RESEARCH ARTICLES

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Conservation of *Saccharum spontaneum* as Defuzzed True Seed

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ABSTRACT

Storing true seed (fluff) of sugarcane germplasm at low temperatures for preserving its germinability was highlighted as an alternative or complementary method for their maintenance and further utilisation in a Sugarcane Breeding Programme. The viability of defuzzed true seed collected from 50 *Saccharum spontaneum* clones stored at −20°C temperature for five years was investigated .The germination potential of the seed was evaluated annually using germination tests. It was found that the seed of the germplasm could be stored safely without much reduction in germinability. The practical advantage of maintaining sugarcane germplasm as defuzzed true seed under cold storage conditions has been highlighted.

Keywords : True seed, sugarcane, defuzzed seed, conservation, storage, germplasm, *Saccharum spontaneum*, fluff

INTRODUCTION

Sugarcane Breeding Institute, Coimbatore, is maintaining one of the world's two Sugarcane germplasm live collections at Kannur, Kerala. The importance of the wild species *Saccharum spontaneum L.* was realized after its successful hybridization with the cultivated species, *S.officinarum*, resulting in the production of first commercial hybrids Co 205 and Co 285 which replaced the indigenous cultivated varieties of northern India. The loss due to genetic erosion is not only confined to cultivated varieties, but also extends to useful genes of wild relatives of cultivated plants. The scale of conservation effort thus becomes enormous and calls for developing technologies and strategies that can meet the requirements of the challenge. The primary objective of conservation is the preservation of at least one copy of each of the different alleles rather than an accurate representation in the sample of the allelic frequencies (Bennett, 1970).

Germplasm may be stored as seeds for short and long periods at low temperature and relative humidity to maintain its maximum vigour (FAO, 1982). Roberts *et al* (1984) suggested the storage of 'orthodox' seeds for long-term storage of plant germplasm. Walker (1980) recommended that true seed maintained in long-term storage should supplement clonal collections of sugarcane. Vegetative maintenance of the large number of *Saccharum spontaneum* clones in the world collection is extremely laborious and costly and subject to perils of man and nature (Tai *et al.*, 1994).

Research on storage behavior of sugarcane true seed was less. A prototype sugarcane seed defuzzer was developed for reducing the volume and weight of seed fluff (Balasundaram, 1991). Procedure for storing defuzzed seed of sugarcane was standardised (Rajendra Prasad *et al.*, 1998) A simple laboratory germination test for sugarcane true seed was developed (Rajendra Prasad and Tripathi, 1999a). Utilising these techniques, investigations were made to store and evaluate the germination behaviour of true seed of *spontaneums*. In the present paper, the results of seed preservation are presented and discussed.

MATERIALS AND METHODS

During the 1995-96 flowering season, open pollinated collections of unbagged arrows were done from about 50 clones of *Saccharum spontaneum*. The seed fluff from arrows was dried in a drying room at 35°C for 48 hs (Rajendra Prasad and Tripathi, 1999 b) to obtain a moisture content of about 7%. The volume and weight of the dried seed fluff was considerably reduced by using defuzzing machines (Balasundaram and Rajendra Prasad, 1993) and winnowed.

Seed Germination Test

Initial seed germination was tested. The number of germinants per 0.1 g of defuzzed seed (on an average 150-200

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seeds) was counted from a replicated germination test in a seed germinator (Rajendra Prasad and Tripathi, 1999a). From this, the number of seedlings per gram of defuzzed seed was determined.

Seed packing and storage

The true seed of sugarcane (*Saccharum spontaneum)* in defuzzed form was packed in multiple numbers of moisture resistant polythene laminated aluminium foil pouches for each clone and stored at -20°C in a deep freezer. The seed was kept in storage for five years.

Monitoring the germinability over years

After every year of storage the seed was taken out from pouches and tested for its germination potential. Thus, the germinability of stored seed was assessed for five years making use of germination test.

The data on the number of germinants per gram of defuzzed seed of 50 clones stored for five years was tabulated and analysed using a completely randomized block design (Panse and Sukhatme, 1954)

RESULTS AND DISCUSSION

Years of storage

A perusal of the data (Table 1) indicates that there was a gradual decrease in the mean germination potential of true seed of *spontaneum* clones in the germination test from 87.66 per 0.1 g of defuzzed seed initially to 74.25 after five years of

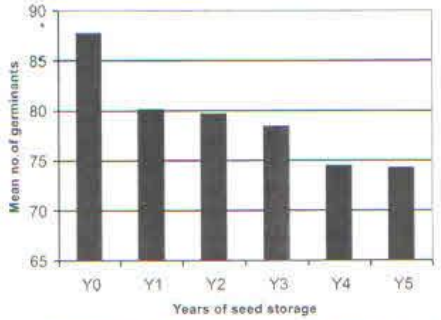

Fig.1. Mean number of germinants per 0.1 g of defuzzed seed from 50 spontaneum clones over five years of storage

storage period (Fig.1). Totally 15.3 % of germinability was lost. The decline had been 8.36%, 0.75%, and 1.68%, 5.05% and 0.20% after each consecutive year (Fig.2). The loss of viability after first year and fourth year of storage was faster. Nevertheless, the defuzzed seed could retain 85.3 % of its initial germination potential. This is in conformity with the storage studies made by Rajendra Prasad *et al* (1998) using defuzzed seed. This implies that the scientific principles of long-term

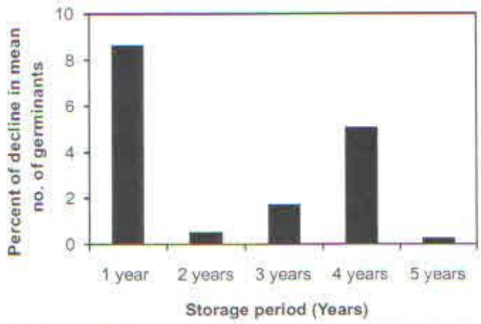

Fig. 2. Year wise percent decline in germination of defuzzed seed

seed storage and the technology are now on firm foundation (Roberts, 1989).

The system of storing the sugarcane true seed at lower temperatures and the possible benefits that could be accrued were stressed by Walker (1980), Rao (1980,1982), Tai *et al* (1994), Martin (1995) and Rajendra Prasad *et al* (1998). This study amply proved that defuzzed Sugarcane seed could be used for Sugarcane germplasm conservation. At this juncture, it is worth mentioning that USDA/ARS has placed seed of *S.officinarum and S.spontaneum* in NSSL at Fort Collins, Colorado (Tai, 2001).

Storability of true seed from different clones

Seed of 50 clones showed marked differences in their response to storage at –20°C during five years of storage (Table 2.) There has been gradual decrease in germinability of seed except two clones viz. SES 74B (+2.1%) and SES 148(+7.6%) wherein a slight increment in germination over initial levels were noticed. The initial seed germination potentials of clones were not maintained in the same order till the end of storage period. The decline in germination levels after five years of seed storage ranged from lowest 2% (SES 121 A) to highest 38.1% (SES 146).

Considering the decline in germinability of clones after five years, the clones could be classified as having good and bad seed storability (Table 2). Of the fifty clones, 41 clones maintained more than 75% of their initial germination. Clones SES 20, SES 137 B, SES 138, SES 150, SES 161, SES 375, SES 147, SES 406, SES 526, SES 567 did not lose more than 10% of their initial viability showing good seed storability, whereas clones SES 408,SES 48,SES 143,SES 565,SES 8,SES 146,SES 268,SES 275 and SES 376 lost more than 25% of their initial germination. Under same conditions of storage, the variable behavior could have been genetical nature. If there were no delay from the time of seed collection till it was tested and stored, the initial germination levels would have been still better.

Table 1. Mean number of germinants per 0.1g of defuzzed seed from fifty clones in germination test during the period of storage for five years

S.No	Clones	Initial	After one year	After two years	After three years	After four years	After five years	Percent reduction(-)/ increase (+) after five years
1	SES 2	163.33	161.33	138.00	152.33	144.67	139.00	-14.9
2	SES 4B	45.00	49.67	45.00	31.33	29.67	31.33	-30.4
3	SES 8	41.67	43.00	42.33	31.67	27.00	26.00	-37.6
4	SES 20	100.67	101.67	99.00	94.67	87.33	95.33	-5.3
5	SES 32C	139.00	143.00	114.00	111.67	125.67	122.67	-12.2
6	SES 55 A	126.67	122.33	106.67	99.67	101.00	99.33	-21.6
7	SES 74 B	175.00	160.33	167.33	179.00	183.00	178.67	+2.1
8	SES 76	26.67	26.00	20.67	18.33	22.00	22.33	-16.3
9	SES 85	88.33	89.67	73.33	68.67	73.33	74.33	-15.9
10	SES 87A	146.67	132.00	132.00	122.33	122.33	119.67	-18.4
11	SES 88C	150.67	140.67	148.00	155.00	141.00	145.67	-2.9
12	SES 93	200.00	189.33	177.00	198.33	161.67	173.67	-13.5
13	SES 96B	161.67	153.67	162.33	169.00	133.67	136.67	-15.5
14	SES 106A	115.00	101.67	98.33	101.67	88.67	92.00	-2.0
15	SES 108B	78.33	77.33	67.67	69.00	61.00	62.00	-20.8
16	SES 113B	127.33	132.33	126.00	115.33	102.00	98.33	-22.8
17	SES 121A	33.33	34.67	40.00	49.00	27.67	32.67	-2.0
18	SES 121C	60.00	48.33	62.33	52.33	56.67	48.67	-18.9
19	SES 137 B	105.67	103.67	102.33	102.00	97.67	100.33	-5.1
20	SES 138	82.33	81.67	74.67	74.67	80.33	79.00	-4.0
21	SES 143	75.33	61.07	59.00	61.33	53.00	50.67	-32.7
22	SES 144	193.67	186.67	115.00	146.00	152.67	145.33	-2.5
23	SES 146	7.00	4.33	4.00	3.33	3.33	4.33	-38.1
24	SES 147	161.67	149.00	151.00	158.33	133.00	149.00	-7.8
25	SES 147A	145.00	137.67	143.33	130.00	134.33	128.67	-11.3
26	SES 148	4.00	4.66	4.00	3.67	2.67	4.33	+7.6
27	SES 150	118.33	120.00	119.67	121.33	117.67	115.67	-2.3
28	SES 160A	98.33	91.67	91.67	84.00	84.00	79.67	-1.9
29	SES 161	10.00	10.00	9.67	10.33	7.00	9.67	-3.4
30	SES 168	66.67	55.67	57.67	60.33	53.67	51.67	-22.5
31	SES 268	15.00	9.33	9.33	10.00	6.00	9.33	-37.8
32	SES 270	5.67	5.00	5.00	4.67	3.00	4.00	-17.6
33	SES 272	65.00	45.00	42.67	47.67	48.00	50.67	-22.1
34	SES 274	151.00	129.00	140.00	133.33	132.00	131.00	-13.3
35	SES 275	86.33	85.33	91.33	79.33	77.33	55.67	-35.5
36	SES 286	167.67	130.67	123.00	135.67	141.67	144.67	-13.7
37	SES 375	86.37	80.67	80.33	89.33	84.67	82.67	-2.4
38	SES 376	9.00	7.67	9.00	9.33	5.33	5.67	-3.7
39	SES 403	78.33	49.33	51.33	51.67	45.33	41.00	-13.4
40	SES 405	9.00	7.00	9.67	9.00	10.00	7.33	-18.5
41	SES 406	78.33	62.00	71.67	72.00	77.00	74.33	-5.1
42	SES 408	88.33	71.00	76.33	77.67	71.33	65.00	-26.4
43	SES 409	10.00	10.00	9.67	6.00	6.66	7.67	-23.3
44	SES 517	160.33	151.00	153.33	139.33	131.67	130.00	-18.9
45	SES 522	35.00	32.00	29.33	34.00	28.33	27.33	-21.9
46	SES 525	108.33	106.00	95.00	85.00	79.00	95.00	-12.3
47	SES 526	85.00	82.33	76.00	81.67	83.67	78.67	-7.5
48	SES 563	14.00	13.00	16.67	14.00	12.67	10.67	-23.8
49	SES 565	8.33	8.67	4.00	5.33	6.33	5.67	-3.2
50	SES 567	74.33	67.67	75.67	66.33	63.67	69.67	-6.3
	General Mean	87.66	81.30	79.73	78.39	74.43	74.25	-15.3
		Initial	One Year	Two years	Three Years	Four Years	Five Years	
CD Value (P=0.05)		7.25**	9.71**	7.98**	6.48**	9.32**	8.18**	
CD Value (P=0.01)		9.59**	12.85**	0.57**	8.58**	12.33**	10.82**	

Table 2. Seed storability of *spontaneum* clones after 5 years

Clones showing increase or decrease in germination after five years								
A+	A	B	C	D	E	F	G	H
Increase >5%	< 5% decrease	>5 to 10% decrease	>10 to 15% decrease	>15 to 20% decrease	>20 -25%decrease	>25- 35 %decrease	>30 - 35% decrease	>35- 38.1% decrease
SES 74B	SES 121A	SES 20	SES 2	SES 76	SES 55A	SES 408	SES 48	SES 8
SES 148	SES 88C	SES 137B	SES 32C	SES 85	SES 108B		SES 143	SES 146
	SES 138	SES 147	SES 93	SES 87A	SES 113B		SES 565	SES 268
	SES 150	SES 406	SES 147 A	SES 96B	SES 144			SES 275
	SES 161	SES 526	SES 274	SES 106A	SES 168			SES 376
	SES 375	SES 567	SES 286	SES 121C	SES 272			
			SES 403	SES 160A	SES 409			
			SES 525	SES 270	SES 522			
				SES 405	SES 563			
				SES 517				
No. of clones 2	6	6	8	10	9	1	3	5

Sugarcane seed loses its germination quickly (Roberts et al., 1984). In the present study, majority of the seeds could retain their germination potential as they were dried to 7% moisture content and packed in moisture resistant polythene laminated aluminium foil pouches at –20°C (Rajendra Prasad *et al* (1998). At low temperatures, metabolic activities are minimal and deterioration is practically non-existent. No silicagel has been added inside the seed pouches.

Though there was some decline in germination after five years, seed storage facilities offered scope for a diminished need for live-maintenance to grow out each year. This true seed could be a backup for vegetative clone maintenance. When considering the overall requirement for effective and efficient conservation of plant genetic resources, it can be concluded that the two conservation approaches are highly oomplementary (Engels and Engelmann, 2002). Another advantage with defuzzed seed was that it occupied less space as compared to seed fluff. The true seed of any clone from any species of sugarcane could be stored implying storing resistant genes that could be exploited in a breeding programme.

Further, there is immense scope for studying the storage behavior of sugarcane defuzzed true seed at ultra-low temperature of –196°C for conservation.

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