production (data not shown).

Other Traits

Fresh and dry biomass yield was highly correlated with plant height and, to a lesser extent, flowering time and stand density–tillering (Table 3). Specifically, height was highly correlated with increased stem biomass (and thus total biomass), moderately correlated with an increase in leaf biomass, and slightly correlated with a decrease in grain biomass, which highly altered harvest indices. These results contrast with those of Quinby and Karper (1954), who reported that the genes controlling height were brachytic, only affecting stem node elongation, and did not affect other traits (Morgan and Finlayson, 2001). Flowering time was correlated with height and had similar but less dramatic effects than height on leaf and stem yield. Stand density and tillering had slight positive correlations with biomass from all tissue with almost no change in harvest indices. Increasing mean stem thickness showed very low negative correlations with leaf and panicle yield with no significant correlation with stem yield. Unlike the yield traits, leaf and stem structural composition generally showed little correlation with height, flowering time, stand density–tillering, or mean stem thickness.

The conditions for lodging were extremely dependent on environment and were best evaluated in CS06. Because of this environmental dependence, trait correlation values, although highly significant, were fairly low. Increased lodging had some of the highest positive correlations with increased height, leaf cellulose, and, surprisingly, increased stem lignin; and the highest negative

Table 3. Pearson correlation coefficients for corrected trait data.

Trait	Fresh stem yield	Fresh leaf yield	Fresh panicle yield	Dry total biomass yield	Dry stem structural yield	Dry leaf yield	Dry panicle yield	Stem harvest index	Leaf harvest index	Panicle harvest index	Grain harvest index	Stem NDF	Stem cellulose	Stem hemicellulose
Fresh total biomass yield, t ha^{-1}	0.97***	0.77***	0	0.92***	0.91***	0.82***	−0.02	0.58***	−0.09**	−0.53***	−0.49***	0.04	0.04	−0.21***
Fresh stem yield, t ha^{-1}	–	0.64***	−0.11***	0.89***	0.95***	0.73***	−0.13***	0.7***	−0.2***	−0.61***	−0.57***	0.0	−0.02	−0.23***
Fresh leaf yield, t ha^{-1}		–	−0.06	0.69***	0.61***	0.93***	−0.08**	0.29***	0.42***	−0.44***	−0.41***	0.12***	0.11***	0.0
Fresh panicle yield, t ha^{-1}			–	0.14***	−0.18***	−0.14***	0.97***	−0.59***	−0.39***	0.76***	0.78***	0.05	0.12***	−0.19***
Dry total biomass yield, t ha^{-1}				–	0.9***	0.78***	0.12***	0.5***	−0.22***	−0.41***	−0.35***	0.0	0.0	−0.28***
Dry stem structural yield, t ha^{-1}					–	0.73***	−0.19***	0.78***	−0.21***	−0.66***	−0.61***	0	−0.02	−0.2***
Dry leaf yield, t ha^{-1}						–	−0.16***	0.42***	0.36***	−0.54***	−0.49***	0.1**	0.09**	−0.05
Dry panicle yield, t ha^{-1}							–	−0.58***	−0.4***	0.76***	0.79***	0.08*	0.15***	−0.17***
Stem dry harvest index								–	−0.15***	−0.88***	−0.84***	0.02	−0.02	−0.06
Leaf dry harvest index									–	−0.21***	−0.23***	0.16***	0.12***	0.34***
Panicle dry harvest index										–	0.98***	0.05	0.11***	0.0
Grain dry harvest index											–	0.04	0.11***	−0.02
Stem NDF, g kg^{-1}†												–	0.97***	0.72***
Stem cellulose, g kg^{-1}													–	0.64***

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
†NDF, neutral detergent fiber.

Table 3. Continued.

Trait	Stem lignin	Stem crude protein	Leaf NDF	Leaf cellulose	Leaf hemicellulose	Leaf lignin	Leaf crude protein	Stand density	Tillering	Mean stem thickness	Plant height	Flowering time	Lodging	Brix
Fresh total biomass yield, t ha^{-1}	0.29***	−0.36***	0.14***	0.06	0.14***	0.2***	−0.1**	0.27***	0.29***	−0.03	0.7***	0.29***	0.11***	0.24***
Fresh stem yield, t ha^{-1}	0.31***	−0.38***	0.13***	0.03	0.16***	0.19***	−0.1**	0.19***	0.23***	0.02	0.8***	0.35***	0.15***	0.34***
Fresh leaf yield, t ha^{-1}	0.06	−0.13***	0.2***	0.11***	0.2***	0.11***	−0.13***	0.4***	0.36***	−0.13***	0.3***	0.13***	0.02	0.06
Fresh panicle yield, t ha^{-1}	0.0	−0.3***	−0.18***	−0.07*	−0.31***	0.09**	0.16***	0.12***	0.12***	−0.16***	−0.2***	−0.41***	0.0	−0.37***
Dry total biomass yield, t ha^{-1}	0.29***	−0.47***	0.05	0.02	0.05	0.18***	−0.14***	0.34***	0.36***	−0.15***	0.68***	0.2***	0.08**	0.33***
Dry stem structural yield, t ha^{-1}	0.34***	−0.4***	0.11***	0.03	0.15***	0.18***	−0.13***	0.21***	0.26***	−0.02	0.79***	0.35***	0.1**	0.44***
Dry leaf yield, t ha^{-1}	0.15***	−0.19***	0.21***	0.14***	0.19***	0.09**	−0.22***	0.42***	0.4***	−0.18***	0.46***	0.17***	0.07*	0.21***
Dry panicle yield, t ha^{-1}	0.04	−0.32***	−0.17***	−0.05	−0.31***	0.09**	0.17***	0.11***	0.11***	−0.17***	−0.18***	−0.4***	0.02	−0.38***
Stem dry harvest index	0.35***	−0.2***	0.19***	0.06*	0.21***	0.17***	−0.12***	0.0	0.05	0.1**	0.73***	0.46***	0.1**	0.46***
Leaf dry harvest index	−0.2***	0.46***	0.18***	0.18***	0.17***	−0.16***	−0.15***	0.17***	0.1**	−0.04	−0.35***	−0.02	−0.12***	−0.17***
Panicle dry harvest index	−0.18***	0	−0.18***	−0.05	−0.31***	−0.04	0.2***	−0.05	−0.05	−0.11***	−0.57***	−0.51***	−0.04	−0.5***
Grain dry harvest index	−0.16***	−0.06	−0.19***	−0.03	−0.33***	−0.05	0.17***	−0.03	−0.02	−0.14***	−0.52***	−0.53***	−0.03	−0.48***
Stem NDF, g kg^{-1}	0.61***	−0.02	0.33***	0.35***	−0.11***	0.26***	0.13***	0.13***	0.16***	−0.1**	0.05	−0.11***	−0.02	−0.53***
Stem cellulose, g kg^{-1}	0.62***	−0.11***	0.33***	0.34***	−0.11***	0.27***	0.11***	0.13***	0.15***	−0.08**	0.04	−0.13***	0.0	−0.56***
Stem hemicellulose, g kg^{-1}	0.22***	0.44***	0.19***	0.16***	0.04	0.02	0.08**	−0.1**	−0.07*	0.08*	−0.23***	−0.07*	−0.17***	−0.36***
Stem lignin, g kg^{-1}	–	−0.46***	0.21***	0.25***	−0.15***	0.31***	0.02	0.12***	0.18***	−0.12***	0.5***	0.06	0.12***	−0.15***
Stem crude protein, g kg^{-1}		–	0.06*	−0.02	0.19***	−0.12***	0.0	−0.23***	−0.26***	0.22***	−0.51***	0.08**	−0.23***	−0.1**
Leaf NDF, g kg^{-1}			–	0.64***	0.42***	0.46***	−0.29***	0.09**	0.07*	−0.02	0.11***	0.09**	0.1**	−0.24***
Leaf cellulose, g kg^{-1}				–	−0.27***	0.14***	−0.43***	0.02	0.06*	−0.07*	0.1**	−0.16***	0.27***	−0.32***
Leaf hemicellulose, g kg^{-1}					–	0.0	−0.12***	0.05	0.03	0.08*	0.04	0.36***	−0.16***	0.2***
Leaf lignin, g kg^{-1}						–	0.2***	0.15***	0.12***	−0.03	0.15***	0.15***	−0.1**	−0.17***
Leaf crude protein, g kg^{-1}							–	−0.16***	−0.16***	0.14***	−0.1**	−0.03	−0.17***	−0.19***
Stand density								–	0.72***	−0.58***	0.05	−0.09**	−0.1**	0.0
Tillering									–	−0.75***	0.13***	−0.15***	−0.07*	0.04
Mean stem thickness										–	−0.02	0.33***	0	−0.03
Plant height, cm											–	0.29***	0.25***	0.37***
Flowering time, days												–	−0.13***	0.39***
Lodging, %													–	−0.11***

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†NDF, neutral detergent fiber.

correlations with stem and leaf crude protein. Also, both lodging and height showed a significant south-facing border effect (Table 2), even though additional border rows had been planted with dwarf grain sorghums. Recombinant inbred lines on the south-facing border had average lodging reduced by 12% in WE05, 2% in CS05, and 50% in CS06 (data not shown). Recombinant inbred lines on the south-facing border also showed an average reduction in height of 22 cm in WE05, 3 cm CS06, and 8 cm in CS06 (data not shown).

Regrowth Potential

Sorghum, a weak perennial, regrows from the stalk base after each harvest, thus generating more biomass and protecting the soil. Regrowth on mown plots was measured only for the CS06 location. Regrowth constituted a large source of additional biomass, with RILs yielding an additional 7.7 to 39 t ha^{-1} fresh or, assuming the same dry matter content of the first cutting, 3 to 16.7 t ha^{-1} dry biomass (includes leaf, stem, grain, and sugar; see Supplementary Table S2). No lines produced as much structural biomass, sugar, or grain as the first harvest, and there was high variation in regrowth ability, yielding between 20 and 80% of the first cutting (data not shown). The lines with the highest biomass on the first cutting had a greater variance in regrowth biomass than lower lines but still outperformed lower biomass types (data not shown). Regrowth fresh biomass had moderate genetic correlation with fresh biomass (0.44***). Regrowth brix showed an average decrease in raw values from primary growth by 20% (Supplementary Table S2) and had low but significant correlation with primary growth brix (0.21***). The highest correlation was between regrowth fresh biomass and primary height (0.71***). The experimental variance for regrowth in the two randomized complete block replicates was highly significant, suggesting that environmental conditions within the field had large effects on regrowth ability (Supplementary Table S3).

QTL Analysis

Corrections on raw data for identifiable sources of nongenetic error were made before performing QTL analyses. For most traits, correcting raw data modestly improved trait normality, heritability, peak likelihood of odds (LOD) scores, and narrowed the marker LOD intervals noticeably but not substantially (data not shown). Using both raw and corrected data, IM and CIM showed potential QTL likelihood peaks in the same genetic location for all traits (data not shown). Composite interval mapping with corrected data (Fig. 1 and Supplementary Fig. S1) identified more of these peaks as significant and with less genetic distance within the 1- and 2-LOD intervals. Many of these QTL were supported by single marker analysis, which also identified additional QTL not significant under the stringent permutation thresholds used for IM and CIM (Supplementary Table S4).

Across the three locations, QTL were identified for all but one of the measured traits (Fig. 1), regrowth juice yield. In all environments, the sweet sorghum parent, Rio, provided alleles for taller plants and increased stem and total biomass. Unlike our previous study on nonstructural carbohydrates, the grain parent, BTx623, did not provide alleles that consistently increased any traits.

Structural biomass yield "hot spots" for QTL colocalization appeared in similar locations as nonstructural carbohydrate QTL. This result was not surprising, since both types of carbohydrates are strongly correlated with height, flowering time, and stand density–tillering, especially in the sorghum midge [*Stenodiplosis sorghicola* (Coquillett)] and rain-stressed environment CS05 (Murray et al., 2008). Although linkage of two separate genes cannot be ruled out for QTL colocalization between traits, it is likely that colocalization was due to the pleiotropic effects of a single gene (e.g., taller plants produce more stem biomass given consistent stem diameters and stand density–tillering ability).

Across the three locations, five regions were responsible for the majority of QTL colocalizations. Height QTL on chromosomes 7 and 9 were consistent across environments, and colocalized with increased stem and total biomass QTL and decreased stem protein QTL. A delayed flowering time QTL on chromosome 6, likely photoperiod-sensitive ma_1, colocalized with increased stem, leaf, and total biomass, decreased grain yield, and changes in leaf and stem composition only in CS05. A stand density–tillering QTL on chromosome 4 colocalized with increased regrowth maturity and decreased mean stem thickness and stem crude protein. Finally, the major brix–sugar concentration QTL on chromosome 3 colocalized to regrowth brix and altered structural stem composition across all three environments (Supplementary Fig. S1). The colocalization of opposite effects between brix and structural stem composition was directly influenced by stem residual sugar concentration. To adjust for residual sugar differences in QTL mapping, the lignin, cellulose, hemicellulose, and protein were expressed as percent structural solids (calculated by dividing by the sum of these components), which controls for residual sugar. After adjustment, stem percent structural cellulose and stem percent structural protein still colocalized to the chromosome 3 brix QTL, suggesting that some change in stem structural composition is pleiotropic with stem sugar concentration (Supplementary Table S1).

Quantitative trait loci for dry measures of leaf, stem, grain, and total biomass generally colocalized with fresh measures and explained similar amounts of variation. Given the higher heritability, it was surprising that QTL for harvest indices did not explain more variance compared to fresh or dry yields. For leaf harvest index, many unique QTL were detected. For panicle and grain harvest

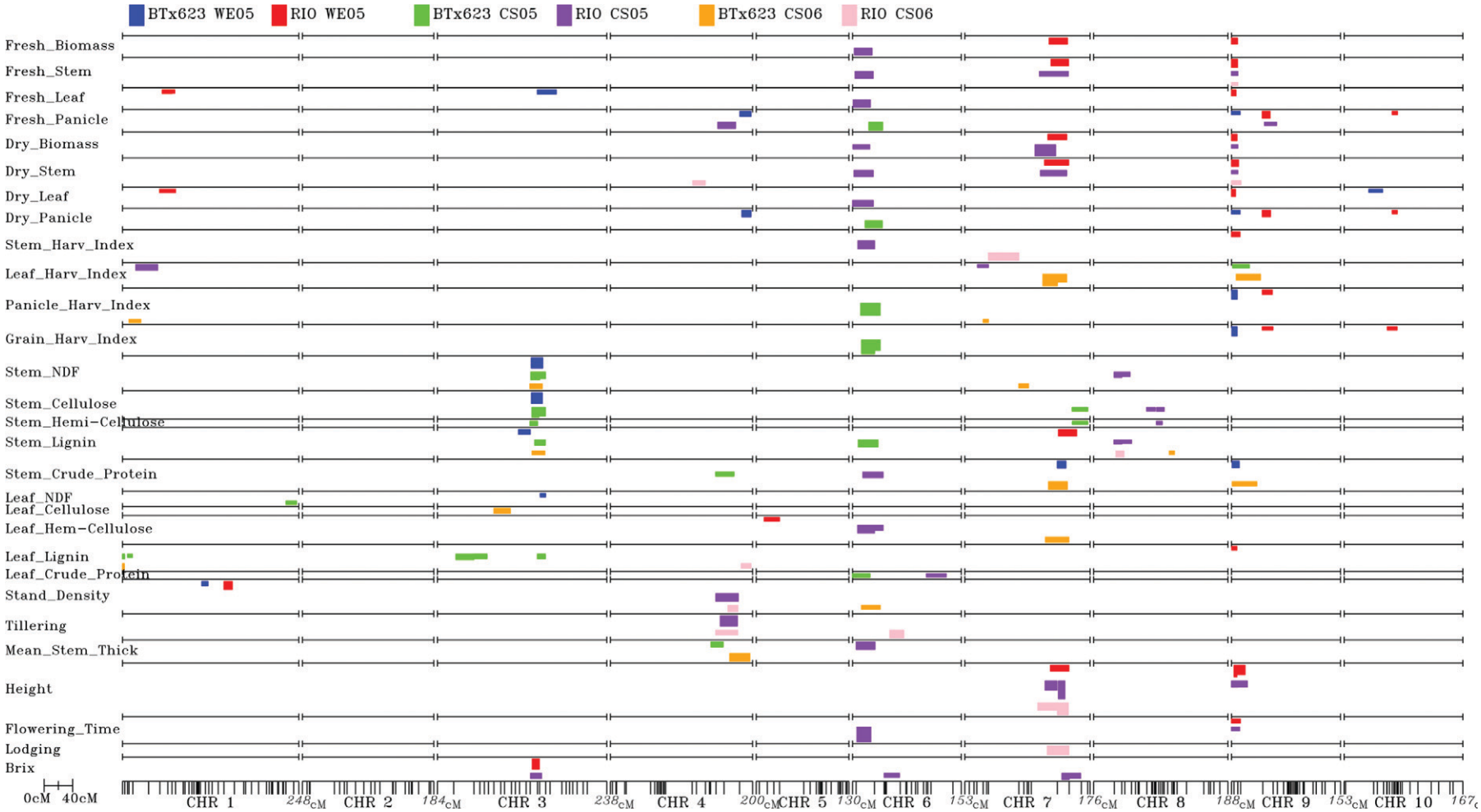

Figure 1. Quantitative trait loci (QTL) positions in three locations in Texas: Weslaco, 2005 (WE05); College Station, 2005 (CS05); and College Station, 2006 (CS06). Colored bars represent QTL detected by composite interval mapping. Alleles that increased trait values inherited from 'BTx623' (grain parent) are shown in blue (WE05), green (CS05), and orange (CS06). Alleles that increased trait values inherited from 'Rio' (sweet parent) are shown in red (WE05), purple (CS05), and pink (CS06). Trait names are shown on the left side; markers are shown as vertical lines on the bottom above chromosome (CHR) numbers. The length of each bar represents the 2-likelihood of odds (LOD) QTL interval, and height represents the variance (R^2) explained by the QTL. QTL for traits were not always detected in all three locations. QTL for regrowth and percent structural solids are presented in Supplementary Fig. S1. Exact QTL locations, LOD scores, and R^2 can be found in Supplementary Table S4.

index, QTL were detected in the same location as fresh or dry measurements but were of larger magnitude.

DISCUSSION

In this study, we evaluated progeny from a cross between an elite grain parent and a high biomass–high stem sugar parent to investigate the genetic basis of traits that might be useful for improving sorghum as a crop residue and/or dedicated biomass feedstock. We also examined genetic correlations between structural (leaf and stem cellulose) and nonstructural (stem sugar and grain starch) carbohydrate yield and composition traits. To our knowledge, this is the first documented study to analyze the genetic relationships among yield and composition traits of all aboveground products (sorghum grain, stem sugar, and biomass). Genetic relationships among traits were identified from both trait correlation and QTL analyses. Results from correlation analyses included the effects of major genetic loci, genetic background (small-effect loci and/or epistatic interactions), and genetic × environment interactions. The QTL analyses, on the other hand, identified genetic tradeoffs in different environments only at major genetic loci (caused either by pleiotropy or genetic linkage). Across all traits, the QTL identified did not fully explain the genetic variation suggested by heritability calculations. Quantitative trait loci colocalization also did not fully explain the correlations between traits suggested by correlation coefficients. Our power to detect QTL was restrained by the use of a stringent statistical significance threshold ($P = 0.01$). However, the stringent threshold increased our confidence that the QTL identified were not false positives.

Relationships between Nonstructural and Structural Carbohydrate Yields Were Primarily Due to Height, Flowering Time, and Stand Density

In Murray et al. (2008), a few positive correlations between stem sugar and grain starch yields were identified, primarily due to genetic differences for height, flowering time, and stem density–tillering. In this study, leaf and stem biomass yields were also found to be strongly correlated with height, flowering time, and stand density–tillering. Increased structural stem yield and biomass yield colocalized to the same height (chromosomes 7 and 9), flowering time (chromosomes 6 and 9), and stand density–tillering (chromosome 4) QTL identified for stem sugar (Murray et al., 2008). Although few leaf yield QTL were identified, these also colocalized with flowering time (chromosomes 6 and 9). Panicle yield, which is mostly nonstructural grain yield, colocalized with flowering time QTL (chromosome 6). Therefore, a simplified general relationship between structural and nonstructural yield is that increasing biomass increases the yield of stem sugar and slightly decreases yield of grain starch.

Stem and Leaf Carbohydrate Compositions Were Independent

Although stem, leaf, and grain yield were highly correlated because of height, flowering time, or stand density–tillering variation, this effect was not observed for structural carbohydrate composition traits. Furthermore, we found composition traits had low genetic correlation between leaf and stem tissues. In addition, there was no colocalization between leaf and stem carbohydrate composition QTL except for total structural carbohydrates (NDF) which colocalized with the major brix QTL on chromosome 3. In forage maize, Krakowsky et al. (2005, 2006) also found that composition of leaf and stem tissues were under separate genetic control. This finding suggests that improvement of whole-plant composition for biofuel production would proceed more quickly by selecting on leaf and stem tissue composition separately. Furthermore, because the stem contributes more to total biomass than leaf tissue, selection for composition alone could potentially change harvest indices.

Protein Levels in Leaf, Stem, and Grain Are Also under Separate Genetic Control

Our results showed that in nonstressed environments, leaf, stem, and grain protein levels were not correlated and QTL for theses traits did not colocalize. Therefore, protein composition across tissues was under separate genetic control. This finding contradicts results of Moyer et al. (2003) who showed that among sorghum hay and forage types crude protein levels in stems and leaves were significantly positively correlated. Because hay and forage sorghums are selected for increased total protein, the latter result could either be an artifact of selection or it is possible that QTL that affect protein levels in different tissues were not evaluated because they were fixed in our experimental population.

We did find some evidence for negative correlations between protein in all tissues and carbohydrate composition and/or yield. Since there appears to be some tradeoff between protein content and carbohydrate yields, improvement of sorghum as a biofuel crop should focus on lowering protein levels in harvested tissues. As with structural carbohydrate composition, this finding suggests that breeding for lower overall protein may be made most quickly with separate selection for grain, leaf, and stem protein.

Regrowth Protects the Soil and Increases Harvestable Energy

A major concern of using crop residues for biofuel feedstocks is that this practice leaves soil bare and prone to erosion and removes organic matter that could be incorporated into the soil. Unlike maize, sorghum continues to produce tillers (ratoon) after it is harvested, given sufficient water and protection from freezing temperatures. Because sorghum does

regrow, vegetative material is available both for erosion control and for providing additional soil organic matter. An additional benefit is that, like sugarcane, regrowth sorghum can also be harvested as an additional source of biomass. In the CS06 location, sorghum plants were allowed to regrow after the initial cutting and the first regrowth was harvested around the time of grain maturity. Although there was large variation in regrowth, this second harvest provided a large additional source of harvestable energy and there was still sufficient time before frost for a third regrowth of tillers to protect the soil. For structural biomass feedstocks, experiments to evaluate the efficacy of harvesting repeated cuttings of regrowth material compared to replanting must be conducted.

Energy Considerations

In the United States, grain starch is currently the primary feedstock of ethanol production. Theoretical yield is 0.72 L of ethanol kg^{-1} starch and actual efficiency (yield) is 85 to 89% of this value for sorghum (Wu et al., 2007). Sugar has a theoretical ethanol yield of 0.68 L kg^{-1} sugar while actual efficiency using raw sugar is about 83% (USDA, 2006). Cellulose has a theoretical ethanol yield equal to starch (0.72 L of ethanol kg^{-1} cellulose). Cellulose conversion efficiency, however, varies widely, although values are improving as the conversion technologies continue to evolve (Hamelinck et al., 2005; USDOE, 2007). From crop physiology and energy production perspectives, therefore, 1 kg of grain starch is approximately equal to 1 kg of stem sugar or 1 kg of cellulose. Figure 2 summarizes dry yield data in WE05 for starch, sugar, and biomass, and theoretical ethanol yields for the parents and selected RILs with extreme phenotypes (high brix, starch, cellulose, etc.).

Our data indicated that, on average, starch composed 63.3% of dry grain yield (53–69%), sugar accounted for 12.4% of juice yield (4.6–17%), and cellulose composed 33% (27–39%) of dry leaf and 35.6% (29–46%) of dry stem yields. Grain is, therefore, more "energy dense" than stem juice or biomass, although there is less total energy produced from grain because of lower yields (Fig. 2). In dry form, stem and leaf biomass is also energy dense. Stem juice sugar is not energy dense but, unlike starch and cellulose, is immediately available for fermentation without supplementation with additional water.

Energy density is important because it directly affects the cost of transporting plant material from the field to ethanol production facilities; as energy density increases, transportation costs decrease. It seems logical, therefore, that breeding strategies should focus on improving composition for high energy density. As shown in Fig. 2, however, data from RILs that are energy dense (high brix, starch, cellulose) and high yielding (high stem juice, grain, leaf, and stem biomass yields) indicate that increased yields are more important than improved composition for ethanol production in this population. Based on mean yield and composition, improving only the starch, sugar, and cellulose composition to the maximum observed levels would raise the amount of theoretical ethanol produced by 17%, improving the yield of grain, juice, and stem and leaf dry biomass would increase theoretical ethanol by 89%, and improving both the maximum composition and yield would increase theoretical ethanol by 124% over the population mean. This finding clearly argues for focusing first on yield increases, and then on composition (assuming that all traits can be improved simultaneously and hybrid heterosis would not affect these relationships).

Strategies for Sorghum Improvement

We suggest two ideotypes as goals for sorghum improvement for energy: a primary grain crop with residue improved for stem sugar and structural biomass composition, and a dedicated biomass crop maximizing cellulose yield. In our RIL population, yields of leaf, stem, and grain biomass contributed more variation than composition. Therefore, yield improvement should be a primary goal for breeding both residue and dedicated biomass feedstocks. With grain sorghum, increases in leaf and stem yields (total biomass) could be achieved by avoiding the height and flowering time QTL on chromosome 9 and the flowering time QTL on chromosome 6. For sweet and dedicated biofuel sorghums, selecting for increasingly tall, late-flowering material with greater stand density–tillering ability appears promising, and plentiful genetic variation is available for these traits. Because height and flowering time affect so many traits, development of molecular markers at these QTL would be highly advantageous for rapid selection of desirable phenotypes (Holland, 2004).

Our results suggest that stem and leaf carbohydrate composition, as well as protein composition in nonstress environments, are under separate genetic control. These traits, therefore, should be evaluated separately in future studies to maximize improvement. Because protein is undesirable in a biomass feedstock, we conclude that crop improvement should focus on lowering protein separately in each tissue harvested. The cost and labor of this approach, however, can only be justified if major QTL for height and flowering time are fixed in the experimental population.

CONCLUSIONS

Demand for agriculture feedstocks coupled with new biofuel processing technologies are creating a major shift from regarding plants as sources of food, feed, and fiber to viewing whole plants as a method to capture and store energy. To our knowledge, this is the first study to simultaneously evaluate genetic variation in yield and composition of the whole plant (stem, leaf, and grain) for biofuel applications. We found that yield contributed more variation to theoretical ethanol than composition in all tissues tested. Although

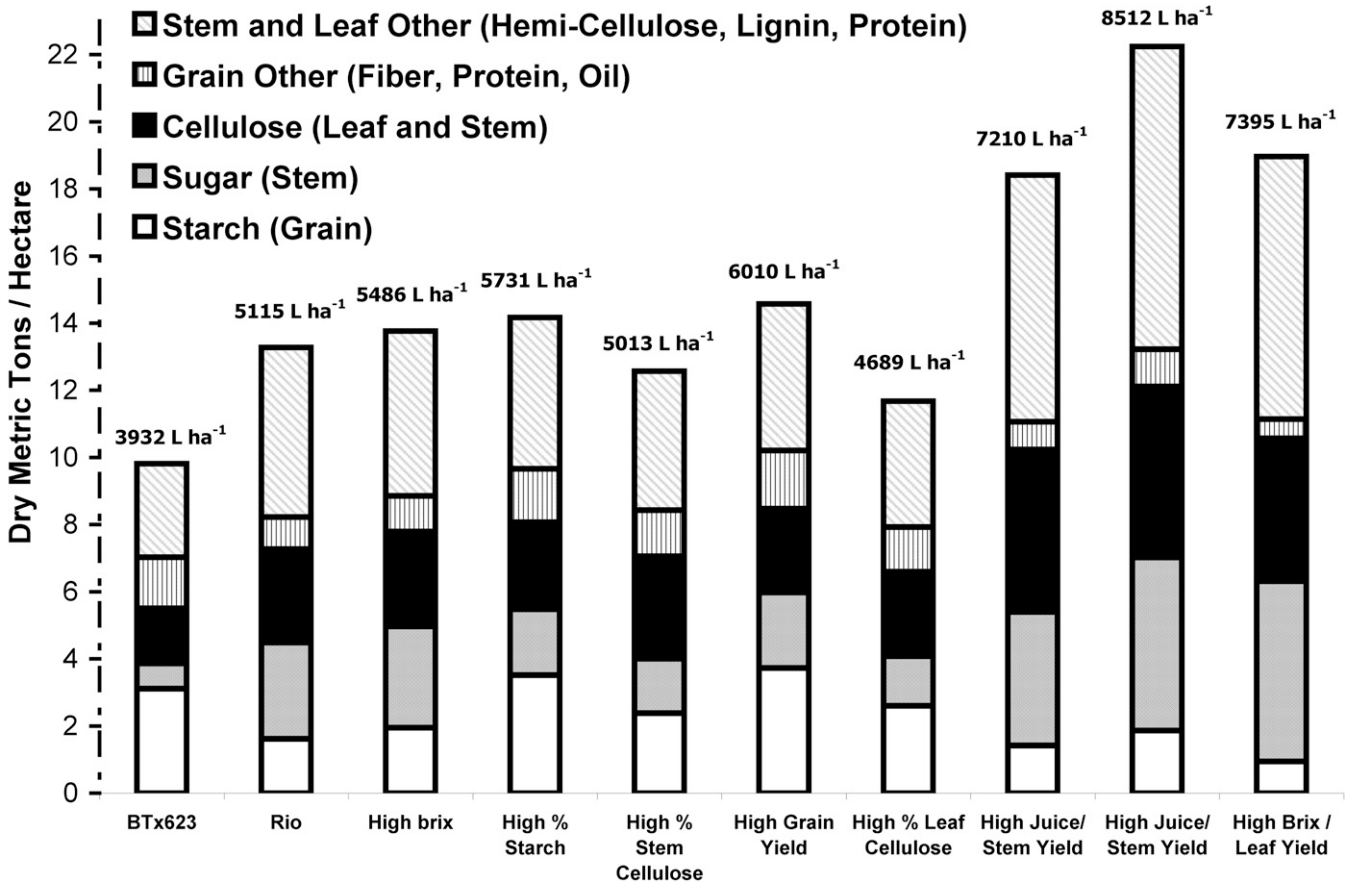

Figure 2. Yield, composition, and theoretical ethanol for parents and eight selected recombinant inbred lines (RILs). Parents and seven extreme RILs for yield and composition (at bottom) are shown for the two replicate average yield of grain starch, stem sugar, leaf–stem cellulose, grain byproducts, and leaf–stem byproducts. Theoretical ethanol yield (shown at top of each bar) was calculated as 1 kg of starch and cellulose is equal to 0.72 L of ethanol (Wu et al., 2007; USDOE, 2007), and 1 kg of sugar was equal to 0.68 L of ethanol (Shappouri and Salassi, 2006). RILs selected on the basis of yield produce more theoretical ethanol than those selected based on composition. Data are from Weslaco, TX, 2005, only.

correlations between estimates of tissue chemical composition from forage methods (NIRS) and ethanol yield have not been established, direct chemical measurement by current laboratory methods is both impractical and prohibitively expensive for assaying large numbers of samples. Therefore, the expense of performing comprehensive chemical analyses is probably not justified before biomass yield is improved in dedicated biofuel sorghums.

Much of the current work in developing biofuel feedstocks has focused on transgenic technologies, both for improved composition and digestibility of cellulosic components (Sticklen, 2006). Presently, it is not clear what genetic diversity exists for biomass traits, and future work should concentrate on evaluating a broader range of germplasm. Discussions of economic viability are premature until improved feedstocks are available and a consensus is reached on location-dependent, feasible digestion technologies.

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Sweet Sorghum Genetic Diversity and Association Mapping for Brix and Height

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Abstract

Sweet sorghum [*Sorghum bicolor* (L.) Moench], like its close relative, sugarcane (*Saccharum* spp.), has been selected to accumulate high levels of edible sugars in the stem. Sweet sorghums are tall and produce high biomass in addition to sugar. Little has been documented about the genetic relationships and diversity within sweet sorghums and how sweet sorghums relate to grain sorghum racial types. In this study, a diverse panel of 125 sorghums (mostly sweet) was successfully genotyped with 47 simple sequence repeats (SSRs) and 322 single nucleotide polymorphisms (SNPs). Using both distance-based and model-based methods, we identified three main genetic groupings of sweet sorghums. Based on observed phenotypes and known origins we classified the three groups as historical and modern syrup, modern sugar/energy types, and amber types. Using SSR markers also scored in an available large grain sorghum germplasm panel, we found that these three sweet groupings clustered with kafir/bicolor, caudatum, and bicolor types, respectively. Using the information on population structure and relatedness, association mapping was performed for height and stem sugar (brix) traits. Three significant associations for height were detected. Two of these, on chromosomes 9 and 6, support published QTL studies. One significant association for brix, on chromosome 1, 12kb from a glucose-6-phosphate isomerase homolog, was detected.

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SWEET SORGHUMS belong to the same domesticated species [*Sorghum bicolor* (L.) Moench] as grain, forage, and broomcorn sorghums but have been selected to accumulate high levels of sucrose in the parenchyma of juicy stems (Harlan and deWet, 1972; Vietor and Miller, 1990). Sweet sorghum sugar accumulation levels can be similar to that in sugarcane (*Saccharum* spp.), a close relative, though studies on enzymatic control and carbon transport suggest that the mechanism of accumulation is different (Lingle, 1987; Tarpley and Vietor, 2007). The stems of sweet sorghum are desired for food-grade syrup (stalks are pressed and juice is subsequently boiled) but also for fresh chewing and alcohol production in Brazil and India (House et al., 2000).

Recent demand for biofuel, in light of perceived Brazilian success with sugarcane, has caused a re-evaluation of sweet sorghums as a source of energy (Rooney et al., 2007; Vermerris et al., 2007). Up to 13.2 t/ha of total sugars, equivalent to 7682 L of ethanol per hectare can be produced by sweet sorghum under favorable conditions (Jackson et al., 1980). Sweet sorghum and other sugar crops have been researched for biofuel production in the U.S. for over 30 years (Lipinsky et al., 1977). Primary research, development, and breeding began in the late 1970s when the high

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Abbreviations: AFLP, Amplified fragment length polymorphism; BLAST, basic local alignment search tool; GLM, general linear model; HPLC, high performance liquid chromatography; LD, linkage disequilibrium; Mb, megabase; MLM, mixed linear model; NIRS, near-infrared spectroscopy; PC, principal coordinate; PCoA, principal coordinate analysis; QTL, quantitative trait locus (loci); RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

cost of oil spurred interest in alternative energy sources. These investigations were ended by 1987 when petroleum costs had decreased (DOE-OSTI, 2008).

Sweet sorghums, also called sorgos, were originally brought to the U.S. as landraces from China (cv. Chinese Amber) and Africa (cvs. Orange, Sumac/Redtop, Gooseneck /Texas Seeded Ribbon Cane, Honey, White African, and others) via France in the 1850s for producing syrup (sirup) and forage (Winberry, 1980; Maunder, 2000). Many of these original sweet sorghum landraces continued to be selected by farmers regionally in the U.S. and were renamed. Other cultivars were introduced later: 'Collier' from South Africa, 'McLean' from Australia, and others with unknown origin such as 'Folger,' 'Coleman,' 'Sugar Drip,' and 'Rex,' referenced as early as 1923 (Sherwood, 1923; Vinall et al., 1936; Maunder, 2000).

Almost all sweet sorghum cultivars improved with modern methods were bred at the USDA-sponsored U.S. Sugar Crops Field Station in Meridian, MS, from the 1940s until it closed in 1983. The Meridian station used landraces for plant improvement and released improved syrup lines. A few lines were also selected for sugar production and energy (biomass tonnage) in collaboration with others across the U.S., notably Texas and Georgia. Of the syrup lines bred and released by the Meridian station, release notes suggest primary improvement was focused on improving disease resistance in high sugar lines. Disease can alter sorghum juice, reducing the desirability of syrup and contributing to lodging. Besides disease resistance, other selected traits include high brix (very few report stem sugar), low purity juicy stalks, high yields, stalk erectness, and good quality syrup.

The Meridian, MS, station additionally curated a "sweet sorghum world germplasm collection." When it closed, materials were transferred to the USDA sorghum collection in Griffin, GA (Freeman, 1979; USDA-ARS, 2008). Many accessions from this collection, used in later breeding, were obtained in a 1945 collecting trip by Carl O. Grassl around the African center of sorghum domestication (Freeman, 1979). Six of these African landraces, specifically MN960, MN1048, MN1054, MN1056, MN1060, and MN1500 were used in the pedigrees of many U.S. released improved sweet sorghum lines (Table 1). This suggests that there may be a narrow genetic base for U.S. sweet sorghum cultivars resulting in close genetic relationships. If the genetic base is too narrow there may be difficulty in breeding from this material to develop energy types.

Although published pedigree information is available for some of the more recent sweet sorghum lines, the relationships with historic sweet cultivars and grain sorghums are poorly documented. A few genetic studies (Anas and Yoshida 2004, Casa et al., 2008) investigated grain sorghum germplasm panels that included some sweet sorghums. Further work by Seetharama et al. (1987) and Ritter et al. (2007) suggested that sweet sorghums are of polyphyletic origin, with relatives among kafir, caudatum, and other grain sorghum types.

Currently, there are no discrete objective criteria, such as a molecular marker or sugar concentration level, to differentiate sweet sorghums from grain sorghums. There are multiple generalized phenotypic differences: sweet sorghums are always tall, have high biomass and juicy stem [juicy versus dry stem is controlled by a major gene (Bennetzen et al., 2001)], and most importantly have high stem-sugar concentrations. Stem-sugar concentration may be quantitatively measured by high performance liquid chromatography (HPLC) or as brix, a measurement of soluble solids which in sorghums is mostly sucrose. Stem-sugar concentration inheritance is not simple; environment, genetic × environment interaction, and the genetic background (epistasis) all play a role. Within mapping populations, few QTL have been identified and they explain little variation given the moderate heritability (0.51 to 0.86) reported for the trait (Schlehuber, 1945; Clark, 1981; Natoli et al., 2002; Bian et al., 2006; Ritter, 2008; Murray et al., 2008a). In two different populations, Natoli et al. (2002) and Murray et al. (2008a), both identified the strongest QTL for stem sugar on chromosome 3, explaining 18, and 25% of the trait variance, respectively. Natoli et al. (2002), in an F_2 population derived from a sweet sorghum × sweet sorghum cross, estimated the chromosome 3 QTL effect was 56% additive and 44% dominant. Murray et al. (2008a) used a recombinant inbred-line population derived from a sweet sorghum × grain sorghum cross, so only additive effects could be calculated. We chose to follow up the stem-sugar QTL on chromosome 3 as a candidate for association mapping in a diverse panel of sorghums.

Association mapping uses diverse material to associate genetic markers with a phenotype of interest, taking advantage of lower levels of linkage disequilibrium than are present in linkage populations. Association mapping has been used to identify genes of interest in many plant species with varying degrees of success (Wilson et al., 2004; Aranzana et al., 2005; Breseghello and Sorrells, 2006). In sorghum, a diverse grain sorghum germplasm panel for association mapping was previously reported by Casa et al. (2008). However, only eight of the 356 accessions could be considered "sweet sorghum" types. Though there likely was variation for brix, the panel was mostly dwarf grain sorghum uncharacteristic of tall and high-biomass sorghums of interest. We therefore assembled a panel that represents historically important U.S. sweet-sorghum cultivars, important sweet-landrace progenitors, and cultivars that would serve as non-sweet controls.

In this study we were interested in addressing three questions. (i) What are the genetic relationships among sweet sorghums in the United States? (ii) What are the genetic relationships among sweet and grain sorghums across grain racial classifications? (iii) Can we confirm the major QTL for total stem sugar (brix), or any of the QTL for height previously identified using association mapping?

Table 1. Sweet sorghum panel cultivar names and associated information.

Name†	Full name	Source‡	Source 2§	Type¶	Parentage or place of origin#	Reference
7035S	7035S	U	PI 552851	?		
Atlas1	Atlas	T	ASA.61	HS		
Atlas2	Atlas Sorgo	T		HS		
Axtel	Axtel	T		HS		
Bailey	Bailey	K	NSL 187557	MS	Wiley, Tracy	Duncan et al., 1984
Brandes	Brandes	T	NSL 29336	MS	Collier 706-C, MN1500	Coleman and Broadhead,1968
Brawley1	Brawley	U	PI 533998	MS	Rex, White-seeded Collier	USDA, 1958
Brawley2	Brawley	T		MS		
CAmber1	Chinese Amber	U	PI 22913	A		Maunder, 2000
CAmber2	Chinese Amber	U	PI 248298	A		Maunder, 2000
CAmber3	Chinese Amber	T	ASA.45	A		Maunder, 2000
Colier1	Collier	U	PI 19770	HS		Maunder, 2000
Colier2	Collier	T	ASA.64	HS		Maunder, 2000
Colier7	Collier 706C	U	PI 563032	HS		Maunder, 2000
Colier3	Collier Meridian	T		HS		Maunder, 2000
Colier4	Collier	T	PI 19770	HS		Maunder, 2000
Colman1	Colman	T	ASA.52	HS		Sherwood, 1923
Colman2	Colman (Young Meridian)	T		HS		Sherwood, 1923
Cowley	Cowley	T		MS	Collier 706-C, MN1054, MN960, MN 1056, MN 1054, Early Folgers Hodo, MN 1060	Kresovich et al., 1985
CnAtlas	Cunningham Atlas	T		HS		
DkAmber	Dakota Amber	T	ASA.48	A		
Dale	Dale	K	NSL 74333	MS	Tracy, MN960	Broadhead et al., 1970
Danton	Danton	T	ASA.65	HS		
Della1	Della	K		MS	BTx622, Dale	Harrison and Miller, 1993
Della2	Della	T		MS	BTx622, Dale	Harrison and Miller, 1993
Della3	Della	U	PI 566819	MS	BTx622, Dale	Harrison and Miller, 1993
EFolger	Early Folger	T		HS		
EllisSo	Ellis Sorgo	T		HS	Leoti, Atlas	Karper, 1949
Folger	Folger	T	ASA.59	HS		
Fremont	Freemont Sorgo	T	Akron, Co	HS		
GaBlueR	Georgia Blue Ribbon	T		HS		Freeman et al., 1973
HoneyS1	Honey Sorghum	U		A		Freeman et al., 1986
HoneyS2	Honey Sorghum	T	PI 181080	A	aka MN2931	
Iceberg	Iceberg Sorgo	T		HS	Orange type	
KColier	Kansas Collier	T	Anthony, Ks	HS		Maunder, 2000
KOrange	Kansas Orange	T	ASA.51	HHS		Maunder, 2000
Keller1	Keller	K		MS	MER 50–1, Rio	Broadhead et al., 1979
Keller2	Keller	T		MS	MER 50–1, Rio	Broadhead et al., 1981
Leoi	Leoi	U	PI 154995	HS		
Leoti	Leoti	T	ASA.58	HS		
M81E	M81E	K	NSL 174431	MS	Brawley, Rio	Broadhead et al., 1981
McLeanS	McLean (Starchy)	T		HS		
McLeanW	McLean (Waxy)	T	ASA.62	HS		
MnAmber	Minnesota Amber	T	ASA.46	A		
Mn1054	MN 1054	U	PI 152965	LMN	Sudan	Freeman, 1979
Mn1056	MN 1056	U	PI 152967	LMN	Sudan	Freeman, 1979
Mn1060	MN 1060	U	PI 152971	LMN	Sudan	Freeman, 1979
Mn1500	MN 1500	U	PI 154844	LMN	Uganda-aka Grassl	Kresovich et al., 1988
Mn2812	MN 2812	U	PI 167093	LMN	Egypt/Turkey	
Mn291	MN 291	U	Grif 14968	LMN	Extra Early Sumac	
Mn3046	MN 3046	U	PI 195754	LMN	China	
Mn3083	MN 3083	U	PI 196586	LMN	India/Taiwan	
Mn410	MN 410	U	PI 145619	LMN	S. Africa	
Mn4125	MN 4125	U	PI 250583	LMN	Egypt	
Mn4466	MN 4466	U	PI 255744	LMN	Turkey, Taslik village	
Mn822	MN 822	U	PI 152694	LMN	Kordofan, Sudan	
Mn856	MN 856	U	PI 152728	LMN	Sudan	
Mn960	MN 960	U	PI 534165	LMN	Sudan	Freeman, 1979
N100	N100	T	PI535785	MS	Waconia, Wray	Gorz et al., 1990
N108	N108	T	PI535793	MS	Saccharum Sorgo	Gorz et al., 1990
N109	N109	T	PI535794	MS	White Collier, Grain Sorghum Line	Gorz et al., 1990
N110	N110	T	PI535795	MS	Red X	Gorz et al., 1990
N111	N111	T	PI535796	MS	Waconia	Gorz et al., 1990
N98	N98	T	PI535783	MS	Rio, Waconia, Fremont, AN39, N4692	Gorz et al., 1990
N99	N99	T	PI535784	MS	Fremont, Theis	Gorz et al., 1990
Orange1	Orange	U	PI 2363	HHS		Maunder, 2000
Orange2	Orange	U	PI 533902	HHS	aka MN 604	Maunder, 2000
Orange3	Orange	T	ASA.50	HHS		Maunder, 2000
PI52606	PI52606	K	PI52606	LMN		
P526905	PI526905	K	PI526905	L- zimB		
P527045	PI527045	K	PI527045	L- zimB		
P550604	PI550604	K	PI550604	?		
Ranchr1	Rancher 3	T	Brookings, SD	A		Karper, 1949
Ranchr2	Rancher 3	T	ASA.93	A		Karper, 1949

Table 1. Continued.

Name†	Full name	Source‡	Source 2§	Type¶	Parentage or place of origin#	Reference
RedAmbr	Red Amber	T	ASA.49	A		
RedTopT	Red Top Tennesse	T		HS		Winberry, 1980
Rex	Rex	U	PI 534163	HS		Sherwood, 1923
Rio1	Rio	T		MS	Rex, MN 1048	Broadhead, 1972
Rio2	Rio	T		MS	Rex, MN 1048	Coleman et al., 1965
RxOrng1	Rox Orange	K		HHS		
RxOrng2	Rox Orange	T		HHS		
WhitMam	White Mammoth	T		G		
Saccaln	Saccaline	T		HS		Vinall et al., 1936
Sapling	Sapling	T	ASA.55	HS		Vinall et al., 1936
Simon	Simon	K		HS		
Smith	Smith	U	PI 511355	MS	MN4004 (Grif 16302), MN 2754, Wiley, MN 48, MN 1056, others	Kresovich and Broadhead, 1988
Sorgras	Sorgrass	U	PI 563222	F		
SucreDm	Sucre Drome	U	PI 197542	LMN		
SgrDrp1	Sugar Drip	U	PI 586435	HS		Freeman et al., 1986
SgrDrp2	Sugar Drip	U	PI 146890	HS		Freeman et al., 1986
SgrDrp3	Sugar Drip	K		HS		Freeman et al., 1986
SgrDrp4	Sugar Drip	T		HS		Freeman et al., 1986
SgrDrp5	Sugar Drip	T	Oklahoma A&M	HS		Freeman et al., 1986
SgrDrp6	Sugar Drip	T	Oklahoma A&M	HS		Freeman et al., 1986
Sumac1	Sumac	U	PI 63715	HHS		Maunder, 2000
Sumac2	Sumac	U	PI 35038	HHS		Maunder, 2000
Sumac3	Sumac	U	PI 534120	HHS		Maunder, 2000
TxDblSw	Texas Double Sweet	K		HS		
Top76	Top 76–6	K	PI 583832	MS	Brandes, Collier 706-C, MN 1500, MN 1056	Day et al., 1995
Tracy	Tracy	T	NSL 4029	MS	White African, Sumac	Stokes et al., 1953
Umbrela	Umbrella	K		HS		
WcAmber	Waconia Amber	T	ASA.47	A		Maunder, 2000
WxAtlas	Waxy Atlas	T		HS		
WhtAfr1	White African	U	PI 52606	G		
WhtAfr2	White African	T	ASA.60	G		
WhtAfr3	White African	T	Oklahoma A&M	G		
WileyRL	Wiley R Line	K		HS		Stokes et al., 1956
WileySo	Wiley Sorgo	T		MS	Collier, MN 822, MN 2046	Coleman et al., 1956
Wiliams	Williams Sorgo	T	Ky. Certified	MS		Freeman et al., 1973
Wray	Wray	T		MS	Brawley, Rio, MN 856	Broadhead et al., 1978
BTx623	B.Tx623	T		G	BTx3197, SC170–6	Miller, 1976
BTx635	B.Tx635	T		G		Miller et al., 1992
BTx631	B.Tx631	T		G		Miller, 1986
BTx642	B.Tx642	T		G	SC35	
P850029	P850029	T		G		
Macia	Macia	T		G		
Sureno	Sureno	T		G	S423,CS3541,E35	Meckenstock et al., 1993
ATx623	A.Tx623	T		G		
EA1083	SC599	T	sc599	G	IS17459C	
EA1074	Rio 9188	T	Rio 9188	G		
EA1084	SC599–6-9188	T	PI 593916	G		
Forag41		T		F		
Forag73		T		F	TX631, TX2910	
Ramada	Ramada	U	NSL 107377	MS	MER 45–45, MN 1056, MN 1054, MN1060	Freeman et al., 1974
Sart	Sart	U	NSL 91616	MS	Sudan	Stokes et al., 1951

†Abbreviated name used in later tables and figures.

‡K: University of Kentucky; T: Texas A&M University; U: USDA/ARS.

§USDA PI number or additional information to distinguish accessions.

¶A: amber; G: grain; HHS: historical sweet 1850s HS: historical sweet by 1923; MS: modern sweet; F: forage; ?: unknown or diverse

#If known, parent lines for modern cultivars with pedigrees, place of origin for collected landrace material. Additional information in reference.

Materials and Methods

Plant Material and Phenotypic Analysis

Two replicates of 125 diverse accessions were planted in College Station, Texas in 2006 (CS06) and 2007 (CS07), and one replicate was planted in Ithaca, NY in 2007 (ITH07). These accessions were primarily historical and modern sweet-sorghum cultivars, though grain, and forage sorghums were also included (Table 1). These accessions will subsequently be referred to as the "sweet sorghum panel." Literature and the GRIN database (USDA-ARS, 2008) were used to identify cultivars as amber, historical sweet, modern sweet, modern sugar and energy, MN landraces (brought to Meridian, MS from Africa by C.O. Grassl), or grain types. We use the term "modern" to denote improved lines that have published pedigree information. Seed was obtained from a variety of sources for CS06 (Table 1), and seed bulked from self pollinated plants was planted for CS07 and ITH07. In CS06 and CS07, 3-m rows with 76 cm spacing (~160,000 plants ha^{-1}) were planted in a randomized complete block design. In ITH07 30 seeds were hand planted in 1.5-m rows with 76 cm spacing.

Some material was photoperiod sensitive and, depending on environment, there was a wide range for time of maturity. Plants were harvested when most accessions were in the soft-dough to hard-dough stage. By harvesting without regard to specific cultivar maturity we minimized the environmental effect, but likely caused biases in stem-sugar phenotypes due to flowering time, which peaks right before the hard dough stage (unpublished data). This would be expected to decrease our power but not create false positives. In each location, 1 m per row was harvested by cutting within 3 cm of the soil. Stems were separated from panicles and leaf tissue. Stem juice was extracted using a three roller mill. Brix was measured using a handheld refractometer. Measurements were collected on 1 m of row in CS06 and CS07. Measurements were collected from three random plants in ITH07. HPLC was performed according to Murray et al. (2008a). No HPLC analysis was performed for CS07 or ITH07. Plant height was averaged across each row from the soil to the top of the panicle for all three locations.

Genetic Analysis

Leaf tissues were collected from plants grown at the CS06 location. DNA was extracted from pooled tissue of five or more plants using a standard CTAB protocol (Doyle and Doyle, 1987). Forty-six polymorphic SSRs, used in the diverse association panel of Casa et al. (2008), were evaluated using the same equipment and published methods (Xcup19, Xtxp065, Xtxp287 were not included). One SSR, Xcup55, was not polymorphic in the sweet-sorghum panel and was excluded from further analysis, resulting in 45 SSRs shared with Casa et al. (2008). Two additional SSRs, Xtxp120 (Menz et al., 2002) and a new SSR were successfully added (Xcup75; primers sequences: TTGCTTCATTCAACGGGAATACA, TTCGATGCAGCGAGCTTTGG). An additional 384 SNP genotypes were collected using an Illumina Goldengate assay (Fan et al., 2006) at Cornell's Life Sciences Core Laboratories Center (Ithaca, NY) using recommended procedures (Illumina Inc., San Diego, CA). These 384 SNP assays were developed from SNPs discovered in previously published (Hamblin et al., 2004, 2005, 2006, 2007a) and unpublished [Murray, this study (sucrose pathways); Salas Fernandez et al., 2009 (carotenoid pathways)] resequencing studies, and were chosen both to provide genome-wide coverage and to survey variation in genes of interest. A total of 226 loci are represented in the panel, of which 39 loci are candidate genes; the remainder is distributed across all ten linkage groups. Genetically mapped loci were chosen from resequencing studies of unannotated restriction fragment length polymorphism (RFLP) probes (see Schloss et al., 2002). Supplemental Table 1 shows the GenBank accession numbers for reference sequences and map position, where available. Of the 384 Illumina SNP assays, 329 were successful, and 322 were polymorphic in the sweet-sorghum panel.

To identify candidate genes for brix, the major QTL for brix in a cross between a grain sorghum and a sweet sorghum from Murray et al. (2008a) was located on the sorghum genome sequence (Phytozome, http://www.phytozome.net/sorghum; verified 26 Jan. 2009) using BLAST analysis with sequence-based markers (Menz et al., 2002; Feltus et al., 2006). More than 100 starch and sucrose metabolism enzymes (Kanehisa et al., 2006) and sugar transport candidate genes from maize (*Zea mays* L.), sugarcane, tomato (*Solanum lycopersicum* L.), and rice (*Oryza sativa* L.) (NCBI, http://www.ncbi.nlm.nih.gov/; verified 26 Jan. 2009) were also placed on the sorghum genome using BLAST to identify co-localization with the chromosome 3 QTL. New SSRs within the chromosome 3 QTL were identified from Phytozome contig sequences using the program Tandem Repeats Finder (Benson, 1999). Primer 3 (Rozen and Skaletsky, 2000) was used to design all primer sequences. All sequencing was performed on sweet-sorghum cultivar Rio at Cornell University's Bioresource Center using a 3730 capillary sequencer. Trace files were investigated for polymorphisms between Rio and grain sorghum 'BT×623' in Sequencher 4.0 (Gene Codes Corp., Ann Arbor, MI).

Genetic Distance and Principal Coordinate Analysis

The program PowerMarker version 3.0 (Liu and Muse, 2005) was used to evaluate F_{ST} (Wright, 1965) and create genetic distance matrices (Nei, 1972). Distance matrices were double-centered, and used to obtain eigenvectors, which were plotted in NTSYS-pc Version 2.02 (Rohlf, 1990).

To compare sweet sorghums with the larger sorghum panel of Casa et al. (2008), Nei's 1972 genetic distance matrix was created in PowerMarker using the polymorphic SSRs that had been scored in all accessions in both studies. Eigenvectors were obtained implementing the cmdscale function (eig = TRUE) and then plotted using R (R Development Core Team, 2005). R cmdscale was used rather than NTSYS-pc for this analysis because the data set was so large. Using smaller test data sets, the two principal coordinate analyses (PCoA) gave identical results (Gower, 1966).

Population Structure, Relatedness, and Association Mapping

To minimize false positives in association mapping it is important to control for population structure and relatedness (Falush et al., 2003; Yu et al., 2006). Three programs were used to estimate the number of populations and assign cultivars' membership in them: Structure, version 2.1 (Pritchard et al., 2000), InStruct (Gao et al., 2007), and NTSYS-pc. Because population structure estimates assume unlinked markers, SNP assays from the same physical locus were converted into 208 haplotypic loci. Phase ambiguities were called as missing alleles and loci with more than 20% missing alleles were eliminated. Excluding brix candidate gene markers on chromosome 3, and including SSRs, a total of 241 markers were used. In both Structure and InStruct, five independent runs having 5×10^5 burn-in and sampling iterations were conducted allowing k (number of populations) to vary between 1 and

15. For Structure, the ancestry model allowed for population admixture and correlated allele frequencies. For Instruct, population structure and individual selfing rates were inferred. Optimal *k* was identified using the marginal improvements in estimated logarithm of the likelihood of the data, greater than 0.5 posterior population assignment probability, and on consistency of the five independent runs. *k* was additionally inferred using the DIC criterion in InStruct. Once *k* had been determined for both Structure and InStruct, a run of 5×10^6 burn-in and sampling iterations were used. PCoA eigenvectors from haplotypes were also used as population assignments.

Using the package SPAGeDi 1.2 (Hardy and Vekemans, 2002), a kinship coefficient estimation matrix was created according to J. Nason (described in Loiselle et al., 1995). Association mapping was performed using the GLM and MLM procedure in TASSEL (Bradbury et al., 2007). Six **Q** (population structure) matrices, with different numbers of populations, were separately tested for model percent variation explained of brix and height phenotypes. Positive tests were reported using a significance threshold of $p < 1.3 \times 10^{-4}$, based on a stringent Bonferonni correction of 0.05 divided by 369 tests.

Results

Genetic Analysis

Between all pair-wise comparisons of SNPs from different loci, linkage disequilibrium (LD) was minimal (Supplemental Fig. 1) in this panel, as expected with this low density of markers. Perfect LD ($r^2 = 1$) was observed between at least two SNPs within each of four genes (SB00037, SB00076, SB00114, SB00130) and between two other pairs of SNPs (SB00124 and SB00027; SB00076 and SB00103) due to close physical distance.

Seventy-seven of the 125 cultivars were heterozygous or heterogeneous at one or more marker loci. Two known to be F_1 forage hybrids segregated at the most marker loci, 41% (Forage 73) and 37% (Forage 41). MN landraces as a group averaged 22% heterozygous markers, with only MN960 having no heterozygous marker loci and Mn1054 having the most (37%). Departure from 1:1 ratios of alleles in some SNP assay results suggested that levels of heterozygosity were increased by pooling tissue from multiple individuals within cultivars, as landraces are often heterogeneous.

Cultivars in the sweet sorghum panel with identical names but different seed sources all had at least one genetic polymorphism (Table 2). With Sugar Drip, of the loci that differed, almost every possible combination of allele sharing across the six lines was observed. A few cultivars had very different names but identical genotypes potentially due to human error. 'N110' and 'Sugar Drip 4' were found to be exactly identical except for one locus with missing data. 'Rox' 'Orange 2,' 'Saccaline,' and 'Sapling' were also genetically identical. The phenotypes of these cultivars were very similar, so it appears possible the seed unintentionally came from the same source in error for the CS06 planting.

PCoA Relatedness

To identify accessions for use in breeding, it is useful to understand the relationships within the sweet sorghums and between sweet sorghum and grain sorghum's racial types. Genetic relationships were most easily seen by plotting the first two PCoA eigenvectors generated with the full SSR and SNP data set (Fig. 1). Three separate groups were observed and delineated based on historical references and breeding objectives. These three groups included a tight cluster of historical and modern syrup cultivars, modern sugar and energy sorghums with MN landraces, and amber types, which were the most diverse. Grain sorghums did not cluster in any one group. The first 12 PCoA eigenvectors explained 35.7, 21.4, 7.2, 6.3, 5.3, 4.4, 4.3, 3.6, 3.2, 3.1, 2.6, and 2.4% of the variation, respectively, totaling more than 100% due to model overfitting. The same three clusters seen in Fig. 1 were also observed when using only SNPs or only SSRs, though a few individuals did shift groups (data not shown). No clear relationships were observed when additional eigenvectors were plotted (data not shown).

To objectively assess sweet sorghum genetic relatedness to grain sorghum racial groups, PCoA analysis of SSR genotypes was used to compare the sweet sorghum panel to Casa et al.'s (2008) pure racial group (138 accessions, Supplemental Fig. 2). Comparing these two panels, the sweet sorghum historical and modern syrup group appeared most similar to kafir and to a lesser extent to bicolor. The modern sugar and energy sweet sorghum group appeared most similar to caudatum and possibly guinea types. The amber sweet sorghum group looked most similar to bicolor racial types but was more divergent than most of the material in the Casa et al. (2008) panel. The sweet panel had little material that was similar to durra types.

Candidate Gene Identification and Sequencing

The primary brix QTL identified in a cross between Rio and BT×623 (Murray et al., 2008a) was localized to a 15Mb sorghum super contig (Phytozome). A sorghum homolog to maize shrunken2—the large subunit of ADP-glucose pyrophosphorylase (Hamblin et al., 2007a), and a rice hypothetical monosaccharide transporter

Table 2. Polymorphism within accessions with shared names.

Cultivar	Accessions	Shared alleles at 369 markers
Rio	2	359
Della	3	286
White African	3	282
Chinese Amber	3	194
Sumac	3	183
Orange	3	150
Sugar Drip	6	157

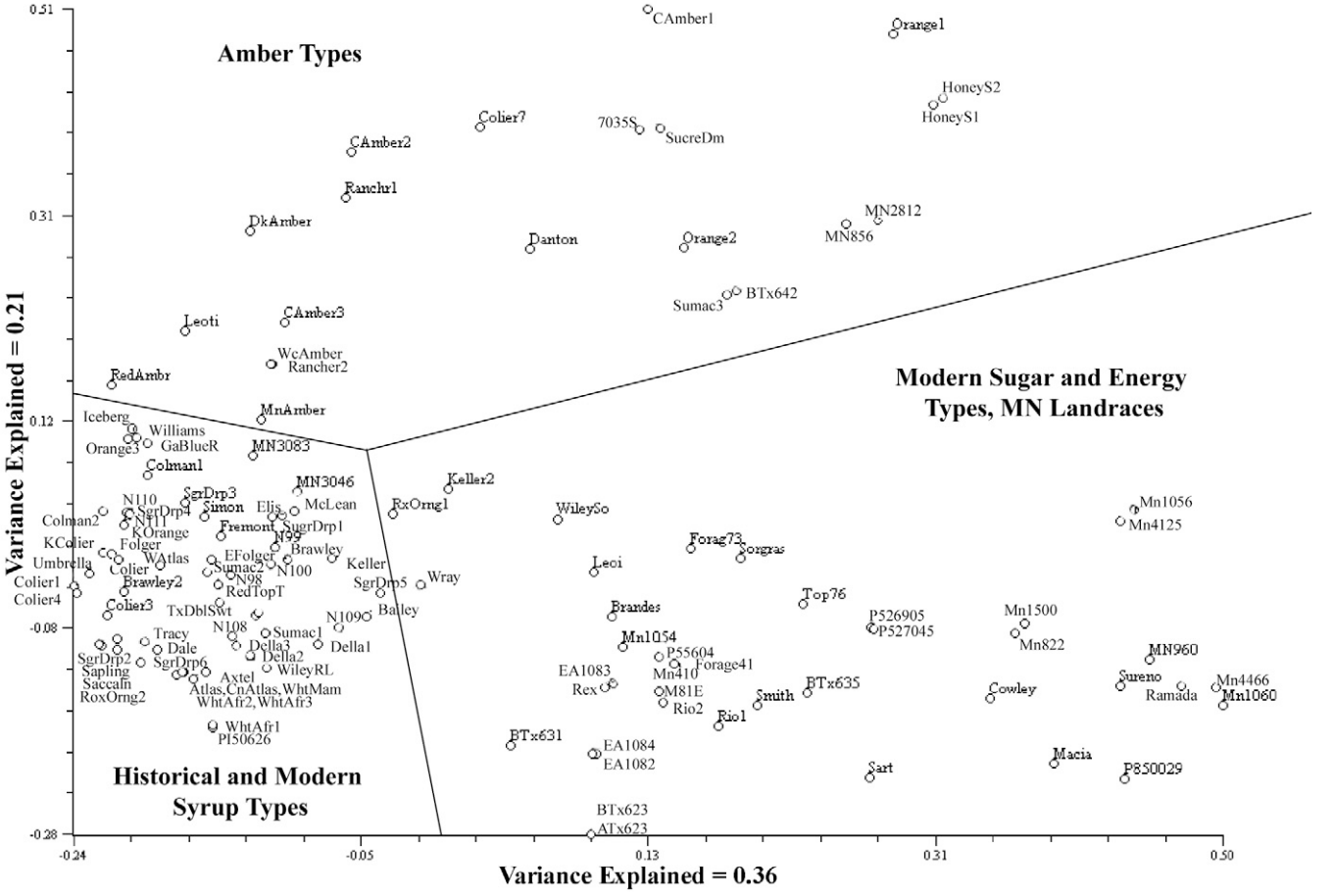

Figure 1. PCoA eigenvector plot of sweet sorghum panel genetic similarity Nei's (1972) genetic distance was calculated from 47 SSRs and 318 SNPs.

(NM_001053738) (NCBI) were the only sugar metabolism genes found to align to this Phytozome contig. Furthermore, these sequences were both located in a 2 Mb region flanked by the SSR marker bordering the QTL on the left, and an SSR marker close to the 2LOD peak border on the right (Supplemental Fig. 3). The full-length genes (as annotated), the 5′ and 3′ ends, and genetically close non-coding sequence were sequenced in Rio (a total of ~20,000bp) and no polymorphisms with BT×623 genome sequence were observed. We then identified nine SSRs spaced through the 2 Mb interval. Only one out of the nine was found to be polymorphic between Rio and BT×623. This marker was included in all analyses (Xcup75).

Phenotypic Analysis

Brix and height values were recorded in three locations. For the sweet-sorghum panel in CS06, brix and HPLC-measured stem sugar had good correlation ($r = 0.73$, $p > 2.2e^{-16}$), with outliers caused by bacterial degradation in HPLC samples. Height and brix were positively correlated across locations (Fig. 2). Height had higher correlations within and across locations than brix in this panel. For brix, ITH07 was more similar to CS06 than to CS07. ITH07 did not correlate well with CS locations for height, due to photoperiod sensitivity which delayed flowering in some cultivars.

Population Structure and Association Mapping

To control for false positives in association mapping, **Q** (population structure) and **K** (kinship) matrices were first constructed (Yu et al., 2006). **K** is unrelated to *k*, the number of populations used in the model for **Q**. Six separate **Q** matrices were calculated using the two most likely population assignments in each of three programs, InStruct, Structure, and NTSYS-pc. InStruct results suggested five or eleven populations were likely with little posterior probability increase after eleven (Fig. 3). InStruct DIC criteria also found eleven populations to be most probable. Structure results suggested either four or eleven populations as most probable. Structure posterior probability continued to increase marginally past eleven populations, but consistency of runs and population assignment decreased. Because the posterior probability is calculated differently in Structure and InStruct, these cannot be directly compared (H. Gao, personal communication, 2008). Using haplotypes for PCoA resulted in eigenvectors very similar to those obtained using individual markers in Fig. 1.

Association Analysis

Association mapping was performed for brix and height using the GLM procedure in TASSEL (Bradbury et al., 2007). Of the six **Q** matrices tested, models with

11 populations as inferred by InStruct and Structure explained the highest percent variation (Table 3). Models based on the smaller number of populations inferred by InStruct ($k = 5$) and Structure ($k = 4$) decreased the percent variation explained; the model with $k = 4$ also had a larger number of positive tests. Models using PCoA eigenvectors explained more variation than those with no **Q** matrix but much less than models based on Structure and InStruct analyses.

The MLM model, which included the kinship matrix, **K**, explained more variation than with **Q** alone. With MLM, results were nearly identical even if no **Q** matrix was added.

Using MLM with a Bonferroni corrected cutoff of 0.05 (1.3×10^{-4}), five significant associations were detected for height, and one was detected for brix (Table 4). One marker, SB00016.1, was most significant for height and nearly significant for brix. For brix the only significant marker was SB00166.1.

F_{ST} of Populations and Markers

Wright's (1965) classical F_{ST} (θ) was used to evaluate genetic differentiation between populations in the panel (Table 4). Four separate methods were used for dividing the material into populations to address different biological questions.

1) Based on the a priori expectation of sorghum types [Table 1 (amber, historical syrup, grain, diverse)]. F_{ST} averaged 0.14 across loci (range: –0.04 to 0.47; negative F_{ST} values are likely due to imprecision in the estimation and should be interpreted as no genetic differentiation). Markers with high F_{ST} would be useful for distinguishing these a priori groups and might also be linked to traits important within only one population.
2) Using the three groups identified in PCoA analysis (Fig. 1). F_{ST} averaged 0.26 (range: –0.02 to 0.77). Markers had higher F_{ST} than our a priori division. Markers with the highest F_{ST} would be useful for assigning germplasm with unknown background to these groups.
3) Using a grouping based on brix. Cultivars in the top half highest brix in CS06, CS07, and ITH07 were in Population 3, cultivars in the bottom half for all locations were in Population 0. F_{ST} averaged 0.03 (range: –0.03 to 0.19).
4) Using the number of times a cultivar was in the top half of average height for a location, similar to divisions for brix. F_{ST} averaged 0.02 (range: –0.04 to 0.23). Markers with high F_{ST} when separated by brix and height may be linked to the phenotype of interest, and useful for characterizing different germplasms.

Relationships between these estimates of F_{ST} and association results may suggest incomplete correction. Markers with high F_{ST} did not have significant associations with traits, except in the case of SB00016.1.

Discussion

From historical publications on sweet sorghum, it initially seemed likely that sweet types might be closely related to each other and distant from grain sorghums. Two recent publications have suggested otherwise. Casa et al. (2008), using 377 diverse sorghums including eight sweet cultivars, found that while a few sweet sorghums clustered together they were generally as diverse as grain sorghums. (A. Casa, personal communication, 2007). This finding was supported by Ritter et al. (2007) who, using amplified fragment length polymorphism (AFLPs), showed that 31 sweet sorghums clustered within three of the five clusters containing 64 diverse grain sorghums.

Harlan and deWet (1972) and others have classified sorghums into five major races: bicolor, caudatum, durra, guinea, and kafir. These divisions are mostly based on panicle and grain characteristics as well as the regions of Africa and India where the races are commonly found. Sweet sorghums have not been bred for panicle or grain characteristics, and the referenced origins of sweet sorghum provide little insight. Therefore, the relationship of sweet sorghum to the traditional classification of major sorghum races was inconsistent.

Our study, like that of Ritter et al. (2007), identified three separate groups of sweet sorghum which often are classified together. We classify these major types as syrup (historical and some modern), modern sugar and energy types with associated landrace parents, and amber types. These divisions were supported by PCoA, measures of F_{ST}, phenotypic observations, and structure analysis. Structure analysis and association results suggested that, within these three sweet sorghum groups, as many as eight additional subpopulation divisions exist (Supplemental Table 2). Population structure analysis is somewhat subjective and depends on the criteria used and the germplasm evaluated. Although InStruct and Structure assigned these subpopulations similarly, we did not observe a historical or biological basis for this further subdivision excepted where noted below.

Historical and Modern Syrup

Within the sweet sorghum panel, the historical and modern syrup population had the best representation but the least diversity. Among sweet sorghums cultivars the historical cultivars are best known, and the modern cultivars are some of the most common for syrup, Orange, Sumac, White African, Collier, Sugar Drip, 'N98' through N110, 'Della,' and 'Bailey.' Phenotypically, this material generally had straight, tall, very juicy, medium-large diameter stalks. Across the cultivars the juice had high average brix, but lower than the sorghums developed for sugar production. Two of the sorghums developed for sugar and having very high brix, 'Keller,' and 'Wray' were near classification in this group based on PCoA. The clustering of the syrup types reflects selections from historical material and shared pedigrees from syrup × syrup crosses. Furthermore, cultivar

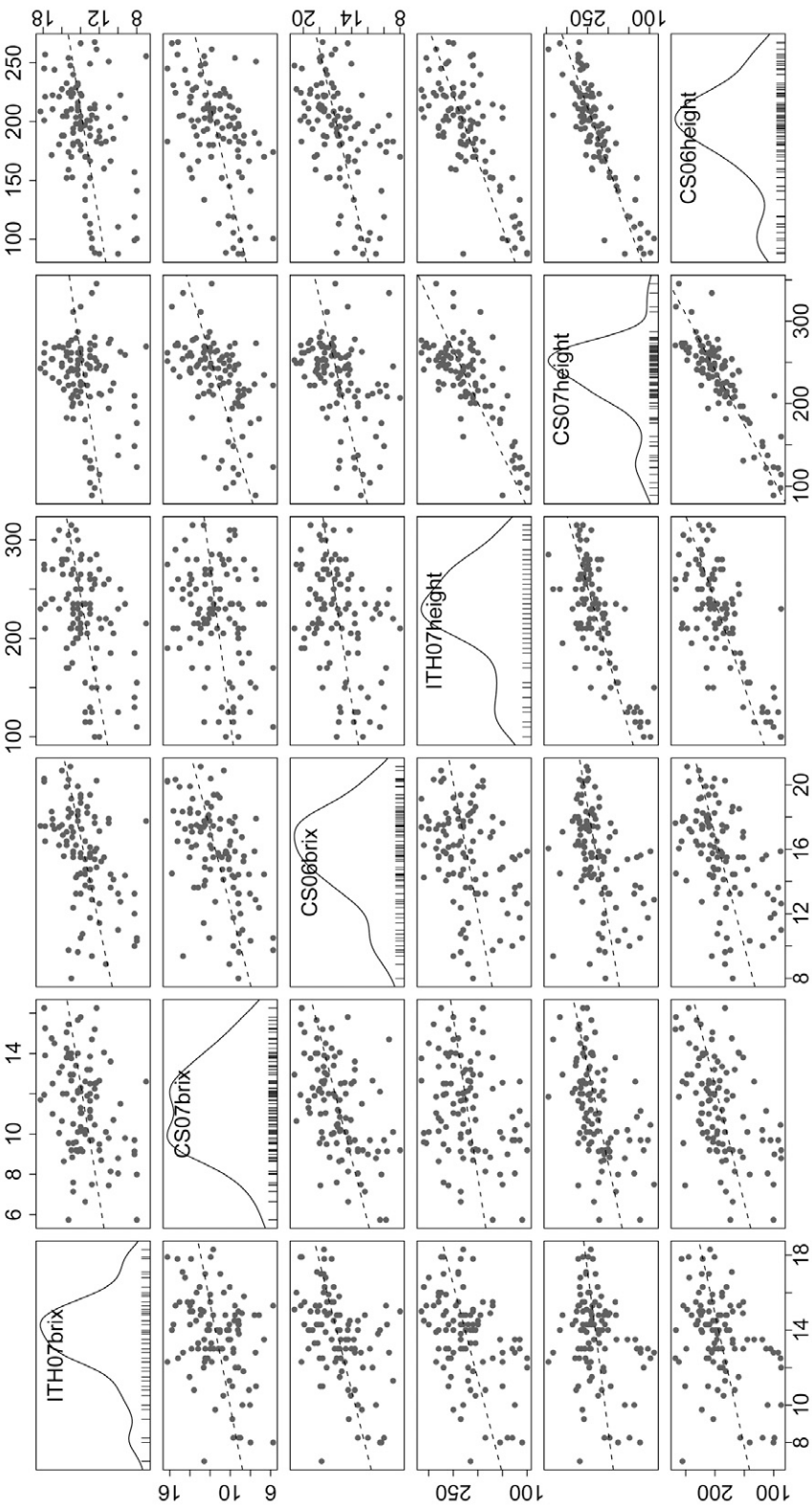

Figure 2. Relationship within and between brix and height across three locations. Units are degree brix for brix and cm for height measured in Ithaca 2007 (ITH07), College Station 2006 (CS06) and 2007 (CS07). Trait names are presented in the center diagonal with a histogram of replicate mean values. Plot regression lines showing positive relationships were observed between all sets of measurements but correlation was best within height, specifically between CS06 and CS07.

release notes show that most modern syrup sorghums were developed within the Meridian, MS breeding program. InStruct and Structure divided this population into 4 subpopulations of 19, 18, 14, and 12 individuals (Supplemental Table 2). An interesting case is Sugar Drip, which is divided into two groups. Based on polymorphism data Sugar Drip was likely heterozygous at many loci, which became fixed as different sets of seeds were isolated and maintained separately.

Sugar and Energy

Modern sweet sorghum cultivars for sugar and energy production such as Rio, 'Ramada,' 'Top76-6,' and 'M81E' tended to cluster together with MN landrace cultivars. Most MN landraces in the panel were specifically chosen because they were in the pedigrees of modern sweet sorghum cultivars. These MN cultivars were also from the center of sorghum domestication around Sudan, Ethiopia, and Uganda. This population was very diverse for brix and height. Nearly all of the cultivars were photoperiod sensitive, and had very thick stalks, some with hard rinds like sugarcane. The modern sugar and energy cultivars had very high brix while the MN landrace progenitors did not. Many of these cultivars, especially MN1500, produced very high biomass. We initially believed that MN1500 was 'Grassl,' a cultivar selected from MN1500, but the high heterozygosity suggested that it is likely the landrace MN1500 and that seed for Grassl are no longer available. In contrast to the expectation that the sweet sorghums derived from MN cultivars would have a narrow genetic base, the heterogeneity in these landraces likely contributed to the diversity seen in the modern cultivars. Population analyses (Supplemental

Table 2) further divided this population into groups of 24 (most sugar energy and MN cultivars), nine (Rio, Keller, Wray), and six (grain and forage).

Amber

Amber and honey sorghums were very distinct from the other two populations but were also very diverse within the population. The weak clustering of amber may be partially the result of a limited number of cultivars being included in this study. Amber sorghums are not included in published pedigrees of modern sweet sorghum but were among the earliest sweet sorghums introduced to the U.S. Unlike most sweet sorghums, amber types tended to senesce in CS06 and CS07 locations, but did not in ITH07. Possibly as a result, amber cultivars had relatively higher brix in ITH07 than in either CS06 or CS07. Amber types among the sweet sorghums also had the least consistency of brix between environments with cultivars having a high brix in only one location. This is why no amber cultivars were identified as top sugar producers. Structure and InStruct (Supplemental Table 2) divided the ambers into subpopulations of 12 (all but one cultivar with amber in the name and 'Sucre Drome'), six (Honey, '7035S'), and three (grain sorghums). PCoA suggested that Honey sorghums were most like race Durra, suggesting geographic genetic relationships, since Honey accessions and Durra are both from India. The amber population also had some of the most unusual cultivars, e.g., 7035S was the tallest cultivar in the panel, had a very large stalk, and was the only cultivar not to tiller at all and to senesce before it flowered in CS06. Sucre Drome was an interesting cultivar in this panel because it was the only one with a "dry" stalk, carrying a dominant gene that reduced stem moisture by 50% of the panel average and may be useful for cellulosic biofuel.

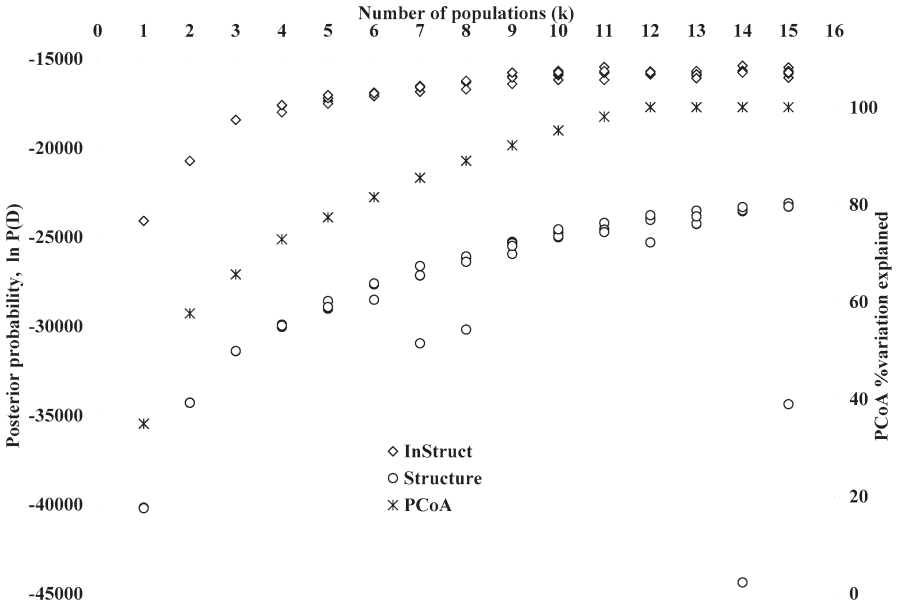

Figure 3. Results of population structure analysis using InStruct, Structure, and PCoA. Using haplotypes created from markers linked at the same locus, each method was run five times to develop population assignment vectors.

Sweet and Grain Sorghum Comparison

PCoA was useful to visualize genetic distances between sorghum races, between our sweet sorghum panel and the panel of Casa et al. (2008), and between individuals. Using PCoA, races tended to cluster together but were not distinctly separated as observed in rice, or maize (rice—Thomson et al., 2007; maize—Liu et al., 2003; Warburton et al., 2008; Hamblin et al., 2007b). Rio and BT×623 appeared to be closely related, and both were fairly distant from much of the other material. This suggests that variation found in the bi-parental population investigated in Murray et al. (2008a, 2008b) was more likely to be functional and not confounded by extreme divergence of genetic backgrounds.

The relationships in the sweet sorghum panel using only SSRs appeared to be similar to what was seen when the 322 SNPs were also included. In contrast, the PCoA eigenvectors explained less genetic variation using only SSRs. This discrepancy likely resulted from more rare alleles per locus, fewer loci, and a larger and more diverse germplasm set. From the combined data sets it appeared that the syrup sweet sorghums clustered best with kafirs,

Table 3. Variation explained by models with population structure (Q matrix) and/or kinship (K matrix) for brix and height in the sweet sorghum panel using TASSEL.

Q matrix	Number of populations (*k*)	R^2 model	
		Brix	Height
GLM†			
InStruct	11	0.39	0.28
InStruct	5	0.28	0.13
Structure	11	0.39	0.30
Structure	4	0.20	0.07
PCoA	12	0.09	0.14
PCoA	5	0.04	0.06
None	0	0	0
MLM‡			
InStruct + K	11	0.45	0.54
InStruct + K	5	0.41	0.49
Structure + K	11	0.49	0.55
Structure + K	4	0.40	0.50
PCoA + K	12	0.39	0.55
PCoA + K	5	0.39	0.55
None + K	0	0.37	0.48

†General linear model.

‡Mixed linear model includes kinship matrix (**K**).

Table 4. Markers with a significant *p*-value at 0.001 or highest F_{ST} in each category.

Name of marker	Name of locus	Chr. number	*p* value height	*p*_value brix	F_{ST} a priori	F_{ST} PCoA	F_{ST} height	F_{ST} brix
SB00016.1	pSB0945	9	1.89E-11†	1.64E-04	0.31	0.21	0.1	0.15
Xgap72	SSR	6	2.98E-09†	0.0027	0.19	0.28	0.06	0.09
Xtxp343	SSR	4	4.01E-06†	7.29E-04	0.07	0.12	0	0.04
Xtxp265	SSR	6	6.57E-05†	0.0684	0.08	0.15	0.02	0.05
SB00014.3	pSB0301	10	1.06E-04†	0.0117	0.14	0.26	0.08	0.03
SB00215.1	psb1812	3	4.55E-04	0.3014	0.03	0.06	−0.01	0.02
SB00156.1	pSB0289	3	5.19E-04	0.0703	0.1	0.08	0.03	0
SB00154.4	pSB0142	10	5.28E-04	0.3493	0.29	0.53	0.05	0
SB00135.1	pSB1224	2	8.56E-04	0.1051	0.05	0.14	0.01	−0.02
SB00166.1	G1R	1	0.0019	2.97E-05†	0.25	0.57	0.03	0.06
SB00053.1	PRC0271	3	0.1231	0.7493	0.36	0.77	0	0.19
mSbCIR276	SSR	3	0.0385	0.4162	0.09	0.07	0.03	0.18
SB00200.1	pSB0122	9	0.0052	0.113	0.24	0.27	0.19	0.18
SB00207.1	C2782	9	0.0235	0.13	0.33	0.49	0.08	0.17
SB00176.3	CrtrB2	0	0.1327	0.5122	0.37	0.68	−0.02	0.16
SB00083.1	pSB1015	6	0.0034	0.0431	0.19	0.54	0.03	0.16
SB00083.2	pSB1015	6	0.0217	0.0476	0.18	0.56	0.03	0.16
SB00028.3	AEST056	7	0.4696	0.1893	0.33	0.42	0.03	0.16
SB00176.5	CrtrB2	0	0.0071	0.005	0.31	0.56	0.02	0.15
SB00109.1	R2266	4	0.9256	0.5007	0.26	0.46	0.23	0.07
SB00084.1	pSB1018	1	0.0148	0.0739	0.17	0.25	0.14	0.04
Xcup42	SSR	10	0.0017	0.0363	0	0	0.13	0.06
SB00022.1	pSB1755	7	0.0015	0.0741	0.07	−0.02	0.11	0.08
SB00094.4	pSB1472	1	0.4345	0.2329	0.28	0.46	0.11	0.05
Xcup71	SSR	4	0.1173	0.1155	0.29	0.53	0.11	0.05
SB00161.1	pSB0716	7	0.0068	0.5694	0.12	0.05	0.1	0.08
SB00049.1	pRC0121	7	0.0105	0.8153	0.08	0.1	0.1	0.07
SB00052.1	pRC0162	0	0.0013	0.0778	0.26	0.78	−0.03	−0.01
SB00060.1	PRC1149	2	0.3466	0.1986	0.3	0.72	−0.01	0
SB00118.3	gpt	7	0.6391	0.4349	0.28	0.69	0.01	0.06
SB00029.1	C0086	3	0.3813	0.1303	0.24	0.67	0.02	0.01
SB00170.3	SPP1	0	0.0128	0.2413	0.22	0.64	−0.02	0.01
SB00131.5	LDreg4	8	0.844	0.5707	0.32	0.63	−0.01	0.1
SB00097.1	pSB1600	5	0.2062	0.8194	0.28	0.63	−0.02	0.06
SB00131.4	LDreg4	8	0.0141	0.1171	0.24	0.62	−0.01	0.09
SB00046.1	HHU62	8	0.3953	0.648	0.47	0.44	0.01	0.09
SB00088.1	pSB1231	3	0.4321	0.1005	0.4	0.23	−0.02	0.04
SB00141.1	pSB1445	4	0.5411	0.3787	0.36	0.4	−0.02	0.02
SB00094.5	pSB1472	1	0.6129	0.9712	0.36	0.37	0.08	0.01
SB00149.2	PHYB	1	0.1176	0.1735	0.35	0.46	0.01	0.04

†At $p < 0.00013$ ($p < 0.05$ corrected for multiple tests) using MLM in TASSEL.

and modern sugar energy sorghums and the landraces cluster best with caudatums. Amber types appeared to be poorly represented in the panel of Casa et al. (2008) but clustered most like bicolor types. In general, the SSR PCoA shows that the panels are structured very differently, the sweet sorghum panel has greater diversity from amber types, the panel of Casa et al. (2008) has much more diversity from durra and caudatum types.

Population Structure and Relatedness in the Sweet Sorghum Panel

We attempted three separate methods for population assignment of cultivars, Stucture, InStruct, and PCoA. Though they use different algorithms for calculation, all three methods suggested that three populations were an absolute minimum, and both four to five and 11 to 12 populations met our selection criteria. Though Structure is widely used for identifying population structure, the

program was developed for natural outcrossing populations. The sweet sorghum panel violates Structure's assumption of Hardy-Weinberg equilibrium and many lines share close pedigrees. InStruct, based on Structure, is a more valid method for a self-pollinated domesticated crop such as sorghum, because it relaxes the assumption of Hardy-Weinberg equilibrium (Gao et al., 2007). It was therefore surprising that Structure and InStruct resulted in nearly identical conclusions in this study. Finally, principal component analysis has been proposed to correct for population structure (Price et al., 2006) and similarly PCoA has been used in association mapping by Cockram et al. (2008). PCoA explained far more variation in this study than in Cockram et al., but the results of this approach were still disappointing in controlling for population structure.

Two main problems with population structure estimates are that they are subjective, on the basis of selection criteria, and they reduce very complex relationships into only a few numbers for population assignment. Thus, it is difficult to completely correct for genetic relationships using structure alone. From our results and model fit, it appears that using the kinship matrix (**K** matrix; Yu et al., 2006) better controlled for relatedness than any measure of population structure (**Q** matrix). In fact, we had better fit and fewer positive tests using **K** without **Q** than with any **Q** alone. It seems likely that this will be true for most bred material where admixed diverse crosses are routine, and closely related material has been selected.

Brix and Height QTL Association

The *Sorghum bicolor* genome is estimated to contain 811Mbp of DNA (Price et al., 2005). With 369 markers, the coverage in this study averaged one marker in 2.2 Mbps. Although sorghum has much greater LD than maize, extending from a few kb to over 35kb, on the basis of the results of Hamblin et al. (2005) we would need at least 55,000 polymorphic markers for a saturated whole genome scan. However, LD is expected to vary greatly across genomic regions and different germplasms investigated. On the basis of the pairwise linkage disequilibrium between markers (Supplemental Fig. 1) the linkage blocks were not saturated in this population.

Given the average extent of LD in sorghum (Hamblin et al., 2005), it is unlikely that any marker locus tested was a causal polymorphism for phenotypic variation, but instead likely linked to the causal polymorphism. Two of the five positive height associations Xgap72, and Xtxp265, were on the same chromosome about 10Mb apart. QTL for height and/or flowering time have been found in this location on chromosome 6, corresponding to the photoperiod sensitivity gene *ma1* (Lin et al., 1995; Rami et al., 1998; Brown et al., 2006; Murray et al., 2008a). This gene has undergone extremely strong selection for temperate adaptation in sorghum and detection over a long physical distance was not surprising.

The most significant QTL in this study was found on chromosome 9 for height. QTL for height in this location have been detected both by QTL linkage analysis (Pereira and Lee, 1995; Lin et al., 1995; Murray et al., 2008a) and by association analysis (Brown et al., 2008). Association analysis in the panel of Casa et al. (2008) detected a peak approximately 400kb away, with significant locus associations on both sides of the marker (SB00016.1) used in this study (Brown et al., 2008). This locus would also be expected to have long range LD given the strength of selection in sorghum for height.

The only significant association for brix, on chromosome 1, has also not been previously reported in linkage mapping studies. However, Murray et al. (2008a) did detect a QTL peak near this region in one location (the closest marker was Txp482, 5Mb away). This peak explained up to 9% of the variation for brix and sugar, but was slightly below the stringent threshold for significance (unpublished data). On the physical genome sequence, a sorghum homolog to glucose-6-phosphate isomerase (EC 5.3.1.9) is located ~12kb away, the third closest predicted gene. Although this enzyme has not previously been implicated in stem sugar accumulation, it is known to convert D-glucose 6-phosphate into D-fructose 6-phosphate, both of which are important for synthesizing sucrose (Kanehisa et al., 2006).

We also attempted to identify additional markers for association mapping to support a QTL for Brix on chromosome 3 detected by Natoli et al. (2002) and Murray et al. (2008a), but were unsuccessful. Furthermore, association analysis using three SSRs and one SNP in this region did not detect any significant associations.

Implications for Germplasm Collection, Conservation, and Breeding

The results of this analysis suggest that for genetic studies, and/or core collection development, as few as five cultivars from the sweet sorghum panel could be selected to represent 90% of the SNP alleles identified. Thus, within the sweet sorghum panel, many of the accessions could be considered redundant for germplasm conservation, especially in the population of syrup cultivars. These differences reflect close pedigrees with similar parentage.

To identify the most informative markers to differentiate the three main groupings, F_{ST} for each marker was calculated between populations defined on the basis of PCoA. A few of the markers having high F_{ST} (PCoA column in Table 4) could be applied to identify a population for sweet sorghums not included in this panel.

The diversity partitioned within sweet sorghum and between sweet and grain sorghum has implications for how this germplasm should be maintained. An interesting observation regarding same named accessions, the six Sugar Drips for example, is that older cultivars were more diverse than the newer ones. There are two obvious explanations, residual heterozygosity would be greater for landraces than for elite cultivars, and over time more outcrossing is likely to occur. Inexpensive DNA markers may make testing easy, but it may be prudent, to reduce redundancy in core collections that duplicates of modern named materials should be removed before historical landraces.

For crop improvement, understanding the diversity present within the three identified groupings and their subgroupings is important. For breeding of syrup cultivars, a larger and less diverse selection of elite material from the modern syrup cultivars would be most useful. For breeding energy types for biofuel (lignocellulose and sugar), further selections from within the sugar and energy population and hybrids across groupings would be most appropriate.

Conclusions

We have identified three major groupings within sweet sorghum, each with multiple subgroupings. This information is beneficial for understanding the origin of sweet sorghums and to identify material for further improvement. These groupings showed some clustering similar to racial types within grain sorghums, but sweet and grain sorghums remain distinct in phenotype and origin. We have identified a marker with significant association for brix and identified a nearby candidate gene, glucose-6-phosphate isomerase, to be tested in the future. Future work within and across these populations may enable molecular cloning of genes responsible for stem-sugar accumulation in sorghum. Understanding the genetic basis for variation in stem sugar may ultimately allow genetic improvement of relatives with more complex genomes such as sugarcane, maize, switchgrass, and miscanthus.

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From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: sweet sorghum association mapping
Date: Monday, April 27, 2009 12:44:00 PM

Bud picked up four hybrids on Friday - that is all. So if that is the four, then yes. If that is not the four, then no.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
Sent: Monday, April 27, 2009 11:39 AM
To: Bill Rooney
Subject: RE: sweet sorghum association mapping
Importance: High

Question: via Walter, there are 4 more sweet hybrids we are getting for trials this season?

J Jefferson Gwyn Ph D
Director of Breeding
Ceres, Inc.
3199 County Road 269 East
Somerville, TX 77879
off 979.272.2265
fax 979.272.2269
cell 805.490.0070
www.ceres.net

From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Monday, April 27, 2009 10:50 AM
To: Jeff Gwyn
Subject: RE: sweet sorghum association mapping

Jeff:

In the presentation on Thursday, I mentioned that we had a mapping population in the field (in cooperation with Veremis at UFL). I also mentioned that this was followup work to research done by Seth Murray (while he was a Ph.D. student at Cornell). From that work, Seth has published three papers (this was the final publication from that work. I've attached the first two (in case you don't have them).

The fieldwork for this paper was completed prior to the Ceres agreement. We have a version of this sweet sorghum panel in the field, mainly for reference and crossing work. We don't have plans to collect specific data on it this summer. If there is an interest from Ceres, you would be able to utilize it as long as it doesn't interfere with any crosses or seed production.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
Sent: Monday, April 27, 2009 10:35 AM
To: Bill Rooney
Cc: Nickolai Alexandrov
Subject: FW: sweet sorghum association mapping

What do we know about this? I don't remember any discussion on sweets and genes and phenology.

Please advise.

From: Nickolai Alexandrov
Sent: Saturday, April 25, 2009 10:40 PM
To: Jeff Gwyn
Subject: RE: sweet sorghum association mapping

Interestingly, we have briefly discussed this paper on our MAB journal club last Wednesday. Jeff, do you think we should collect this kind of data for our internal breeding program?
Nick

From: Steven Thomas
Sent: Sat 4/25/2009 11:25 AM
To: Jeff Gwyn; Edgar Haro; Walter Nelson; Nickolai Alexandrov; John Bouck
Cc: Bonnie Hames; Tanya Kruse; Joon-Hyun Park; Roger Pennell; Richard Flavell; Spencer Swayze
Subject: sweet sorghum association mapping

See attachment for more detail (from The Plant Genome). st

Making Sweet Sorghum Sweeter

Submitted by James Giese on Fri, 04/17/2009 - 14:48

- Feature

Sweet sorghum, like its close relative, sugarcane, has been bred to accumulate high levels of edible sugars in the stem. Sweet sorghums are tall and produce high biomass in addition to sugar. However, there is little documentation about the genetic relationships and diversity within sweet sorghums and how sweet sorghums relate to grain sorghum racial types.

Researchers from Cornell and Texas A&M genotyped with simple sequence repeats and single nucleotide polymorphisms a diverse panel of 125 (mostly sweet) sorghums. Using

both distance-based and model-based methods, the researchers identified three main genetic groupings of sweet sorghums. Based on observed phenotypes and known origins, these were classified as historical and modern syrup, modern sugar/energy, and amber types.

Three significant associations for height were detected. Two of these, on chromosomes 9 and 6, support published studies. One significant association for brix, on chromosome 1, was detected.

****

Steven R. Thomas, Ph.D.
Director of Bioproducts
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320

ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net

****

From: Bill Rooney
To: "Jeff Gwyn"
Subject: RE: sweet sorghum association mapping
Date: Monday, April 27, 2009 1:22:00 PM

No, they are the same females using Umbrella as the pollinator.

Walter asked for an early maturing hybrids to include in Ceres trials - most that you folks made were mid season and full season hybrids and these are earlies.

Does that help?

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
> **Sent:** Monday, April 27, 2009 1:03 PM
> **To:** Bill Rooney
> **Subject:** RE: sweet sorghum association mapping
>
> But where did they come from? Is this entirely new material that didn't exist last fall or are they checks or what?
>
> J Jefferson Gwyn Ph D
> Director of Breeding
> Ceres, Inc.
> 3199 County Road 269 East
> Somerville, TX 77879
> off 979.272.2265
> fax 979.272.2269
> cell 805.490.0070
> www.ceres.net
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Monday, April 27, 2009 12:58 PM
> **To:** Jeff Gwyn
> **Subject:** RE: sweet sorghum association mapping
>
> Bud picked up four hybrids on Friday - that is all. So if that is the four, then yes. If that is not the four, then no.
>
> bill
>
> Dr. William L. Rooney
> Professor, Sorghum Breeding and Genetics
> Chair, Plant Release Committee
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> College Station, Texas 77843-2474
> 979 845 2151
>
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To: Bill Rooney
Subject: RE: sweet sorghum association mapping
Importance: High

Question: via Walter, there are 4 more sweet hybrids we are getting for trials this season?

J Jefferson Gwyn Ph D
Director of Breeding
Ceres, Inc.
3199 County Road 269 East
Somerville, TX 77879
off 979.272.2265
fax 979.272.2269
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From: Bill Rooney [mailto:wlr@tamu.edu]
Sent: Monday, April 27, 2009 10:50 AM
To: Jeff Gwyn
Subject: RE: sweet sorghum association mapping

Jeff:

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The fieldwork for this paper was completed prior to the Ceres agreement. We have a version of this sweet sorghum panel in the field, mainly for reference and crossing work. We don't have plans to collect specific data on it this summer. If there is an interest from Ceres, you would be able to utilize it as long as it doesn't interfere with any crosses or seed production.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Jeff Gwyn [mailto:jgwyn@ceres-inc.com]
> **Sent:** Monday, April 27, 2009 10:35 AM
> **To:** Bill Rooney
> **Cc:** Nickolai Alexandrov
> **Subject:** FW: sweet sorghum association mapping
>
> What do we know about this? I don't remember any discussion on sweets and genes and phenology.
>
> Please advise.

From: Nickolai Alexandrov
Sent: Saturday, April 25, 2009 10:40 PM
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Interestingly, we have briefly discussed this paper on our MAB journal club last Wednesday. Jeff, do you think we should collect this kind of data for our internal breeding program?
Nick

From: Steven Thomas
Sent: Sat 4/25/2009 11:25 AM
To: Jeff Gwyn; Edgar Haro; Walter Nelson; Nickolai Alexandrov; John Bouck
Cc: Bonnie Hames; Tanya Kruse; Joon-Hyun Park; Roger Pennell; Richard Flavell; Spencer Swayze
Subject: sweet sorghum association mapping

See attachment for more detail (from The Plant Genome). st

Making Sweet Sorghum Sweeter

Submitted by James Giese on Fri, 04/17/2009 - 14:48

- Feature

Sweet sorghum, like its close relative, sugarcane, has been bred to accumulate high levels of edible sugars in the stem. Sweet sorghums are tall and produce high biomass in addition to sugar. However, there is little documentation about the genetic relationships and diversity within sweet sorghums and how sweet sorghums relate to grain sorghum racial types.

Researchers from Cornell and Texas A&M genotyped with simple sequence repeats and single nucleotide polymorphisms a diverse panel of 125 (mostly sweet) sorghums. Using both distance-based and model-based methods, the researchers identified three main genetic groupings of sweet sorghums. Based on observed phenotypes and known origins, these were classified as historical and modern syrup, modern sugar/energy, and amber types.

Three significant associations for height were detected. Two of these, on chromosomes 9 and 6, support published studies. One significant association for brix, on chromosome 1, was detected.

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Steven R. Thomas, Ph.D.
Director of Bioproducts
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Thousand Oaks, CA 91320

ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net
****

From: Bill Rooney
To: "Walter Nelson"; "Bud Wylie"
Subject: RE: Umbrella hybrids
Date: Wednesday, April 29, 2009 7:30:00 AM

Walter and Bud:

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Tuesday, April 28, 2009 7:06 PM
> **To:** Bill Rooney
> **Subject:** Umbrella hybrids
>
> Hi Bill,
>
> I need to re-code the umbrella hybrids you gave Bud for our trials. Can you send me the codes and pedigrees for the hybrids you gave him?
>
> Thanks!
>
> Walter
>
> ---
>
> **From:** Bud Wylie
> **Sent:** Tuesday, April 28, 2009 3:26 PM
> **To:** Walter Nelson
> **Subject:** Umbrella
>
> Walter,
>
> What are the codes for the Umbrella hybrids?
>
> Bud Wylie

Manager, Commercial Trials
Ceres, Inc
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
210-882-7257
bwylie@ceres-inc.com

From: Bill Rooney
To: "Bud Wylie"
Subject: RE: Umbrella hybrids
Date: Wednesday, April 29, 2009 1:36:00 PM

Bud:

I am not up to full speed on what Jurg has planned - I need to get so, but it won't be until next week before I can do so.

I am coordinating a stagger planted continuous harvest trial in College Station this year. It is not planted in Lacassine this year. This project was submitted to the South Central SunGrant Project for funding beginning NEXT summer. If it is funded we will be doing the work in both CS and Lacassine.

If you have questions just give me a call at the office after 4 pm.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Bud Wylie [mailto:bwylie@ceres-inc.com]
> **Sent:** Wednesday, April 29, 2009 8:34 AM
> **To:** Bill Rooney
> **Subject:** RE: Umbrella hybrids
>
> Bill,
>
> What type of trials does Juerg have planned for this year? Jeff just mentioned that you or Juerg was doing a maturity yield trial with the sweets maybe in Lacasseine and here? If you have time, would you give me a call so I can understand what is being done as part of our agreement?
>
> Bud Wylie
> *Manager, Commercial Trials*
> *Ceres, Inc*
> *1535 Rancho Conejo Blvd.*
> *Thousand Oaks, CA 91320*
> *210-882-7257*
> *bwylie@ceres-inc.com*
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Wed 4/29/2009 7:30 AM
> **To:** Walter Nelson; Bud Wylie
> **Subject:** RE: Umbrella hybrids

Walter and Bud:

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
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>
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>
> Bud Wylie
> *Manager, Commercial Trials*
> *Ceres, Inc*
> *1535 Rancho Conejo Blvd.*
> *Thousand Oaks, CA 91320*

210-882-7257
bwylie@ceres-inc.com

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Visit Monday
Date: Tuesday, June 16, 2009 12:45:00 PM

Walter:

I'll be here on Monday so just give me a call. Almost certain I'll be in the field.

As for Tuesday, the answer is maybe it depends on how thing progress in the nursery. I've got to get a trip to weslaco in the plans as well.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Tuesday, June 16, 2009 8:45 AM
> **To:** Bill Rooney
> **Subject:** Visit Monday
>
> Bill,
>
> Looks like I'll be out on Monday to meet with the licensing folks for a few hours. Would like to stop by the fields at some point if ok with you. Can get a map or something from you or Delroy or whomever if you're not around.
>
> Also, still had in my mind an interest in visiting the LA Greenfuels guys with you at some point. Interested in going on Tuesday? I will probably go visit with Spencer sometime in July, so not urgent in any way...but thought it would be useful to go with you at some point as well. Just a thought....
>
> W

Walter E Nelson
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
voice: (805)376-6548
www.ceres.net

From:	Bill Rooney
To:	"Walter Nelson"
Cc:	"dustin borden"
Subject:	RE: Visit to CS on Monday
Date:	Thursday, July 09, 2009 7:44:00 AM

Walter:

I'll be here early on Monday, but must leave by 9 am. So I can start a visiti with you; I would recommend Dustin to come along and finish the visit as necessary.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Wednesday, July 08, 2009 4:04 PM
> **To:** Bill Rooney
> **Subject:** Visit to CS on Monday
>
> Bill,
>
> I will be arriving in College Station on Sunday and will be there for one day. Would like to visit the fields on Monday morning. Would someone over there be available to show me around? Am flexible on time, but will be asking Juerg the same question.
>
> Walter

Walter E Nelson
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320
voice: (805)376-6548
www.ceres.net

From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Visit to CS on Monday
Date: Thursday, July 09, 2009 10:58:00 AM

Walter:

Are you coming in early or late on Sunday? If early, then we could meet Sunday evening. If not, then we'll meet on Monday morning by 7 am.

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Thursday, July 09, 2009 8:50 AM
> **To:** Bill Rooney
> **Cc:** dustin borden
> **Subject:** RE: Visit to CS on Monday
>
> That sounds great. What time would you like to start?
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Thursday, July 09, 2009 5:44 AM
> **To:** Walter Nelson
> **Cc:** 'dustin borden'
> **Subject:** RE: Visit to CS on Monday
>
> Walter:
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> I'll be here early on Monday, but must leave by 9 am. So I can start a visiti with you; I would recommend Dustin to come along and finish the visit as necessary.
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> regards,
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> bill
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> Dr. William L. Rooney
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Subject: RE: Visit to CS on Monday
Date: Thursday, July 09, 2009 10:58:00 AM

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Walter

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From: Bill Rooney
To: "Walter Nelson"
Subject: RE: Visit to CS on Monday
Date: Thursday, July 09, 2009 11:31:00 AM

That would be fine.
bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

> -----Original Message-----
> **From:** Walter Nelson [mailto:wnelson@ceres.net]
> **Sent:** Thursday, July 09, 2009 11:12 AM
> **To:** Bill Rooney
> **Subject:** RE: Visit to CS on Monday
>
> Going to have to do Monday morning...don't get into CS until probably 9-10 pm.
>
> Want to meet at the farm?
>
> W
>
> ---
>
> **From:** Bill Rooney [mailto:wlr@tamu.edu]
> **Sent:** Thursday, July 09, 2009 8:58 AM
> **To:** Walter Nelson
> **Subject:** RE: Visit to CS on Monday
>
> Walter:
>
> Are you coming in early or late on Sunday? If early, then we could meet Sunday evening. If not, then we'll meet on Monday morning by 7 am.
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> > **Cc:** dustin borden
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That sounds great. What time would you like to start?

W

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voice: (805)376-6548
www.ceres.net

From: Bill Rooney
To: "Steven Thomas"
Subject: RE: CPBR letter
Date: Tuesday, June 16, 2009 4:05:00 PM
Attachments: 2010 CPBR Proposal Rooney.DOC

Here is a first draft, obviously subject to change...

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

-----Original Message-----
From: Steven Thomas [mailto:sthomas@ceres-inc.com]
Sent: Tuesday, June 16, 2009 1:08 PM
To: wlr@tamu.edu
Cc: Walter Nelson
Subject: CPBR letter

Hi Bill,

Walter tells me that you have requested a letter of support from

If so, I'd like to take advantage of that and include it in the letter. Also, when do you need the letter?

If you think we should talk through this, let me know when you are available and I will call you.

Thanks and best regards, Steve

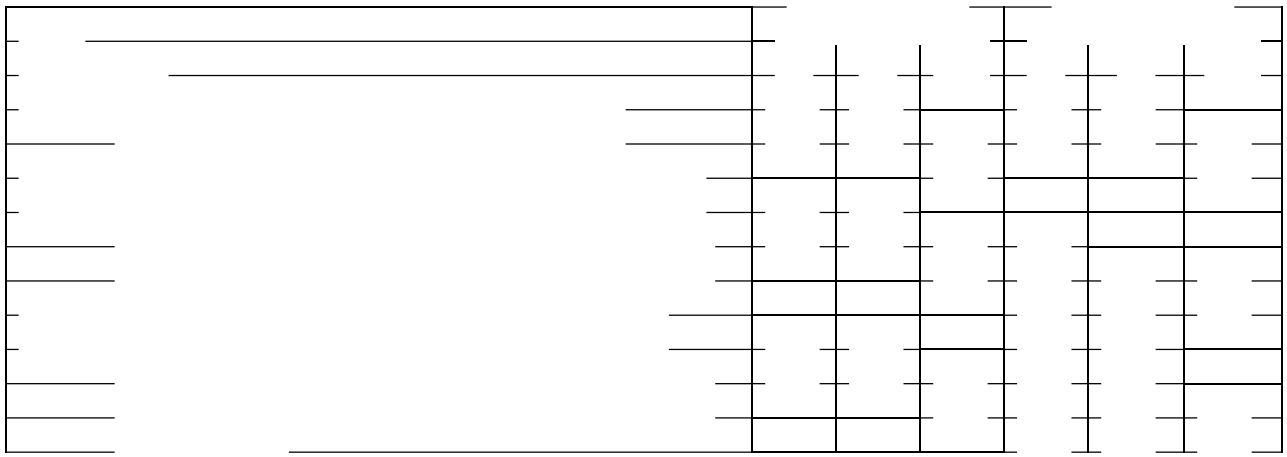
****
Steven R. Thomas, Ph.D.
Director of Bioproducts
Ceres, Inc.
1535 Rancho Conejo Blvd.
Thousand Oaks, CA 91320

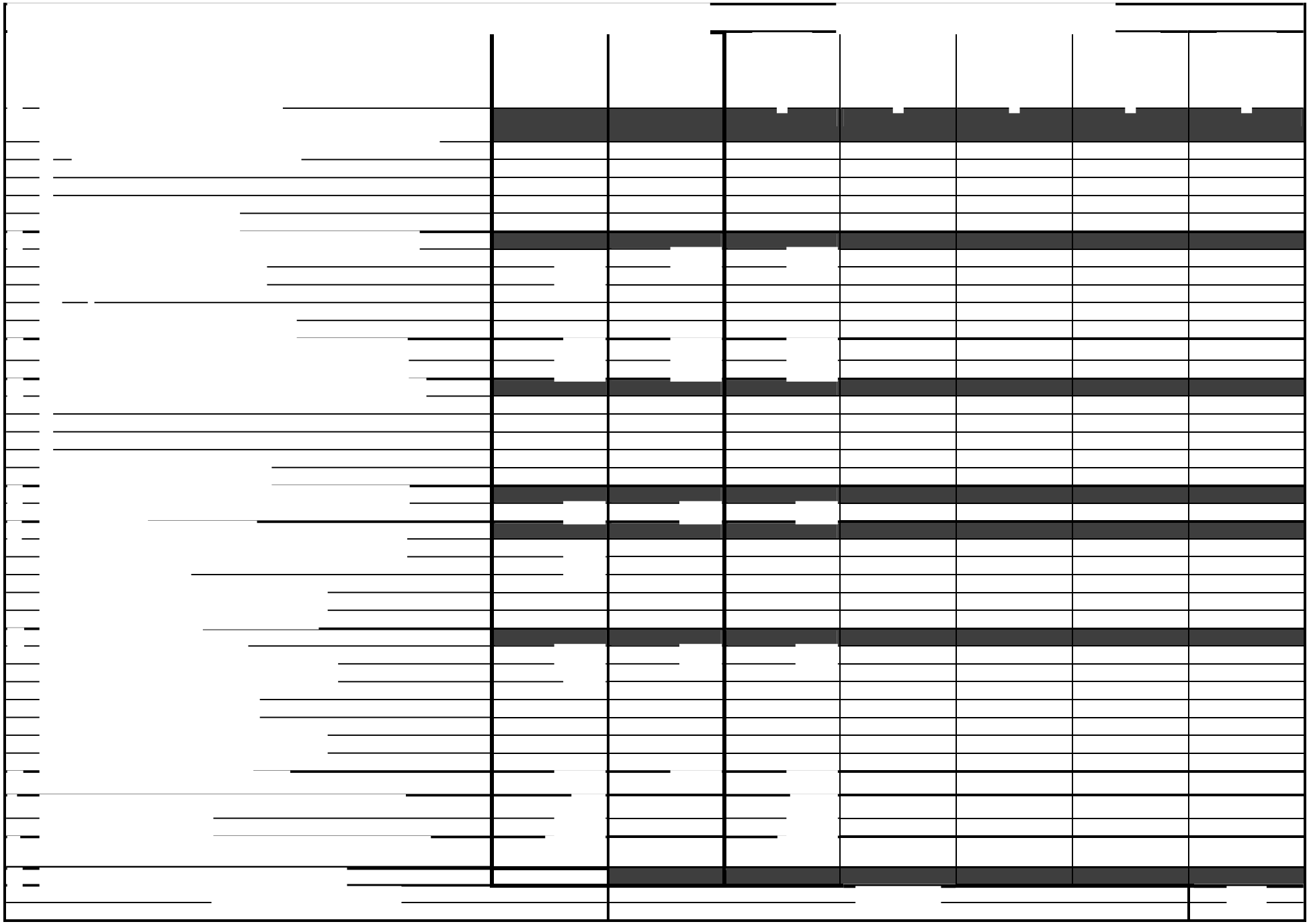
ph: (805) 376-6514
cell: (805) 807-6412
email: sthomas@ceres-inc.com
web: http://www.ceres.net
****

Executive Summary:

Alternate Title:

Anticipated Economic and Environmental Benefits:





AUTHORIZED INSTITUTIONAL OFFICIAL	SIGNATURE	DATE

From:	Bill Rooney
To:	"Walter Nelson"
Subject:	sorry
Date:	Thursday, May 07, 2009 8:15:00 AM

Walter:

FYI, I just gave a fellow your name as a potential contact for sweet sorghum and energy sorghum seed (I told him you would not give out sweet sorghum seed, but I wasn't sure about energy sorghum).

He's from Maryland, claimed he hasn't talked to you, but he may have. He's one that has all the answers and big plans, but nobody to fund it.

Sorry about this, but thought deserved a heads up before you get the phone call.

regards,

bill

Dr. William L. Rooney
Professor, Sorghum Breeding and Genetics
Chair, Plant Release Committee
Texas A&M University
College Station, Texas 77843-2474
979 845 2151

From: Bill Rooney
To: "Steven Thomas"
Subject: we got the letter and all is well
Date: Thursday, June 25, 2009 5:26:00 AM

Steve:

Everything is good, received the letter while finalizing the document and it is now in processing.

regards,

bill

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