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Screening of SSR Primers and Evaluation of Salt Tolerance in 20 Sweet Sorghum Varieties for Silage

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Introduction

Sweet sorghum belongs to the genus Sorghum in the family Gramineae. It is a variant of common grain sorghum, with characteristics of resistance to drought, flood, barren soil and soil salinity and alkalinity (Zhan *et al*. 2008). Since the stem of sweet sorghum is rich in sugar, it is usually harvested as silage fodder in grasslands. Often arable land used for forage production is salt-affected. Chinnusamy *et al*. (2005) have screened and identified a large range of different varieties for salinity tolerance, but there are no published reports of studies screening SSR primers and evaluating the salt tolerance in sweet sorghum varieties for silage. This study aimed to analyze the response of different sweet sorghum varieties to salt stress through observations of biological traits and examinations of SSR molecular markers.

Methods

To screen sweet sorghum varieties suitable for growing in saline soils, and provide a foundation for mapping and cloning of salt tolerance genes, 20 sweet sorghum varieties for silage were selected for analysis through observations of biological traits and examinations of SSR markers (Table 1). This experiment was located in the costal region of Jiangsu Province, China, where saline soil has a NaCl concentration of 3.0 g/kg NaCl. This was used as the control treatment (CK).

Ten seeds of each of 20 sweet sorghum varieties were sown in pots and treated with three salinity levels (3.0 g/kg, 6.0 g/kg, and 9.0 g/kg NaCl) in a complete randomized design with 2 replicates. Germination and growth were assessed by counting germination potential, germination rate from the fourth and eighth day and measuring the plant height, top 2nd leaf length and top 2nd leaf width on the twenty-sixth day. The differences between 3.0 g/kg (CK) and 6.0 g/kg in germination potential, germination rate, plant height, top 2nd leaf length and top 2nd leaf width were designated as d1, d2, d3, d4, d5, respectively, for example, d1= the germination potential at 3.0 g/kg NaCl – the germination potential at 6.0 g/kg NaCl, and so on. The average is as m1. The different between 3.0 g/kg (CK) and 9.0 g/kg is as d6, d7, d8, d9, d10 in above biological traits, *i.e*. d6= the germination potential at 3.0 g/kg NaCl – the germination potential at 9.0 g/kg NaCl, etc. which is average as m2. The differences between 6.0 g/kg (or 9.0 g/kg) and 3.0 g/kg NaCl (Ck) were analyzed using t-test method. The multiple comparisons among 20 varieties were used by Duncan's new multiple range test. SSR primers were screened in the highest salt tolerance and most sensitive varieties. DNA extraction and SSR PCR reactions were performed as described (Zhan *et al.* 2008).

Table 1. Names, origins, biological trait values of 20 sweet sorghum varieties tested

No.	Name	Origin	d1	d2	d3	d4	d5	m1	d6	d7	d8	d9	d10	m2
1	Liaotian No. 11	Liaoning	5	5	21.0	6.0	0.44	7.49a	55	35	31.0	17.0	1.14	27.83a
2	Liaotian No. 8	Liaoning	5	0	27.5	0.2	0.13	6.57a	10	5	27.5	4.2	0.15	9.37bcd
3	Jintian No. 2	Liaoning	10	5	9.5	0.0	-0.10	4.88a	20	-5	17.0	11.0	0.20	8.64bcd
4	Liaotian No. 10	Liaoning	-5	-5	14.5	1.8	0.16	1.29a	5	5	15.0	15.8	0.21	8.20bcd
5	Ketian No. 3	Beijing	5	5	19.0	2.4	-0.04	6.27a	-15	25	29.0	14.4	0.16	10.71bcd
6	Jintianza No. 2	Shanxi	5	5	3.5	0.5	-0.05	2.79a	10	5	5.0	2.5	0.04	4.51cd
7	Liaosiza No. 1	Liaoning	20	15	8.0	-0.7	0.10	8.48a	15	20	20.0	12.3	0.14	13.49bcd
8	Jitianza No.1	Shandong	0	5	9.5	1.0	-0.03	3.09a	10	0	27.0	18.0	0.37	11.07bcd
9	Shensiza No. 11	Liaoning	10	10	13.5	0.9	0.05	6.89a	5	5	26.0	15.4	0.45	10.37bcd
10	Liaotian No. 9	Liaoning	-5	0	14.0	2.0	0.02	2.21a	5	-5	23.0	12.0	0.42	7.09bcd
11	Jilintianza No.1	Jilin	20	-10	4.5	-1.9	0.01	2.52a	50	10	16.5	12.1	0.61	17.84abc
12	Ketian No. 2	Beijin	-10	5	15.5	2.3	0.01	2.56a	-5	0	16.0	5.3	-0.75	3.11d
13	Jitian No.1	Shandong	5	-5	7.5	2.8	0.08	2.08a	20	10	17.0	12.8	0.68	12.10bcd
14	XCTG	Anhui	0	0	10.0	3.5	-0.01	2.70a	10	-15	15.0	14.3	0.60	4.98cd
15	TGL	Anhui	10	10	13.5	17.0	0.52	10.20a	35	25	20.5	20.4	0.72	20.32ab
16	THTG	Anhui	5	10	1.0	0.0	0.20	3.24a	10	10.5	2.0	1.0	1.20	4.94cd
17	M81E	USA	5	5	14.5	4.2	0.02	5.74a	0	0	16.5	7.2	0.20	4.78cd
18	Rio	USA	10	10	7.5	-3.9	0.16	4.75a	15	15	17.0	5.1	0.48	10.52bcd
19	Roma	USA	-10	0	7.5	0.8	0.27	-0.29a	20	10	11.0	5.8	0.62	9.48bcd
20	ZYTG	Anhui	10	0	15.0	-0.2	0.09	4.98a	30	25	17.5	5.8	0.19	15.70abcd

Note: Values followed by the same small letter are not significantly different (P>0.05) in same column.

Results

There were significant differences in germination potential, germination rate, plant height and top 2nd leaf width between 6.0 g/kg and 3.0 g/kg NaCl (CK) at $P<0.05$, but not in top 2nd leaf length. There were highly significant differences in all traits between 9.0 g/kg and 3.0 g/kg NaCl (ck) at $P<0.01$. There were no significant differences among 20 varieties at medium salinity levels (6.0 g/kg NaCl), but there were significant differences among 20 varieties at high salinity levels (9.0 g/kg NaCl) as sown in Table 1. The results indicated that Ketian No. 2 and Jintianza No. 2 had the strongest salt tolerance, whereas Liaotian No. 11 and TGL were sensitive to salt stress.

Nineteen SSR primers were screened with polymorphism in the above mentioned 4 varieties using SSR molecular marker technology. The Primer AH24 (5' GCAAGGGCAAGGTGATGGAG 3', 3' GCTGATGACGGGCAGGGACT 5') was designed using the program Primer 5, and showed obvious complementary bands between the highest salt tolerance and most sensitive varieties (Fig. 1).

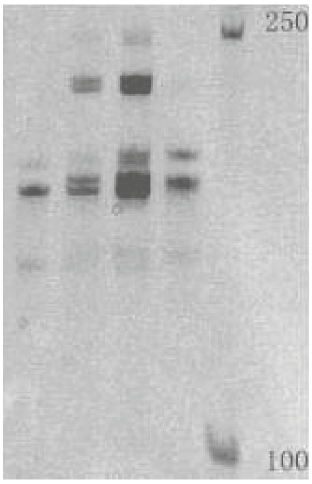

Figure 1. The amplified results by SSR primer AH24 in 4 sweet sorghum varieties (from left to right, TGL, Jintianza No. 2, Ketian No. 2, Liaotian No. 11, Marker)

Conclusion

In 20 varieties, Ketian No. 2 and Jintianza No. 2 had the strongest salt tolerance. The SSR primer AH24 could be used to distinguish the salt tolerance of the varieties.

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