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International Journal of Plant Biology & Research

Research Article

Abiotic Factors Affect in Germination of Sugarcane Seeds (*Saccharum Spp*)

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Submitted: 05 August 2016

Accepted: 28 September 2016

Published: 30 September 2016

ISSN: 2333-6668

Keywords

- Sugarcane
- Caryopsis
- Temperature
- Substrate

Abstract

Sugarcane is a vegetative propagated crop and hence the production of seed and its fate in the environment has little been studied. This study contributes to understanding by defining the abiotic limits for sugarcane seed germination. Using seed from multiple genetic crosses, germination was measured under different substrate regimes (Medium, Soil with Cow dung, Sand and Paper) and temperatures (25 °C and 30 to 39 °C); cardinal temperatures and suitable medium for germination were estimated based on the rates of germination. We found that sugarcane seed could germinate over a broad range of temperatures with optimum ranging from 30°C to 39 °C depending on source of seed. Beside this sugarcane true seed germinate well in paper substrate followed by sand substrate. Regarding germination among four progeny field cross (I 33-97 x I 24-07) showed better.

INTRODUCTION

Sugarcane is asexually propagated for commercial exploitation and seeds (caryopses) through hybridization are essential for improvement. Genetic improvement through hybridization followed by selection and cloning from segregated populations obtains the superior progeny [1]. Floral induction, hybridizations and seed production in sugarcane occur only under specific conditions of temperature and photoperiod. In tropical regions, flowering occurs naturally whereas in sub-tropical and temperate regions, the photoperiod must be managed using growth chambers [2]. Although the seeds are the principal source of genetic variability for sugarcane improvement, there are very few studies on their production and an analysis of their viability. Developing a method which can evaluate seed germination could help in determining the ideal harvesting point and result in obtaining material with a better physiological quality.

Due to the lack of information on the terminative potential of sugarcane seed, more seeds are needed when sowing and since few seeds are produced by each hybridization, their potential wastage can result in the loss of promising material. Therefore, knowledge of seed viability is fundamental before starting plant production.

Standardized methodology is used for testing germination in many crop species and is described in the Rules of International Seed Testing Association. However, for sugarcane, this test procedure is not described with no specifications on pure seed characteristics, substrate, temperature and test duration. Besides this, there are no detailed descriptions in the literature of the morphology of sugarcane seed or seedlings, which makes analyses in the seed laboratory more difficult. Therefore, due to the importance of seed in the genetic improvement of sugarcane, the objective of the present study was to develop a methodology for evaluating sugarcane seed viability.

MATERIALS AND METHODS

The fuzz (true seed) used in the study were from sugarcane crosses carried out in the breeding division at Bangladesh Sugar crop Research Institute. After harvest, the seeds were preserved at -20 ºC for four months (February, 2015 to July, 2015). For the study, four sugarcane progenies were tested, two from biparental crosses of which one is Marcott Cross (I 140-00 x I 101-66), one field Cross (I 33-97 x I 24-07), one self (I 204-08), and one progeny from polycross (I 61-07 x ?).

Once the pure seed was characterized, the test to determine the weight of 1,000 seeds could be done. For each lot of pure seed, eight sub-samples of 100 seeds were randomly separated and weighed on an analytical balance with a sensibility of 0.0001 g. Due to the fact that the dispersal units of *Saccharum* spp. are not easily moved around, a manual separation method was used as described for this type of seed in the International Rules for Seed Testing [3], and the material from each progeny was tipped uniformly onto a table and mixed manually. This homogenized portion of seeds was then successively divided into two equal

parts using a ruler until eight portions divided into two rows of four were obtained. From the combination of the 1st and 3rd portions of the 1st row, with the 2nd and 4th portions of the 2nd row, another lot was formed. By using the same procedure, a sub-sample was formed containing around 100 seeds for carrying out the test. After this, the weight of 1,000 seeds was calculated by multiplying the mean weight of the sub-samples of 100 seeds by 10, and a coefficient of variation of ≤ 6% was adopted [4]. With these results it was possible to calculate the minimum weight of the study sample so that it contained at least 2,500 seeds. Four sub-samples, each of 2,500 seeds for each cross, and obtained from the lot of pure seeds, were used for the germination test, totaling 100 seeds per treatment (Figure 1). The seeds were sown in plastic boxes (16.0 x 14.0 x 2.5 cm) containing four types of substrates: Medium (40% Pressmud, 40% garden soil, 20% Sand), Garden soil with cow dung (each 50%), Sand and paper. For the paper substrate, quantitative kitchen tissue paper (17.0 x 17.0 cm) was used and four leaves were placed in each plastic box. The leaves were weighed and 10 ml of distilled water added to each repetition, which is a volume equivalent to 2.5 times the weight of dry substrate. For medium, soil-cow dung and sand substrate, normal garden soil, two year rotten press mud, rotten sundry cow dung and Fine River sand normally used in building construction was used. All had been sterilized in an autoclave at 121 ºC, 15 PSI for 15 minutes.

Two constant incubation temperatures were tested (25 and 30-39 ºC), in presence of constant light from fluorescent lamps and an alternating temperature (20-30 ºC), with 16 h light and 8 h darkness were applied.

The first count of normal seedlings was done on the 5 days interval after sowing and the final count at 20th day, with evaluations every five days when the abnormal and dead seedlings were then removed. The experimental design was a completely random 4 x 2 factorial, represented by four substrates (medium, soil with cow dung, sand and paper) and two temperatures (25, and 30-39 ºC), which were tested with the four progeny under the study and 1000 seeds were in each observation.

Treatment means were compared using the Tukeys test at the 5% probability level. The data were analyzed using the STAR *Nebula* statistical program 2013.

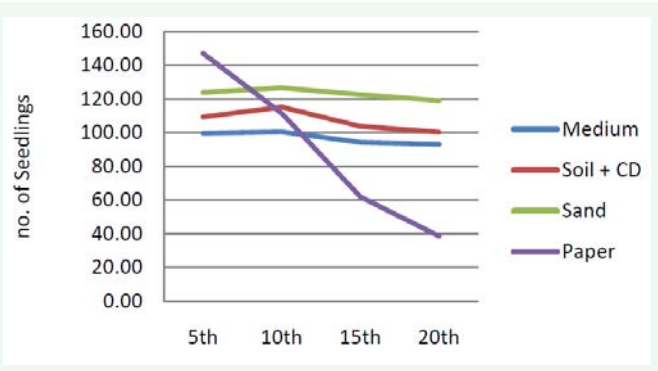

Figure 1 Seedling mortality state on various substrates.

RESULTS AND DISCUSSION

According to seed testing rule a sample contain at least 2,500 seeds regarding the purity analysis [4], the weight of the study sample for the progeny tested was calculated by multiplying the 1000 seed weight for each progeny by 2.5 (Table 1). Based on the weight of 1,000 seeds from the four progeny studied, a work sample for sugarcane of approximately 2.0 g can be suggested, because the heaviest seeds (I 33-97 x I 24-07) by 1.96, and this makes sure that the sample does not have less than the 2,500 seeds necessary.

All the substrates and the temperature are basic components of the germination test since the physiological response of seeds varies according to both these factors [5], and therefore, studying their effects on sugarcane seed germination is important.

The results from the Analysis of Variance (ANOVA) showed significant differences for the temperature factor in the four progenies analyzed. In (Table 2). Show the results of seed germination at varying temperature. There was significance difference observed between the temperatures. In temperature treatment 30-39 ºC showed the better performance and seedlings are healthy too. Pierre et al. [6], found that sugarcane seed could germinate over a broad range of temperatures (from 11 °C to 42 °C) with optima ranging from 27 °C to 36 °C depending on source of seed. In general, the maximum temperature for the seed germination of many species is in the range of 35 to 40 °C and optimum temperature between 15 and 30°C [7]. However, Marcos-Filho et al. [8], Borges and Rena [9] observed that the 20 to 30 °C range is adequate for the seed germination of tropical and sub-tropical species.

Cuenya et al. [10], observed that the best temperature for the germination of sugarcane seeds occurred between 35 and 38 ºC. However, Cesnik and Miocque [1] declared that the ideal temperature for sugarcane seed germination is around 32 ºC, but no official temperature has been established.

Table 1: Estimate of the weight of the study sample for the analysis of purity of four progeny of *Saccharum* spp.

Progeny	Weight of 1000 seeds (gm)	Work sample for purity analysis
I 140-00 x I 101-66	0.683	1.708
I 204-08	0.594	1.485
I 33-97 x I 24-07	0.785	1.962
I 61-07 x ?	0.636	1.590

Table 2: Results of the seed germination test in sugarcane on four substrates and at two incubation temperatures.

Substrates	25 ºC	30-39 ºC	Mean
Medium	94.67	163.35	129.01 b
Soil + CD	114.36	180.61	147.48 ab
Sand	127.59	207.29	167.44 a
Paper	86.52	151.23	118.88 b
Mean	105.79 b	175.62 a	

Table 3: Germination test of sugarcane seed on four substrates and at four consecutive days apart.

Substrates	5th	10th	15th	20th	Mean
Medium	99.73 d	100.54 c	94.21 c	93.05 c	96.88
Soil + CD	109.28 c	115.19 ab	103.67 b	100.34 b	107.12
Sand	123.76 b	126.50 a	122.58 a	118.94 a	122.95
Paper	147.01 a	111.48 b	61.77 d	38.53 d	89.70
Mean	119.94	113.43	95.56	87.72	

Table 4: Performance of varying type of progenies on different substrate.

Progeny	Medium	Soil+CD	Sand	Paper
I 140-00 x I 101-66	117.85 b	110.59 b	170.37 b	128.10 a
I 204-08	63.48 c	64.60 c	69.05 c	43.81 b
I 33-97 x I 24-07	160.88 a	222.69 a	226.12 a	163.82 a
I 61-07 x ?	45.32 c	30.61 c	26.24 d	23.06 b
Mean	96.88	107.12	122.95	89.70

The high temperature, associated with the high relative humidity inside the plastic boxes, caused burn-type lesions on the seedlings which made interpretation of the test difficult, principally for those seeds with a slower germination, which needed to be evaluated after the tenth day (Figure 1). Shows a normal sugarcane seedling in germination test being decline in most of the substrate as gradual days apart but it observed that mortality rate is significantly higher in paper substrate seedlings. Same results were by Caieiro et al. [11],

Germination percent of sugarcane true seed is too poor. In this study the maximum was observed 24% for I 33-97 x I 24-07 at sand substrate. The germination of the seeds of the sugarcane progenies tested was low, reaching a maximum value of 49%. In a study of sugarcane seed germination at a temperature of 30 ºC, also found low percentages with a maximum of 59% of normal seedlings [12]. According to this author, sugarcane is a species which forms few seeds and these have a low viability. Rao [13] believes this occurs because, different from cereals which are selected for the greatest fertility with the aim of increasing seed production, sugarcane is selected for sterility since the flowering process reduces the amount of sugar stored in the stem.

Germination and full development of the four progenies tested started from 5th days and the tests were extended until the 20th day at five days intervals. Although few germination was observed in this period except paper substrate test. It is recommended that the counts for the sugarcane germination test be done on the 10th day after starting the test. But a healthy seedling was observed at 15th to 20th days and significant no of healthy seedlings were exists in sand substrate. So, sand substrate can be chosen for mass sowing.

Significant statistical differences were observed among the substrates used in this study for germination of the four sugarcane progeny tested. From this study, comparing all the substrate, paper is the better and followed to sand for germination test of sugarcane true seed. Caieiro et al. [11], were observed between the sand and paper substrates used in this study for percentage germination of the three sugarcane progeny tested and test for small seed size, compared to sand, paper is better because it is easier to handle and standardize in the laboratory

Test on wide range of substrates four progenies shows significant difference on germination performance among the progenies and in all cases I 33-79 X I 24-07 shows the best performance though all types of progenies are necessary for increased degree of variability.

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Cite this article

Ganapati RK, Alam R, Rahman MM (2016) Abiotic Factors Affect in Germination of Sugarcane Seeds (Saccharum Spp). Int J Plant Biol Res 4(1): 1053.