EFFICIENCY OF THE GERMINATION TEST FOR PREDICTING SUGARCANE SEEDLING NUMBERS AT MOUNT EDGECOMBE

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Abstract

The sugarcane selection programme at Mount Edgecombe requires 250 000 seedlings annually. To ensure that sufficient crosses are sown, it is essential to have accurate estimates of the number of viable seed per cross. Overall, estimates are reasonably reliable, but the variation between estimated and actual germination in individual crosses is high. In this paper, the efficiency of germination tests over the last 15 years is reported and procedures for possible improvements are suggested. The viability of sugarcane seed, retained in cold storage for up to seven years, is confirmed.

Keywords: sugarcane, germination, seed storage

Introduction

In any crossing season, a number of physiological and environmental factors influence seed set (Nuss, 1979) and germination (Heinz, 1974, Breaux and Miller, 1987). Variable seedling numbers obtained after germination may be caused by loss of seed viability during storage and handling, or poor materials and conditions for germination (Breaux and Miller, 1987).

Seed for raising seedlings for the selection programme is obtained from the seed bank and is sown annually in seed boxes. The resulting seedlings are planted outdoors into clay bricks on concrete slabs (terraces) so that seedcane is available for planting in the field. Two hundred and fifty thousand seedlings are planted each year, to enable breeders to select 37 500 clones for planting in Stage 1 trials at each of five selection sites. Therefore, it is important to ensure that germination is estimated as accurately as possible. At Mount Edgecombe, the mean germination of all crosses is close to the estimated mean. However, when individual crosses are examined, the germination percentages vary widely. In this paper, the accuracy of the germination test currently being used and the viability of sugarcane seed after different storage periods are reported. Possible improvements to the germination test and the germination procedure are examined.

Materials and method

Seed storage

When the topmost portion of a tassel begins to fluff, the panicle is collected from the glasshouse; the 'seed' (fuzz) is stripped from the panicle and placed in a drying oven at 30ºC for 12 h to remove excess moisture. The fuzz is then weighed and a sample is taken for a germination test. Sample size depends on the mass of fuzz: 0 to 4 g, a 5% sample is taken; 4.01 to 6.50 g, a 3% sample is taken; and 6.51 g and above, a 2% sample is taken. The remaining fuzz, along with a label and sachet of silica gel, is then packed into a plastic bag and sealed. The bags of seeds, each representing a cross, are stored in racks in a cold room at a temperature of –20ºC.

Germination test

The sample of fuzz from each cross is placed onto a filter paper disc (Whatman® No. 1, 90 mm diameter) inside a disposable plastic petri dish (90 mm diameter) and moistened with tap water. The petri dish is covered and placed in an incubator at a temperature of 30ºC. Six days later, the number of germinated seedlings is counted. The number of viable seeds (estimated germination) for the entire panicle is then calculated by multiplying the number of seedlings obtained by the corresponding sample factor (e.g. the value from a 2% sample will be multiplied by a factor of 50).

Germination

Aluminium seed boxes (40 cm x 24 cm) are sterilized one month before use by dipping in 10% (v/v) Jik® (a.i. sodium hypochlorite). For each cross, one box is used per 400 estimated seedlings. The seed boxes are lined with coarse gravel. This is covered by a layer of fine gravel, a layer of river sand and finally a layer of peat moss (Nirom) mixed with river sand (1:1 mix). The seed boxes are soaked for one hour in deionised water to allow water to penetrate the various layers. The fuzz is then spread evenly over the surface and lightly covered with peat moss. Each seed box is watered thoroughly with a fine spray of deionised water and placed in the heated glasshouse (30 to 35ºC). The glasshouse mist system maintains atmospheric humidity by alternating a one-hour mist period with a two-hour 'dry' period. A watering can is used to supply additional water to the seed boxes. Germination is observed after three days. Thereafter, the seedlings are hardened off by three days of exposure outdoors, while being kept in the glasshouse overnight. After hardening off, the seedlings are left permanently outside under shade cloth and are watered two to three times daily. A mixture of Chemicult® (hydroponic nutrient powder containing essential macro and micro elements; 10 g) and ammonium sulphate (10 g), dissolved in water (10 l), is used to fertilise the seedlings once a week from one week after sowing.

Germination counts

The number of germinated seedlings is counted six days after planting. A piece of transparent Perspex, with a random pattern of 11 holes (3 cm diam), which together represent 10% of the

area of the seedling box, is used to count the seedlings. The Perspex template is placed over the seed box and a cardboard roll is placed over one of the holes. The numbers of seedlings observed through the holes are summed and multiplied by ten. The Perspex sheet is then inverted and a second count is made. The mean of the two counts gives an estimate of the number of seedlings in the seed box.

Data collection

Data accumulated over the last 15 years were extracted from the Crossing database. For each cross the following data were collected: cross number, crossing date, sowing date, proportion of the bag of seed utilized, germination test result and actual germination count. All non-utilized crosses were removed from the data, as well as crosses made for other countries. The data were separated according to the year the cross was made (i.e. by series) and then further separated into two sets: (a) data for seed utilized one year after the cross was made and (b) data for seed utilized two to 10 years after the cross was made.

Data manipulation

(a) Seed utilized one year after the cross was made

The estimated germination for each cross was determined by multiplying the estimated germination (from the germination test) by the proportion of the tassel used. The germination percentages were then calculated from the ratio of actual to estimated germination. The mean values of estimated and actual germination as well as germination percentage, were then calculated for each series. The standard deviation for germination percentage was determined. The numbers of crosses which had germination percentages of between 0 to 10% germination, 10 to 20% germination, and so on up to 190 to 200% germination, were counted. This categorizing was done for each series separately. An assessment was made of the normality of this germination percentage data through the use of skewness and Kurtosis formulae. Correlation coefficients were calculated between estimated and actual germination, estimated germination and germination percentage and actual germination and germination percentage. All statistical manipulations were performed using Microsoft Excel.

(b) Seed utilized two to 10 years after the cross was made

Estimated germination and germination percentage were calculated for each cross. The data was then sorted according to the number of years the seed was stored in the cold room. The mean germination percentage of each group was then determined. The standard deviation (germination percentage) and confidence intervals (95%) were calculated for each group.

Results and Discussion

Data from a total of 9 132 crosses were collected for this investigation. Of these, 9% had a germination percentage in the range of 200 to 3 000%. These crosses were excluded from the analysis, as they would have introduced significant bias. The high germination percentages were a result of actual germination results being far greater than estimated by the germination test. It has been found that there is a gradient in seed setting from the top of the panicle downward, with more seed being set in the upper portion (Anon., 1998). Because the sample size for the germination test is small (2 to 5%), if the fuzz is not mixed, the sample may not be representative of the entire panicle. This may be the cause of the extremely high germination percentages recorded in some instances.

(a) Seed utilized one year after the cross was made

The overall germination obtained each year was reasonably close to the estimated germination (mean 80,8%; Table 1). However, the high standard deviation (mean 45%) indicates the wide range of germination percentages obtained.

The number of crosses in each germination category over the entire 15-year period is illustrated in Figure 1. A germination percentage of approximately 100% would have been desirable. However, the majority of crosses had a germination percentage between 40 and 100%. The germination data was positively skewed and platykurtic (Table 2), i.e. the distribution had a broader peak than normal (Lynch and Walsh, 1998), thus verifying the fact that a wide range of data was obtained for germination percentage. This was consistent for each year. Thus, even though a mean of 80.8% germination was achieved, the majority of crosses had a germination percentage below the mean.

As the germination data did not fit a normal distribution, the correlation coefficients obtained must be regarded with caution. Overall, a high correlation between estimated and actual germination was obtained (mean 0.96, Table 3). Thus, the germination test gives a good indication of the number of viable seeds obtained, i.e. those crosses with a high estimated germination have high actual germination. Conversely, those crosses with poor estimated germination have poor germination. The germination test can therefore be used as a reliable indication of the number of seedlings that will be obtained from each cross, provided that the underestimation of germination percentage is considered.

No correlation was found between estimated germination and germination percentage (Table 3). Therefore, the tassels with high or low seed set are not being consistently over- or underestimated by the germination test. The factors influencing the accuracy of the germination test thus affect all crosses consistently. These factors will be investigated in the future and could

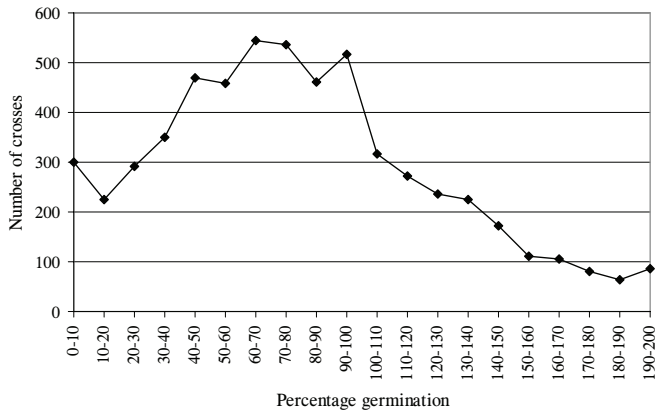

Figure 1. Number of crosses in each particular germination category for 15 years accumulated data.

Table 1. Mean germination results for crosses produced at Mount Edgecombe (1985 to 1999).

SERIES	YEAR	ESTIMATED GERMINATION	ACTUAL GERMINATION	GERMINATION PERCENTAGE (%) (ACTUAL/ESTIMATED)	STANDARD DEVIATION (GERMINATION PERCENTAGE)
V	1985	143 586	85 674	59.7	40.7
W	1986	137 640	120 047	87.2	45.1
X	1987	98 535	83 546	84.8	41.4
Y	1988	189 220	157 262	83.1	42.1
Z	1989	122 285	94 167	77.0	37.9
AA	1990	109 941	97 341	88.5	41.3
BB	1991	162 517	122 468	75.4	44.2
CC	1992	122 391	115 839	94.6	51.2
DD	1993	157 355	139 202	88.5	46.9
EE	1994	108 282	94 470	87.2	52.3
FF	1995	83 885	54 759	65.3	41.4
GG	1996	243 091	197 644	81.3	41.2
HH	1997	73 637	48 813	66.3	46.5
II	1998	85 730	60 287	70.3	41.7
JJ	1999	197 415	174 180	88.2	42.5
TOTAL		2 035 508	1 645 699	80.8	45.0

include temperature differences during incubation; moisture stress during fertilization, seed set and maturation; incorrect handling of the seed and insufficient mixing of the fuzz before the germination sample is taken.

There is a slight correlation between actual germination and germination percentage (mean 0.55, Table 3), but this is confounded by the strong correlation between estimated and actual germination, the ratio of which is used to calculate germination percentage. This therefore means that there is a slight tendency for a cross with good seed set to have a good germination percentage and vice versa.

(b) Seed utilized two to 10 years after the cross was made

Seed viability remains fairly consistent for the first seven years of cold storage before declining significantly (Figure 2). The confidence intervals increase over time as more crosses were used during the first years of storage, than were used after longer periods of storage. Several sugarcane breeding stations have also reported good seed viability after storage at sub-freezing temperatures for seven to 10 years. Low temperature and low moisture content are apparently complementary in maintaining viability and are the two factors that must be taken into consideration in storing sugarcane seed (Breaux and Miller, 1987).

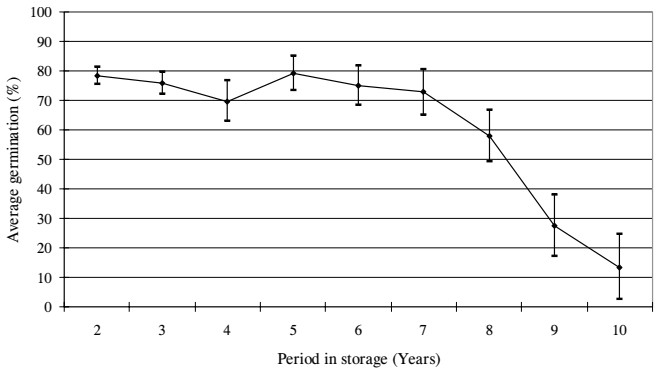

Figure 2. Decline in seed viability during storage.

Conclusions

The germination test used at Mount Edgecombe is efficient at determining the number of viable seeds produced during the crossing season and gives a good indication of the number of seedlings that are expected to germinate. However, actual germination is consistently lower than estimated by approximately 20%. The germination procedure itself therefore requires improvement. One reason for this could be that during the germination test in the incubator the seed is subjected to ideal germination conditions. However, during germination of the crosses in the glasshouse the seed is subjected to varying conditions. These include temperature, humidity, moisture stress and the amount of peat moss covering the fuzz, all of which may be less than ideal. An investigation will therefore be conducted to determine the factors influencing germination, so that the germination procedure can be improved.

Sugarcane seed can be stored in the cold room for up to seven years before a significant loss in viability is expected. As seed viability decreases, the chances of finding good varieties also decrease. This is important for the breeders to remember, so those crosses with good parent combinations, i.e. 'good' crosses, are used before seven to eight years, to avoid the loss of potential commercial varieties during seed storage.

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Table 2. Assessment of normality of germination data.

Series	Skewness	Kurtosis
V	1.033*	1.275*
W	0.312*	-0.477*
X	0.430*	0.024*
Y	0.481*	0.117*
Z	0.808*	0.731*
AA	0.367*	-0.136*
BB	0.444*	-0.186*
CC	0.175	-0.667*
DD	0.298*	-0.445*
EE	0.437*	-0.632*
FF	0.877*	0.459*
GG	0.534*	-0.033*
HH	0.639*	-0.341*
II	0.568*	-0.020*
JJ	0.436*	-0.355*
Total	0.462*	-0.234*

Table 3. Correlation coefficients calculated between various germination criteria.

Series	Correlation coefficients between:		
	Estimated and actual germination	Estimated germination and germination percentage	Actual germination and germination percentage
V	0.843	-0.028	0.301
W	0.729	-0.068	0.377
X	0.924	-0.106	0.143
Y	0.861	-0.151	0.204
Z	0.894	-0.109	0.163
AA	0.935	0.083	0.313
BB	0.749	-0.021	0.374
CC	0.882	0.089	0.389
DD	0.815	-0.171	0.421
EE	0.862	0.148	0.446
FF	0.885	0.109	0.389
GG	0.853	-0.000	0.378
HH	0.830	-0.471	0.355
II	0.851	0.026	0.429
JJ	0.783	0.073	0.574
Total	0.959	0.300	0.549