IAEA Project RAS - 5070

Year-1 and2 Progress Report - July 2017

Developing Bioenergy Crops to Optimize Marginal Land Productivity through Mutation Breeding and Related Techniques (RCA)

Sweet sorghum

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Outlines

- Background information
- Objectives
- Field design and data collection
- Available infrastructure and facilities
- Main progress
- Future plan

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Background information

- Why Sweet Sorghum
- History of Sweet Sorghum in China
- Sweet Sorghum Breeding
- Cultivation of Sweet Sorghum

Biology of Sweet Sorghum

Stems & Spikes
of Sweet Sorghum

Seeds and Root System

Sweet Sorghum Seeds

Juicy Mid-juicy Dry

甜高粱保持系和不育系608A甜秆特征

Why Sweet Sorghum?

Salinity tolerance

Sweet sorghum in saline/alkaline region in Hebei Province

2006年8月海冰水灌溉试验地及周围地区沥涝淹半个月甜高粱仍正常生长

Sweet sorghum growth after two weeks of logging in tidal land.

A Good Energy Crop--Bioethanol

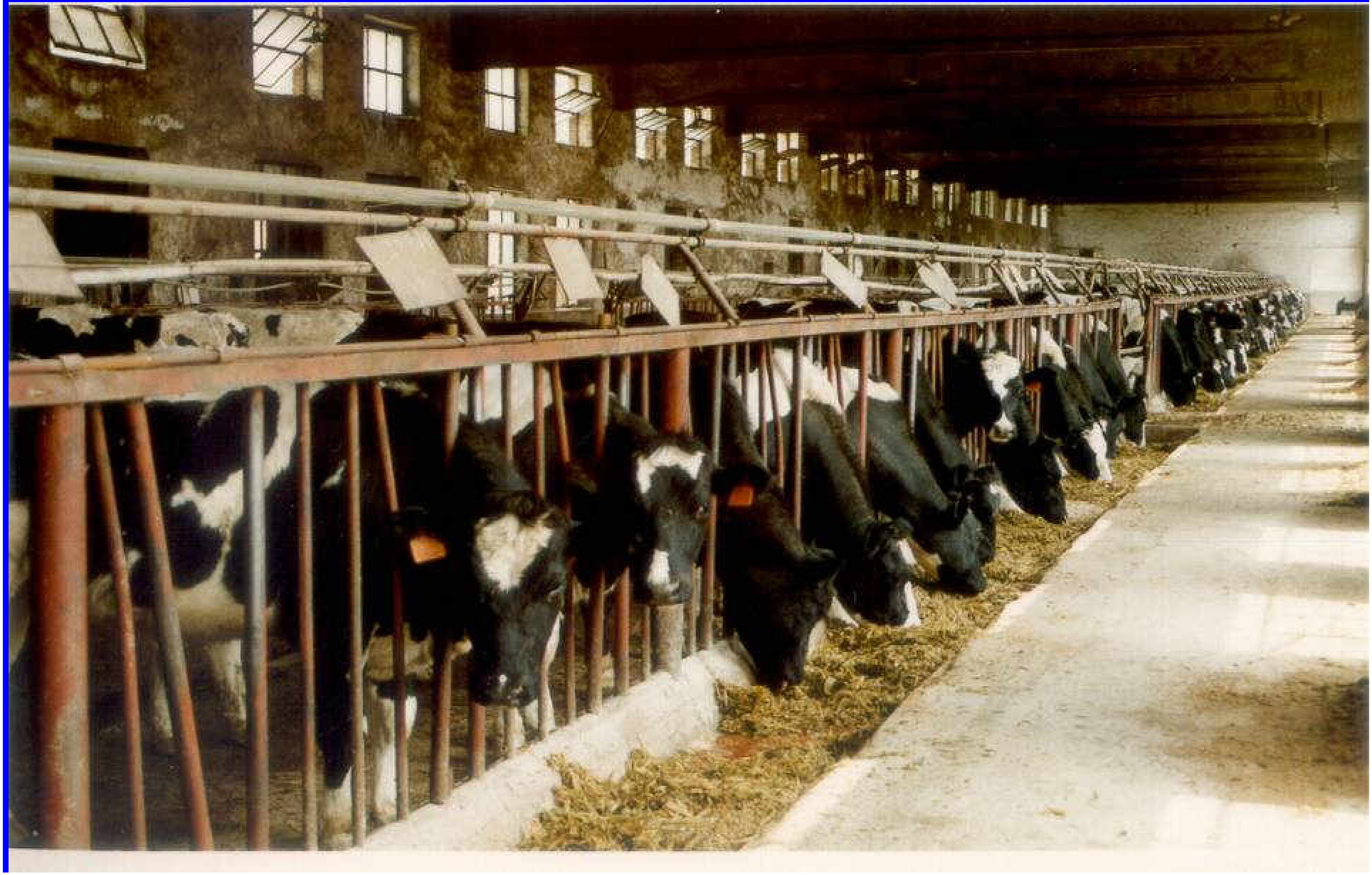
--High biomass yield, high sugar content;

-- Wide adaptation, can be grown on non-arable marginal land ;

--High potential for comprehensive utilization-grain, ethanol, syrup, vinegar, fodder, paper.

Combine harvesting of sweet sorghum for silage making

Cows eating sweet sorghum silage



The Potential and Advantages of Development of Energy Crops

- **Abundant marginal land and non-cultivated land in China:130-136 million ha**
- **Saline-alkali soils : 33.5 million ha**
- **Arable saline-alkali soils : 6.7 million ha**

History of Sweet Sorghum in China

- More than 5000 years of history ????
- Planting in a small scale for chewing the stem juice.
- 1853: a famous variety “Amber” introduced into USA via France embassy in Shanghai— the first sweet sorghum in the USA.
- 1970s-1980s: attempt to produce sugar, not large scale use.
- 1980s-1990s: UNDP project--energy use/feed use
- 1995,1997: 1st and 2nd sweet sorghum conference
- 1998-2000: silence
- 2001- today: FAO project, several national projects for energy.
- 2007: 3rd national sweet sorghum conference and first workshop on its industrialization.

Sweet sorghum in hometown of “Amber”- Chongming Island of Shanghai

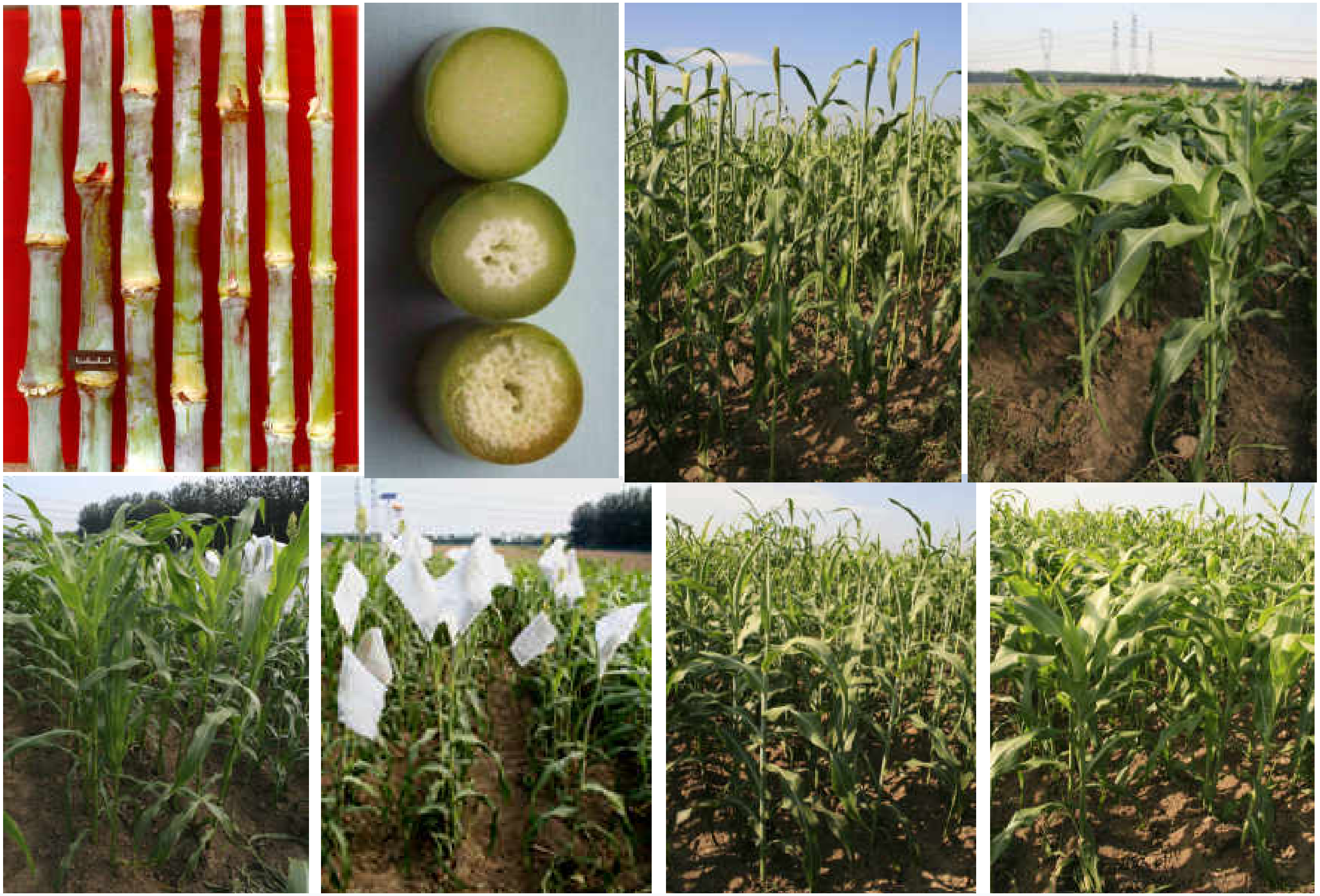
A Mini-market of Sweet Sorghum

Sweet sorghum producer in Chongming Island of Shanghai

Mutation Breeding in Sweet Sorghum

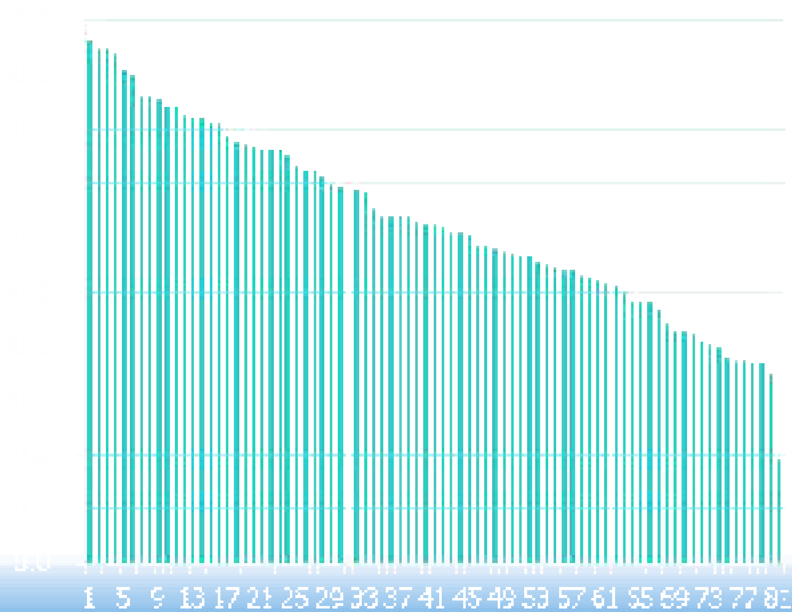
- Germplasm collection and evaluation
- New variety development and application

➢ Diversity of 206 sweet sorghum germplasm



Salt tolerance of sweet sorghum and physiological effects of salt stress

Performance of 81 genotypes in saline land, with biological production from 19.1 to 96.4t/ha.



New CMS lines of sweet sorghum and F1 hybrid test

Bio-effect of Proton (P) Implantation and Synchrotron (SR) Irradiation Compared with Gamma Rays

Damage effect:

P<SR< **γ<CK**

8002	**SR**	**γ**	**P**
CK	**86mA/5'**	**200Gy**	**300Gy**

Spike Variations of sweet sorghum after proton irradiation

CK

M_3 generation

Extension of New Mutant Variety Yuantian No.1

Yuantian No.1


Field evaluation of new mutants / hybrids

Development of sweet sorghum in saline alkaline region for biomass energy

Development of sweet sorghum in saline alkaline region for biomass energy

Extension:

Ca. 1000 hectares

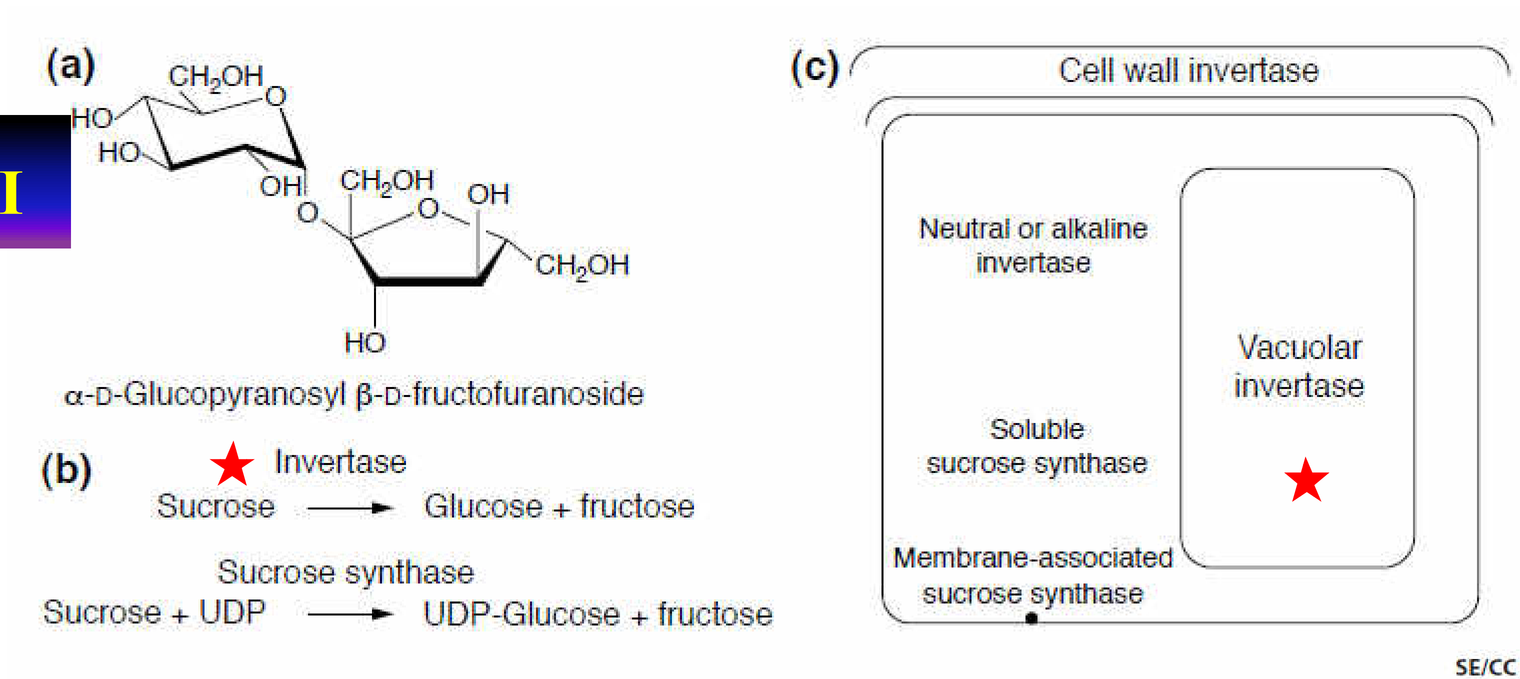
能饲1号在盐碱地上的生长情况

Research on molecular breeding

1、Research on Sugar metabolism related gene

2、Sorghum breeding and germplasm enhancement

Roles of SAI



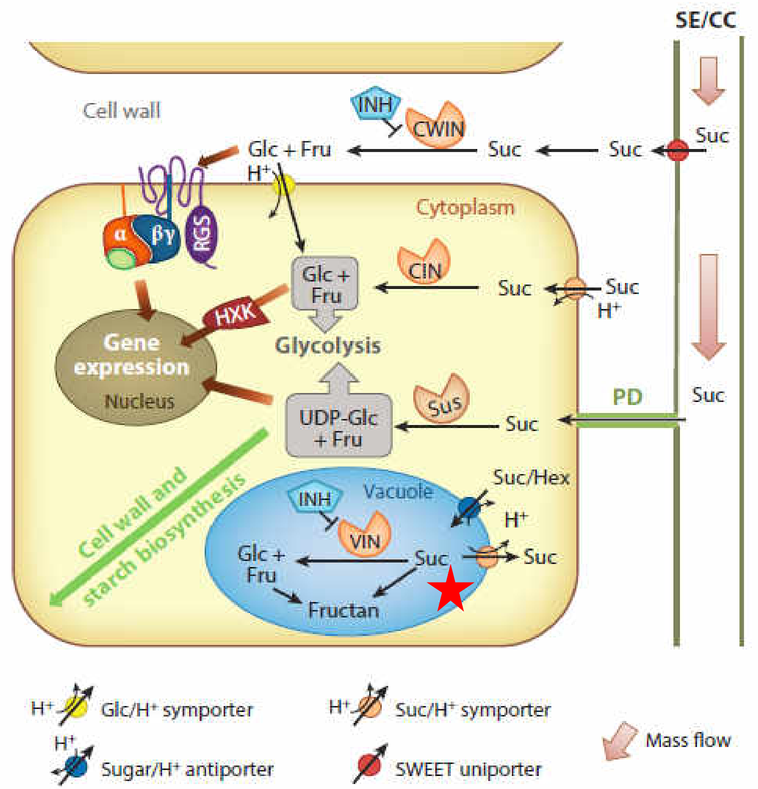
Box 1. Proposed functions of invertases and sucrose synthase

Cell wall invertase
- Sucrose partitioning between source and sink organs.
- Response to wounding and infection.
- Control of cell differentiation and plant development.

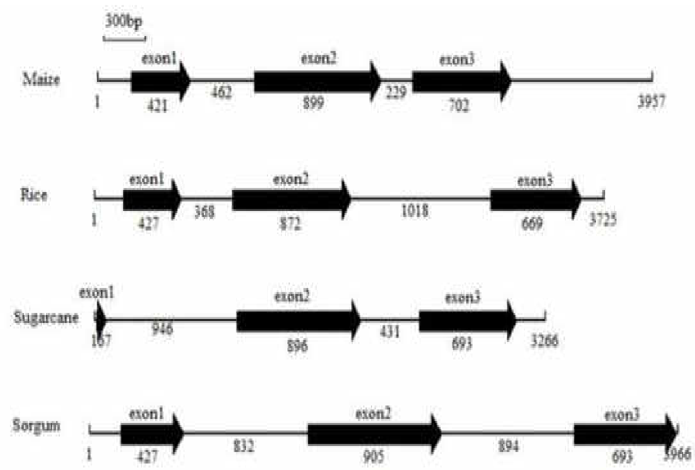
Vacuolar invertase ★
- Osmoregulation and cell enlargement.
- Control of sugar composition in fruits and storage organs.
- Response to cold (cold sweetening).

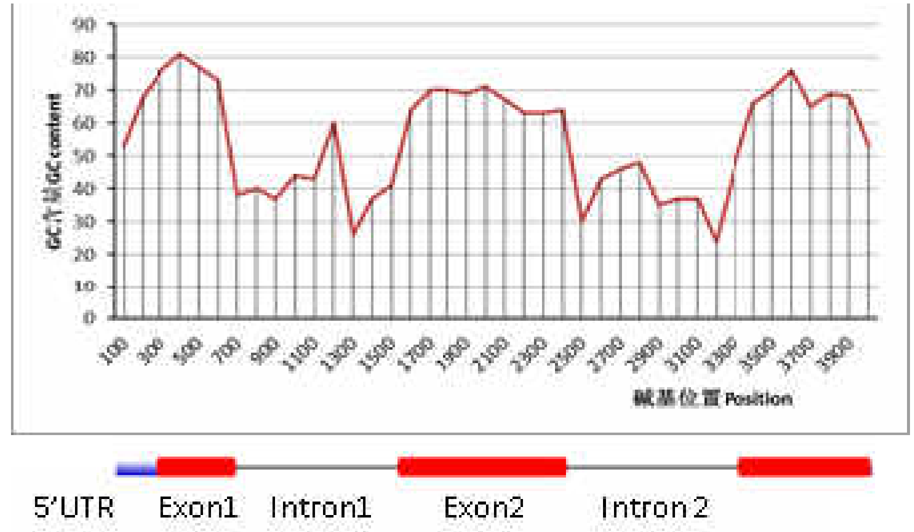
Cytoplasmic invertase
- Largely unknown but is probably involved in channeling sucrose into metabolism (catabolism).

Sucrose synthase
- Channeling of sucrose into metabolism (anabolism).
- Sucrose partitioning between source and sink organs.
- Response to anaerobiosis and cold.

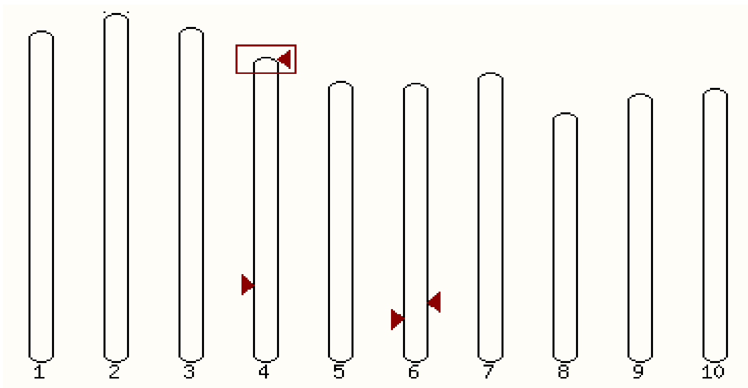


Characterization of *SAI* Gene

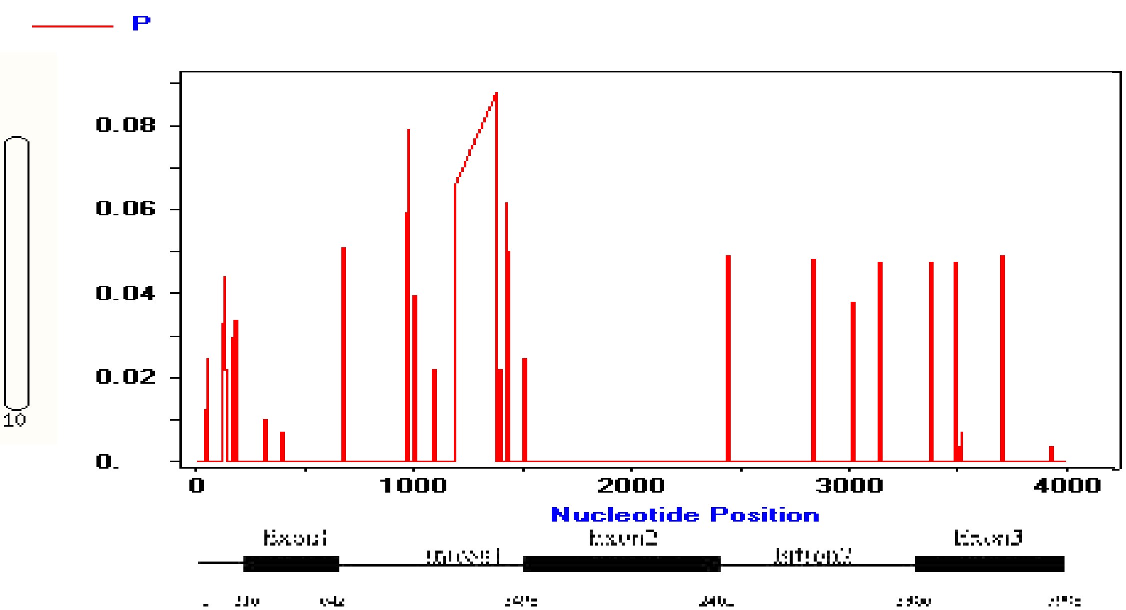




Distribution of GC content of *SAI-1* sequence.

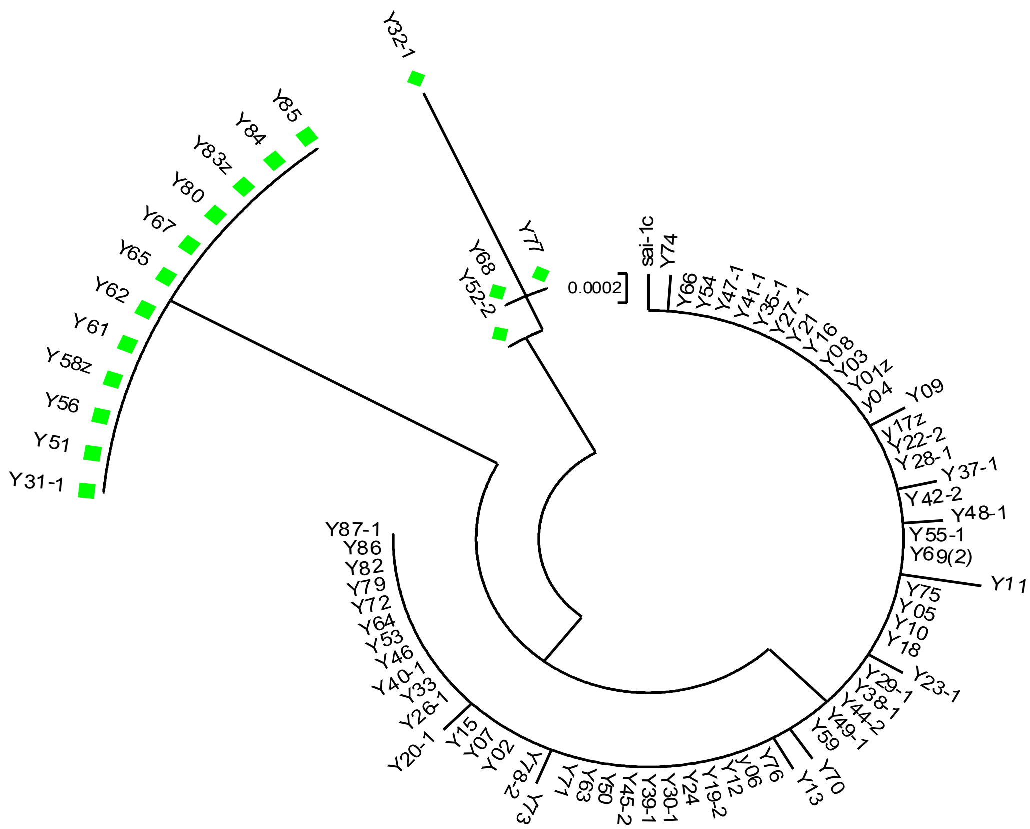


Location and copies of *SAI-1* gene on sorghum chromosome



Slide window of sequence variation of *SAI-1*

Phylogenic tree of *SAI* gene in sweet sorghum and grain sorghum



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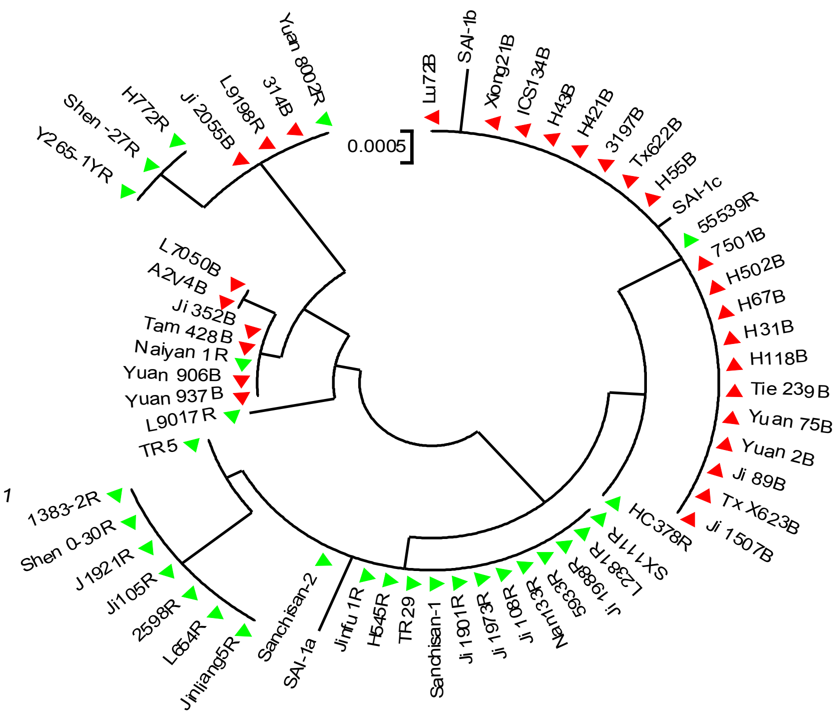
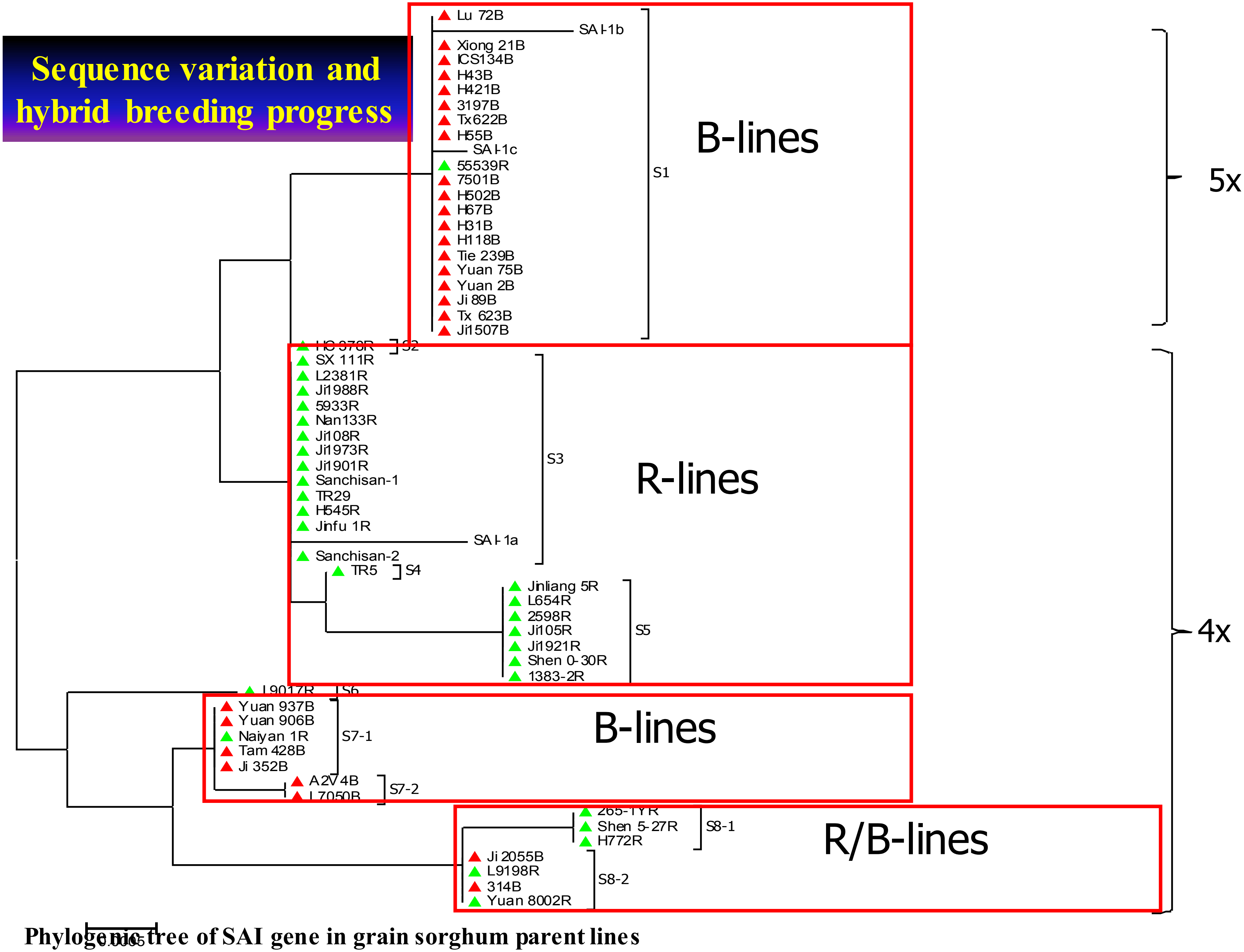
Sweet sorghum



Stand for B， R lines

Grain sorghum

Sequence variation and hybrid breeding progress



Phylogenic tree of SAI gene in grain sorghum parent lines

甜高粱*SAI-1*等位变异与农艺性状间的相关性

甜高粱*SAI-1*等位变异与主要农艺性状的关联性，R^2(上)，*p*(下)

变异位置 Loci-pos	变异类型 Variation type	生育期 Growth period	株高 Plant height	茎粗 Stem diameter	茎节数 Numbers of Node	糖 锤 度 Brix %	单株茎鲜重 Fresh weight /Stem	单株鲜重 Fresh weight /Plant	单株干重 Dryweigh t /Plant
760	1/2b Ins	0.0525 0.0409			0.0785 0.0118			0.0740 0.0146	0.0750 0.0139
985	0/1/2/3/4/5 bIns	0.2232 0.0019		0.1629 0.0198	0.1996 0.0049		0.2198 0.0022	0.2676 0.0003	0.2370 0.0010
1105	1/2 b Ins	0.1077 0.0124	0.0771 0.0456		0.1394 0.0031		0.0995 0.0177	0.1359 0.0036	0.1504 0.0019
2892	1/2 b Ins	0.2370 0.0000		0.1562 0.0014	0.1734 0.0007	0.1871 0.0003	0.1709 0.0007	0.2500 0.0000	0.2108 0.0001
2964	5/10 b Del	0.2579 0.0000	0.1195 0.0075	0.2945 0.0000	0.2253 0.0001	0.2479 0.0000	0.2298 0.0000	0.2913 0.0000	0.2123 0.0001
3257	3 b Del	0.1789 0.0001		0.1258 0.0012	0.1108 0.0025	0.1560 0.0003	0.1260 0.0012	0.1952 0.0000	0.1545 0.0003

Correlation coefficient of *SAI-1* Length and agronomic traits

Region length	Correlation coefficient								SD	CV %
	Growth period	Plant height	Stem diameter	Numbers of Node	Brix %	Fresh weight /Stem	Fresh weight /Plant	Dryweight /Plant		
Full length	-0.3425	-0.1974	-0.4393	-0.2955	0.5675	-0.3304	-0.3690	-0.2608	2.7724	0.0700
Intron 1	0.4441	0.3035	0.3658	0.4269	-0.1491	0.3953	**0.4782**	**0.4722**	1.4513	0.1743
Intron 2	-0.4761	-0.3047	-0.5047	-0.4290	0.5136	-0.4472	**-0.5099**	**-0.4276**	3.4917	0.3909
Intron1+intron 2	-0.3512	-0.2151	-0.4248	-0.3030	0.5439	-0.3407	-0.3748	-0.2786	2.8990	0.1680

Agronomic traits of different haplotypes

Haplotype	No of Genotypes	Plant height (cm)	Stem diameter (mm)	Brix (%)	Plant fresh weight (g)	Plant dry weight (g)
SAI-1a1	5	288.2±50.7	20.47±2.59	14.38±2.45	824.73±259	216.92±73.56
SAI-1a2	11	312.6±54.2	21.23±1.27	15.67±1.58	999.4±171.34	272.66±48.68
SAI-1b	54	286.7±31.9	19.45±1.53	16.73±1.37	738.93±149.94	205.02±46.23
SAI-1c	10	260.3±31.6	17.61±2.29	18.21±1.49	596.33±207.75	173.63±45.59

Germplasm enhancement

Plant height:
1-1.3m
Green-Stay:
Excellent

Grain yield:
500-700kg/mu

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Objectives

(i)Plant breeding:

Mutant lines with desired traits selected and disseminated with best fit soil, nutrient and water technology guidelines produced.

(ii)Soil:

Effective utilization of the marginal lands under soil, water and nutrient management using nuclear techniques

Crop species： sweet sorghum, sweet potato

National Project Teams

- Institute of Crop Science, Chinese Acad of Agric Sci
- Institute of Botany, Chinese Acad of Sci
- China Agriculture University
- Qingdao Agriculture University
- Xuzhou Regional Research Inst of Agric Sci
- Enterprises/Company

Specific Works

- Development of potential advanced mutant lines suitable for marginal land
- Assessment of adaptation and yield potential under stress conditions
- Evaluation of soil and nutrient requirements for advanced mutant lines and varieties.
- Promotion and adaptation of technology packages to the end-users, and enhancement of collaboration in the country

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Field design

Variety（line）：

S1601： 2 B（ sorghum mt） / Cowley. Biomass 102t/ha，Plant height 4.0m，Brix 19.3%

S1602: 906B （ sorghum mt) /Yuantian 1. Biomass 97.5t/ha，Plant height 3.8m，Brix 21.1%

Density： 60000/ha，90000/ha，12000/ha，15000/ha，18000/ha

Sowing date： Spring, Summer

Fertilizer： Base fertilizer + Special fertilizer with for treatments: 0 kg/ha,150 kg/ha，225kg/ha, 300Kg/ha and 375kg/ha

Tiller： Tillers, tiller removal

Data measurement

1. Sowing date
2. Emerging date
3. Harvesting date
4. Plant height
5. Stalk diameter
6. Stalk yield per plot
7. Brix

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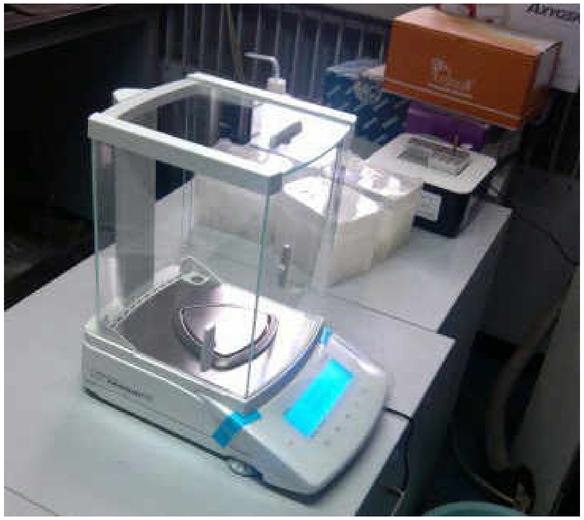
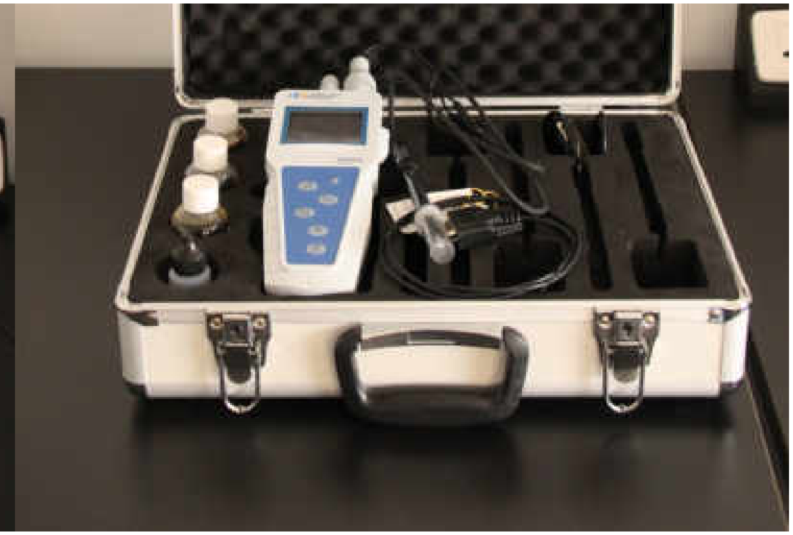
Experimental field
Lab facilities
Greenhouse

Electric scale/PCR/Low tem refrigerator/Fluorescent quantitative PCR



Seed storage
Root Scanner
LC/GC
Canopy analyzer
SPAD502/Chlorophyll meter

Fast soil nutrient analyzer
pH-meter
Neutron moisture meter
Stem strength meter
Elec conductivity meter



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Sorghum EMS Mutants for Basic Research

Sorghum EMS mutants


Brown veins


Leaf distortion


Stem diameter


Tillering


Dwarf


Polished rod (no wax)


Early maturity


Plant height

Aphid-senstive mutant

Aphid-resistant mutant

Leaf color mutant

Leaf shape mutant

Spike mutant

Salt , drought-resistant mutants

Main results of sweet sorghum breeding research (2016)

•**Basic research: the** other soluble acid invertase gene (SAI-2) was sequenced in 16 accessions. And great sequence variation was found the third intron. There are three groups of SAI-2 sequence，Group 1including accessions 9, 11, 20,25, it is long 1954bp (only for this segment) , group2, including accession 4,14, 32, 34, 47, 49,50, long 1888bp. Group 3 including accessions 1,2,19,28 and 51, long 1954bp. So great variation may mean a lot for breeding, because SAI take important role in regulating plant growth.

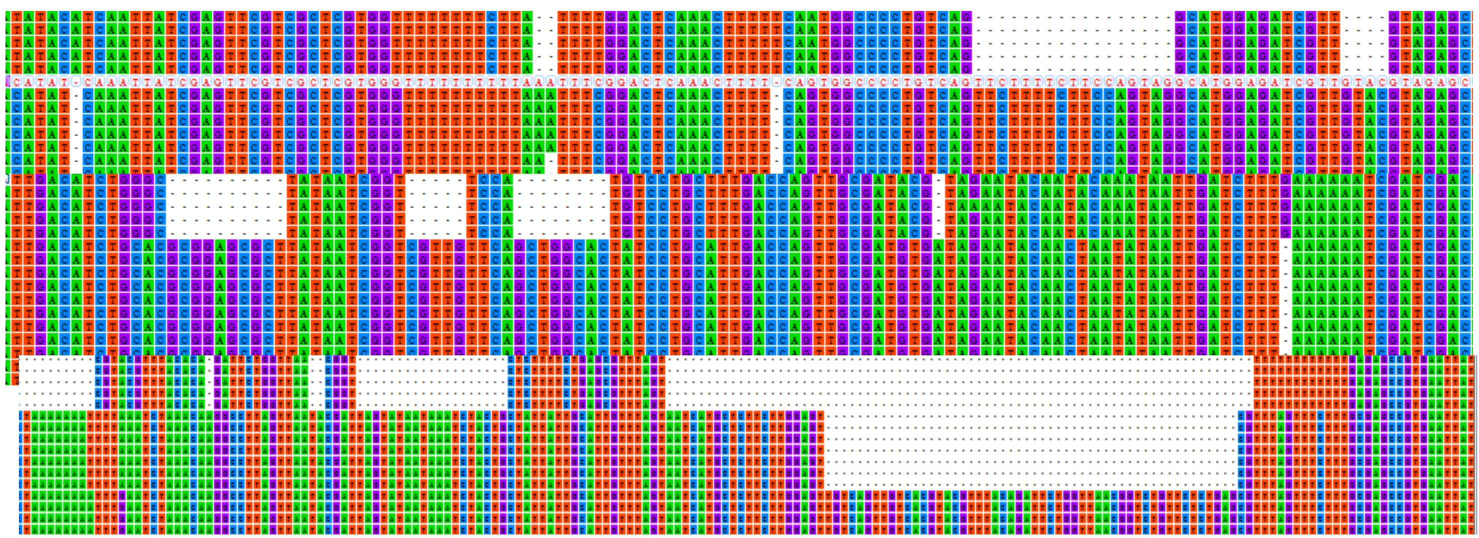

Fig 1 Partial sequence variation in the third intron of SAI-2 Gene.

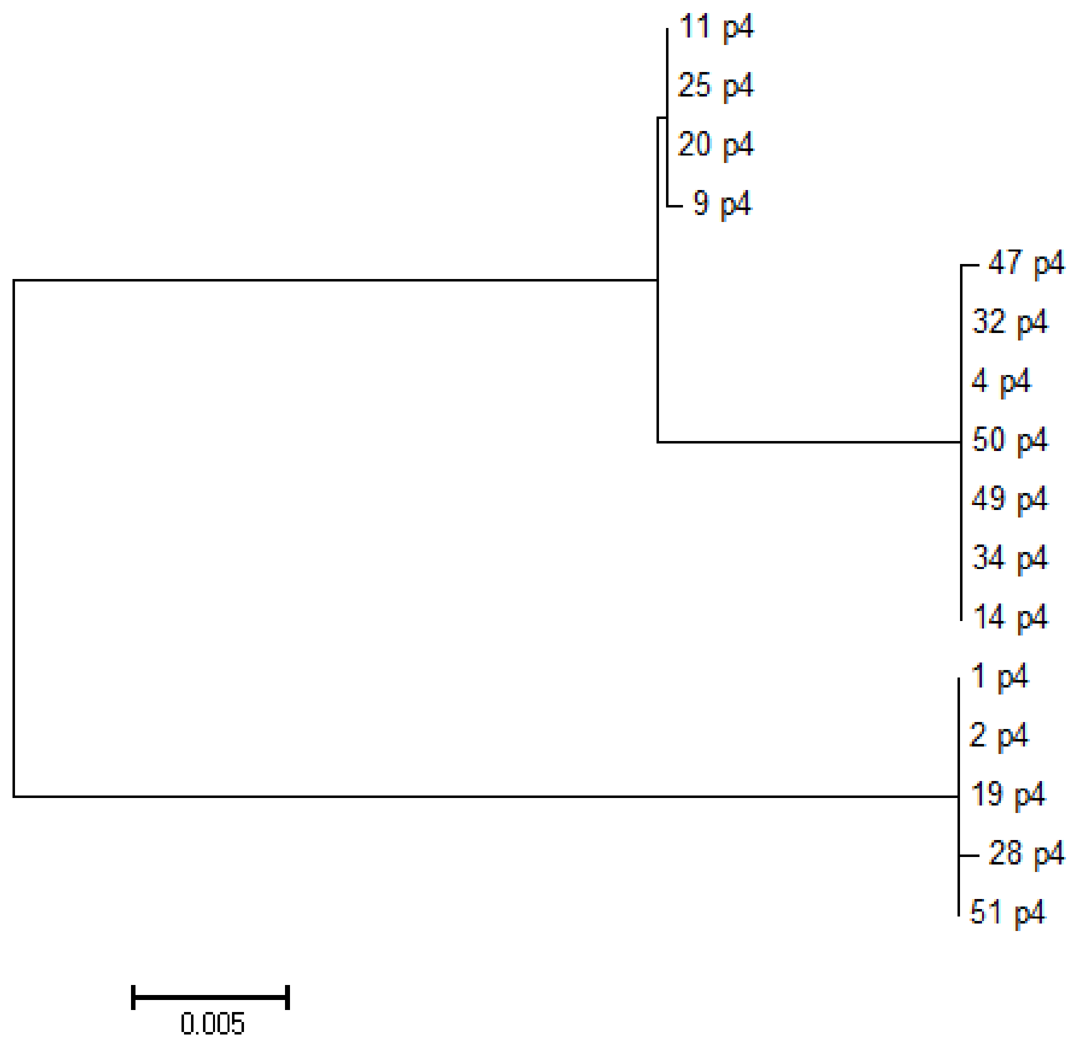

图2Phylogenetic trees of SAI-2 gene by intron3 sequence

- # Cross screening:

72 crosses were grown in Shandong province in June 29, 2 weeks later than plan, due to drought. Fortunately, they performance not too bad as imaged. When we investigate in the end of September, the plant height reached 3.5m and brix reached 20% in some cross, and the fresh yield reached more than 150 tones/hectares. Some better cross listed as follow.Most cross developed in to milk stage and early dough stage, which is suitable for silage.

cross	Plant height	Brix	Fresh yield
CP130067(134A）/611-3	3.44	15.8	199.8
CP130002(623A）/339-1	3.37	18.2	170.496
CP130032(2A）/1583-1	3.1	15.1	170.163
CP130022(623A）/1589-2	3.4	17.4	161.505
CP130043(2A）/493-1	2.8	12.9	147.852
CP130051(31A）/278-1	2.7	13.6	138.195
CP130001(623A）/329-1	3.18	11.6	135.864
CP130016(623A）/1599-4	3.4	18.4	133.2
CP130012(623A）/1171⑤	2.36	16.1	130.536
CP130070(134A）/1585-1	3.15	10.3	129.87
CP130026(623A）/1171①	2.35	9	128.538
CP130032(2A）/291-2	2.96	20.2	123.876
CP130033(2A）/407-1	3.2	16.2	122.544
CP130004(623A）/127-1	2.65	16.2	116.883
CP130001(2A）/358-1	3.4	16.4	116.217
CP130041(2A）/801-1	3.8	19.8	115.218
CP130032(2A）/460-2	3.1	13.2	111.222
CP130045(2A）/1589-5	3.5	20.6	109.3905
CP130033(2A）/460-1	3.47	17.8	109.224
CP130001(2A）/358-2	3.2	17.3	105.894
CP130008(623A）/491-1	2.77	15.8	103.896
CP130037(2A）/918-4	2.2	14	103.23
CP130001(2A）/1588-6	2.7	16.2	102.897
CP130011(623A）/1203①	1.86	16.9	102.564

- **Yield comparison of sweet sorghum line**

24 sweet sorghum lines were grown in Shandong in a random plot with three replications. Some better lines were listed as follow.

Lines	Plant height/m	Brix%	Fresh yield t/h	Increased by CK
DY1621	3.26	16.9	161.0	22.9
DY1621	3.12	14.7	150.0	14.5
DY1618	3.03	14.8	149.3	14.0
DY1618	3.12	17.4	145.3	10.9
DY1613	2.65	19.1	136.7	4.3
GN 11 ck	3.5	12.7	131.0	

- **Conventional works in breeding program**

More than 300 cross were manual made with A-line and sweet sorghum R-line. It is expected to screen better one in 2017.

New A-line development by backcross A-line/ (B-line/sweet R-line) every year.In 2016 50 A-typed lines were backcrossed.

- **Use of sweet sorghum in grain sorghum Gempalsm enhancement**

By cross sweet sorghum and grain sorghum, lots of dwarf grain sorghum with sweet sorghum characteristics, such green-stay, high juice, have been developed.

Molecular assisted breeding

Select parent as per SAI-1haplotyes, and hybridization




Key results of experiments on cultural practices for sweet sorghum mutant varieties

Field experiments on cultural practices for sweet sorghum mutant varieties, S1601, and S1602. It was designed into four treatments as follows:

- Fertilizer application: A commercial sorghum mixed fertilizer (YJZ2, N:P2O5:K2O=100:50:40) was selected in this experiment. Doses were applied in 100,150,225, 300, 375kg/ha, with no fertilizer application as control.
- Plant density: 60000，67500，75000，90000，9750060000plant/ha；
- Tiller handling: tiller removal and no-removal.

All treatments were arranged in a random plot design, with three replications.The plot size was 3m long and 3.6m wide.

- Sowing dates, two treatment: spring sown (May 6), summer sown (June 15)

• Yield performance of two varieties

All plots for each variety was calculated to get the average of tillers/plant, plant height, Fresh yield and brix as table 1.

It was showed that for both varieties, summer sowing tends to higher plants than spring sowing, but less tiller and lower in sugar content(Brix). The fresh biomass didn't show significant deference. Lower Brix meant higher moisture content in summer sown sweet sorghum.

It is noted that spring sowing can obviously lead to higher Brix, but unfortunately, it is susceptible to lodging, make it difficult to harvest.

variety	Sowing time	Tiller/plant	Plant height	Freshyield（tons）	Brix(%)
SS1601	Spring sown	1.0	316.8	104.6	19.1
	Summer sown	0.6	328.2	104.7	16.0
SS1602	Spring sown	1.1	326.4	103.8	15.0
	Summer sown	0.5	378.3	106.1	14.0

•Tiller handling

It was showed that tiller removal seemed affect the growth of sweet sorghum. The plant height showed a slight lower than no removal. And brix also didn't increase. So for these two varieties, **it seemed that tiller removal was not necessary.**

Sowing time	varieties	Tiller handling	Tiller/plant	Plant height	Fresh yield （tons）	Brix(%)
spring	SS1601	No removal	0.39	304.3	141.5	19.1
		Tiller removal	0.00	272.7	93.8	18.0
	SS1602	No removal	0.50	332.0	144.9	19.0
		Tiller removal	0.00	318.0	145.4	18.4
summer	SS1601	No removal	0.08	308.6	78.81	17.5
		Tiller removal	0.00	305.4	87.69	17.6
	SS1602	No removal	0.13	361.2	92.4075	18.0
		Tiller removal	0.00	371.7	89.6325	17.4

•Effect of Plant density on sweet sorghum performance

It was showed that from 60000-97500 plants/ha, higher density would lead to higher yield. And brix didn't change too much. The tiller number decreased as density increase.

The variety SS1602 showed a much lower brix in some treatment, it was caused by aphid infection.

Sowing time	Variety	Density	Tiller/plant	Plant height	Fresh yield（tons/ha）	Brix(%)
Spring	SS1601	60000	2.00	333.7	93.8	20.4
		67500	1.64	332.0	98.8	19.6
		75000	1.27	330.3	105.5	20.1
		90000	0.86	313.3	109.9	20.8
		97500	0.31	311.7	114.3	19.9
		Mean	1.21	**324.2**	**106.4**	**20.2**
	SS1602	60000	1.43	326.0	95.5	16.3
		67500	1.73	327.3	105.0	11.2
		75000	0.96	326.3	106.4	19.0
		90000	0.73	333.3	113.2	5.8
		97500	0.59	318.3	118.2	17.8
		Mean	**1.09**	**326.3**	**107.7**	**14.0**
summer	SS1601	60000	1.0	315.0	103.5	18.8
		67500	0.9	312.3	106.6	17.2
		75000	0.5	326.0	107.1	17.9
		90000	0.2	345.7	109.6	17.8
		97500	0.7	328.3	126.0	17.1
		Mean	**0.7**	**325.5**	**110.6**	**17.8**
	SS1602	60000	1.0	378.3	96.6	17.9
		67500	0.9	386.7	101.0	17.5
		75000	0.9	386.3	104.9	16.0
		90000	0.4	390.0	104.9	16.0
		97500	0.4	380.3	106.6	17.0
		Mean	**0.7**	**384.3**	**102.8**	**16.9**

- **Effect of Fertilizer application on sweet sorghum performance**

It showed that only the 100kg/ha treatment led to yield increase. It may be due to the higher fertility of experimental field.

Sowing time	Variety	Density	Tiller/plant	Plant height	Fresh yield（tons）	Brix(%)
Spring	SS1601	ck	0.6	336.5	119.0	18.4
		100	1.3	299.5	127.4	20.0
		150	0.8	313.5	97.4	19.0
		225	1.0	295	106.6	21.3
		300	1.0	318.5	72.4	10.3
		375	0.6	293	83.3	19.0
			0.9	**309.3**	**101.0**	**18.0**
	SS1602	ck	0.7	309	94.7	18.5
		100	1.6	318	118.2	17.7
		150	1.5	328	99.1	18.2
		225	1.2	343	100.1	18.2
		300	1.4	335	96.6	17.4
		375	0.9	326	102.4	16.6
			1.2	**326.5**	**101.8**	**17.8**
Summer	SS1601	ck	0.5	337.0	100.5	12.8
		100	0.4	350.0	115.6	14.4
		150	0.9	328.0	112.7	14.6
		225	0.5	324.3	110.0	15.1
		300	0.3	327.3	82.7	15.1
		375	0.4	318.3	78.3	13.6
			0.5	**330.8**	99.9	**14.2**
	SS1602	ck	0.4	377.3	111.8	11.1
		100	0.8	384.7	118.0	11.1
		150	0.5	355.0	102.7	11.8
		225	0.6	354.3	101.0	9.1
		300	0.5	377.7	110.0	10.7
		375	0.2	344.0	111.6	13.5
			0.5	**365.5**	109.2	**11.2**

Effects of Nitrogen Fertilization on Soil Nitrogen for Energy Sorghum on Marginal Land in China

To investigate the effects of N fertilizer application rates on temporal–spatial changes in soil inorganic N and N surplus levels on marginal land. Biomass sorghum (variety GN-11) and sweet sorghum (variety GT-8 were planted under five N application rates (0, 60, 120, 180, 240 kg ha^{-1}) in Inner Mongolia, China

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Fig. 1. Monthly precipitation and average temperature during the growing seasons in 2014 and 2015 at the study site.

Table 1. Soil properties of 0- to 30-cm soil depth and bulk density of 0- to 90-cm soil depth at the study site in 2014 and 2015.

Year	Sand†	Silt†	Clay†	pH	SOM‡	Total N	Olsen P	Available K	Bulk density
	%				g kg^{-1}		mg kg^{-1}		g cm^{-3}
2014	86.1	13.1	0.8	8.1	2.3	0.6	6.2	55.3	1.5
2015	90.7	9.8	0.7	8.0	2.3	0.2	5.8	35.1	1.5

† Soil texture classification was according to USDA classification system: sand, 0.05–2.0 mm; silt, 0.002–0.05 mm; and clay, <0.002 mm.

‡ Soil organic matter.

The results showed , as with increases in N application rates during each growing season, nitrate nitrogen (NO_3^-N) content in the 0-to 90-cm soil layer for two **varieties significantly increased** on elongation, anthesis, and harvest dates, while ammonium nitrogen (NH_4^+–N) remained stable. **The N fertilizer of 120 kg ha^{-1} improved dry matter yield and relatively reduced NO_3^-–N accumulation**. Thus, an N rate of 120 N kg ha^{-1}, with an increasing proportion of topdressing N on the elongation date, and a decreasing proportion of basal N on the pre-seeding date and topdressing N on the anthesis date, could improve biomass yield and reduce N accumulation to achieve more efficient sorghum production on marginal land in Inner Mongolia.

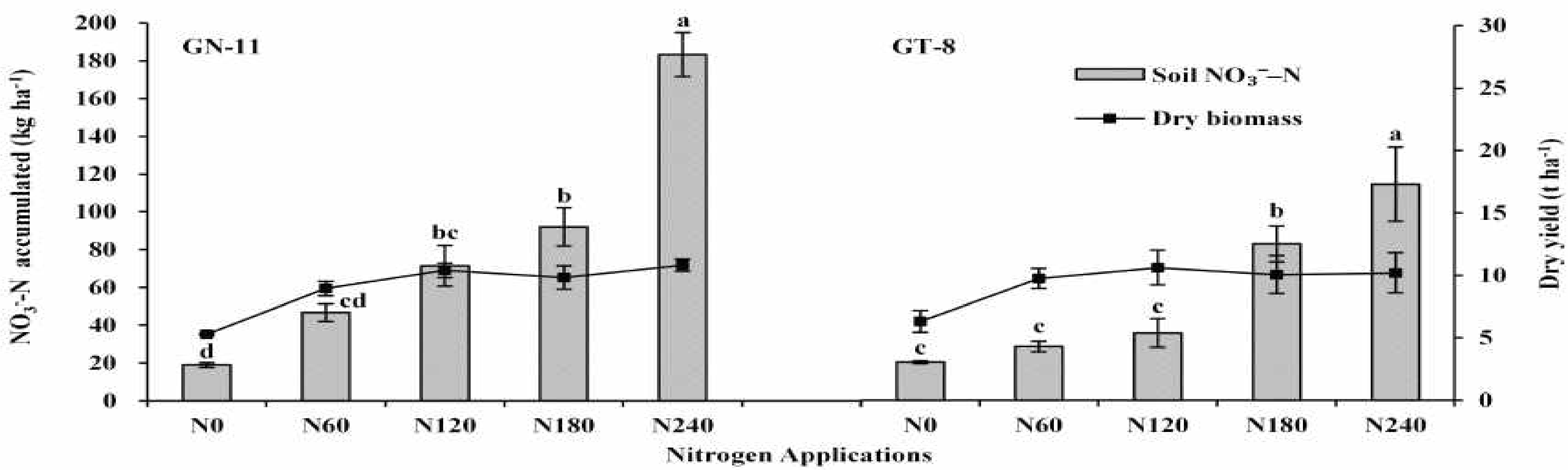

Fig. 3. Annual average nitrate nitrogen (NO_3^-–N) accumulation (2014 and 2015) in the 0- to 90-cm soil depth at harvest for biomass sorghum (GN-11) and sweet sorghum (GT-8) with different N applications. The different small letters indicate significant differences within each soil layer at $P < 0.05$.

The NO^{3-}–N accumulation in the 0- 90 cm soil layer at harvest significantly increased from 0 to 240 N kg ha^{-1}. Larger inorganic N additions were required in the pre-anthesis period to maximize growth of both varieties compared to the post-anthesis period.

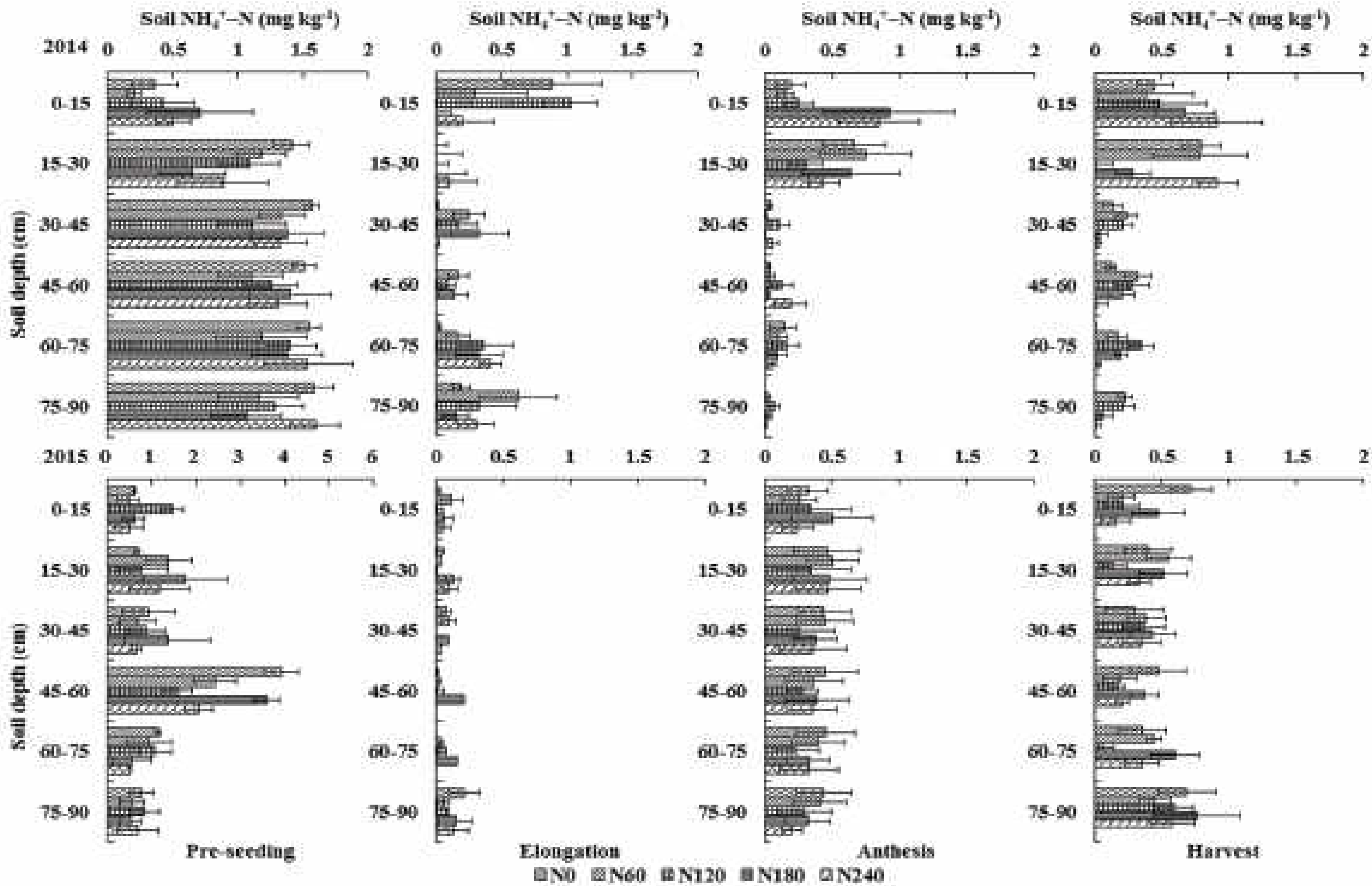

Fig. 4. Average ammonium nitrogen (NH_4^+–N) concentration of biomass sorghum (GN-11) and sweet sorghum (GN-11) in different soil layers at pre-seeding, elongation, anthesis, and harvest dates with different N applications during the 2014 and 2015 growing season. The different small letters indicate significant differences within each soil layer at $P < 0.05$.

Outlines

- Background
- Objectives
- Field design and data collection
- Main progress
- **Future plan**

Future plan

- Assessment of adaptation and yield potential of new hybrids or lines under stress conditions
- Future evaluation of soil and nutrient requirements for advanced mutant lines and varieties using nuclear techniques .
- Economic water management for sweet sorghum under marginal drought conditions.
- Promotion and adaptation of technology packages to the end-users, and enhancement of collaboration in the country


Thank you