

Performance of cotton cloth substrates in laboratory sorghum seeds germination tests

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ABSTRACT

Sorghum (Sorghum bicolor L.) seed germination tests were conducted at Tanzania Official Seed Certification Institute (TOSCI) laboratories, Morogoro, Tanzania. The aim of this study was to investigate the suitability of cotton cloth substrates in laboratory seed germination tests. Sorghum seed samples for this study were collected from three agro-ecological zones of Lake, Central and Eastern represented by Mwanza, Dodoma and Morogoro regions of Tanzania respectively. Cotton cloth substrates were used as treatments; single layer cotton cloth substrates (SLCCS), double layer cotton cloth substrates (DLCCS), and both single and double layer cotton cloth substrates washed and sterilized at 121°C for 15 minutes before re-use. ISTA paper was included as control. The germination results were 82.5%, 82.4%, 82.4%, 82.1% and 82.0% for DLCCS, ISTA paper, SLCCS, re-used DLCCS and re-used SLCCS, respectively. There was no significant ($P < 0.01$) difference in all substrates used in this study. Therefore, cotton cloth substrates were found as suitable as International Seed Testing Association (ISTA) papers for conducting sorghum seed germination tests in the laboratory. Since cotton cloth substrates are locally manufactured they could replace ISTA papers which must be imported. Further work however, is required to standardize this cotton cloth substrate, prepare protocol or standard operating procedures before adopting them for TOSCI routine laboratory sorghum seed germination testing.

Key words: ISTA papers, agro-ecological zones, seed production

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a tropical crop which belongs to grass family; order Poaceae, and genus *Sorghum*. Sorghum is the fourth major cereal crop in the world after wheat (*Triticum* spp.), rice (*Oryza sativa*) and maize (*Zea mays*). It is believed to be native in Africa especially in the south of Sahara desert, where several closely related wild species are found. It is an important staple cereal food in the drier parts of Africa and Asia and it occupies the fourth place among the world's cereals after wheat, rice and maize [20]. In Tanzania sorghum is an important subsistence food crop, mainly grown in arid and semi-arid regions [18] although nowadays with climate variability it can be found produced in regions which were not considered to be semi-arid. Sorghum is considered as a drought resistant crop in Tanzania [15] with estimation of production been between 557,323 - 622,400 ha and with yield of 461,400 - 498,500 [18]. Sorghum grain has various uses in different areas, which include flours for both stiff and non-stiff porridges, malted for local and distilled beers and beverages [19]. Sorghum needs a minimum of 300-380 mm of rainfall during growth.

Seed germination refers to the emergence and development of the seedling to a stage where the aspects of its essential structures indicate whether or not it is able to develop further into a satisfactory plant under favourable conditions in the soil [9, 10]. Laboratory seed germination tests therefore, aim to provide accurate and reproducible guidance, rather than absolute answers or predictions. Germination tests produce results that are usually greater than or equal to how the seed will actually perform in the field [20]. An appreciation of what germination measures can help maximize the understanding of the planting value or storage potential of seed [1, 17]. Several seed growing

media also known as substrates: top of paper, between papers, pleated paper, sand and organic growing medium are recommended by International Seed Testing Association (ISTA) for conducting laboratory seed germination tests [2, 9], Table 1. Laboratory sorghum seeds germination test is carried out on or in paper substrates only according to ISTA [2, 9]. Recommended ISTA papers for laboratory sorghum seed tests are not manufactured in Tanzania and therefore, Tanzania Official Seed Certification Institute (TOSCI), the agricultural seed quality control institute has to import them every time laboratory sorghum seed testing is required. Elsewhere, it has been suggested to use various substrates other than ISTA recommended germination papers and sand which are suitable for conducting laboratory tests of various seeds. Local materials like jute mat (used in making gunny bags) or cotton fabric have been reported to be suitable for rice seed germination test [12]. Other substrates such as soil or artificial compost are not recommended primarily as testing substrates because they are rich in nutrients [12]. This study intended to determine the alternative cotton cloth substrate to be used in conducting laboratory sorghum seed germination test.

Table 1: Types of substrates used in laboratory seeds germination tests

Type of substrate	Applicable method
Paper substrate	Top of Paper (TP)
	Between Paper (BP)
	Pleated paper (PP)
Sand/Organic growing media	Top of sand (TS)
	Top of growing medium (TO)
	Sand (S)
Paper and Sand	Organic growing medium (O)
	Top of paper covered with sand (TPS)

Source: [10].

MATERIALS AND METHODS

Twenty certified sorghum seed samples each were obtained from seed dealers in Kwimba, Chamwino and Kilosa districts of Tanzania. The seed sampling trier was used to obtain 1 kg of composite seed sample where by in TOSCI laboratories conical Boerner divider (made in India by Kartar Scientific industries) was used to obtain 90 g of working sample to undergo purity test to obtain pure seed before germination test is carried out. Pure seed refers to seeds of each kind and/or cultivar, or kind(s) and variety, under consideration which is free of inert matter and free of other seeds distinguishable by appearance or by test [10]. Pure seed portion obtained after purity analysis is the one that was used for germination test [9]. Halving method was then used to obtain 400 seeds for germination tests according to ISTA Rules [9]. One hundred seeds were used per replicate and therefore, 4 replications per sample.

Five different seed germination substrates were used in this study: ISTA papers (as a control), single layer cotton cloth substrate (SLCCS), double layer cotton cloth substrate (DLCCS), re-used single layer cotton cloth substrate (SLCCSr) and re-used double layer cotton cloth substrate (DLCCSr). The SLCCSr and DLCCSr were obtained by wash and sterilize at 121°C for 15 minutes the SLCCS and DLCCS respectively before re-use. The imported ISTA papers were obtained from TOSCI laboratories and cotton cloth substrates were purchased from local fabric shops in Morogoro town. Cotton cloths were 100% cotton locally produced by Polytex fabric industries, Morogoro, Tanzania. Single and double cotton cloth substrates were cut off by using fabric scissors in a desirable size of 30 cm x 20 cm. All treatments (substrates) were moistened with dH₂O in a volume equivalent to 2.5 times the mass of such dry substrate according to Gasparin *et al.* [8]. Four replicates of 100 pure sorghum seeds each were placed uniformly apart on moist substrates and covered with another similar wet substrate. The substrates were rolled, slightly tightened with rubber band and placed in plastic rack. The racks were then placed in the germination room maintained at a temperature of 25°C for ten days. The experiment was laid out in a completely randomized design. Moisture was maintained throughout the experiments by water the substrate using watering can after every two days. The counting of germinated seeds was daily performed, starting from the fourth day after seedling started to emerge (first count) and ending at the tenth day, when all seeds were all supposed to germinate according to ISTA rules [7, 9]. The seedling evaluation was carried out according to ISTA Seedling Evaluation Guide [10, 14] and Brasil [4]. The seedlings and non-germinated seeds were evaluated into five groups by percentages as normal seedlings (those which showed potential for continued development into satisfactory plants when necessary conditions are provided), abnormal seedlings (those which did not show the potential to develop into normal plant when grown in good quality soil and under favourable conditions of moisture, temperature and light), hard seeds (those seeds which remained hard at the end of the test period because they could not imbibe water), fresh seeds (those seeds which fail to germinate under the condition of germination test but remain clear and firm and had the potential to develop into the normal seedling) and dead seeds (seeds which at the end of the test period were neither hard nor fresh nor have produced any part of a seedling). The percentages were calculated as follows:

$$\text{Normal Seedlings (\%)} = \frac{\text{number of normal seedlings counted}}{100}$$

$$\text{Abnormal Seedlings (\%)} = \frac{\text{number of abnormal seedlings counted}}{100}$$

Similar formulae were used for other portions of hard, fresh and dead seeds. The percentages were rounded to the nearest whole number after calculation of tolerance for germination test [9]. In order to find the maximum tolerated range, the average percentage germination of the four replicates was calculated to the nearest whole number. The averages were then located in either of the first two columns and the maximum tolerated range was read in the third column [9],(Table 2).The experiments were repeated three times. Data were analysed by using GenStat computer package 14th edition and analysis of variance was used to draw conclusion. The calculated value (f-value) was compared with tabulated value at 0.05 level of significance. Mean separation test was done by using Turkey's Test.

Table 2: Maximum range of tolerance between four replicates of 100 seeds tests

If average germination percentage		The maximum tolerated range between replicates is:
Is:	Or:	
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93 to 94	7 to 8	10
91 to 92	9 to 10	11
89 to 90	11 to 12	12
87 to 88	13 to 14	13
84 to 86	15 to 17	14
81 to 83	18 to 20	15
78 to 80	21 to 23	16
73 to 77	24 to 28	17
67 to 72	29 to 34	18
56 to 66	35 to 45	19
51 to 55	46 to 50	20

In order to find the maximum tolerated range, the average percentage germination of the four replicates was calculated to the nearest whole number. The averages were then located in either of the first two columns and the maximum tolerated range was read in the third column.

Source:[10].

RESULTS

The normal sorghum seedlings obtained from Kwimba were SLCCS 74.3%, DLCCS 74.4%, SLCCSr 74.1%, DLCCSr 74.1% and ISTA papers 74.3% and were not significantly different ($P < 0.05$) different. The similar trend of the results was obtained from other locations of Chamwino and Kilosa (Figure 1). The Chamwino had SLCCS 87.7%, DLCCS 88.3%,SLCCSr 87.0%, DLCCSr 87.6% and ISTA papers 87.8%; while Kilosa normal seedlings were SLCCS 85.0%, DLCCS 84.7%, SLCCSr 84.9%, DLCCSr 84.3% and ISTA papers 85.0% (Fig. 1).

The abnormal sorghum seedlings obtained from three locations of Kwimba were SLCCS 5.1%, DLCCS 5.0%,SLCCSr 5.4%, DLCCSr 5.1% and ISTA papers 4.9% and were not significantly different ($P < 0.05$). The similar trend of the results was obtained from other locations of Chamwino and Kilosa (Fig. 2). The Chamwino had abnormal seedlings from SLCCS 3.7%, DLCCS 3.5%, SLCCSr 3.5%, DLCCSr 3.9% and ISTA papers 3.7%; while Kilosa abnormal seedlings were SLCCS 4.2%, DLCCS 4.3%, SLCCSr 4.6%, DLCCSr 4.6% and ISTA papers 4.4% (Fig. 2).

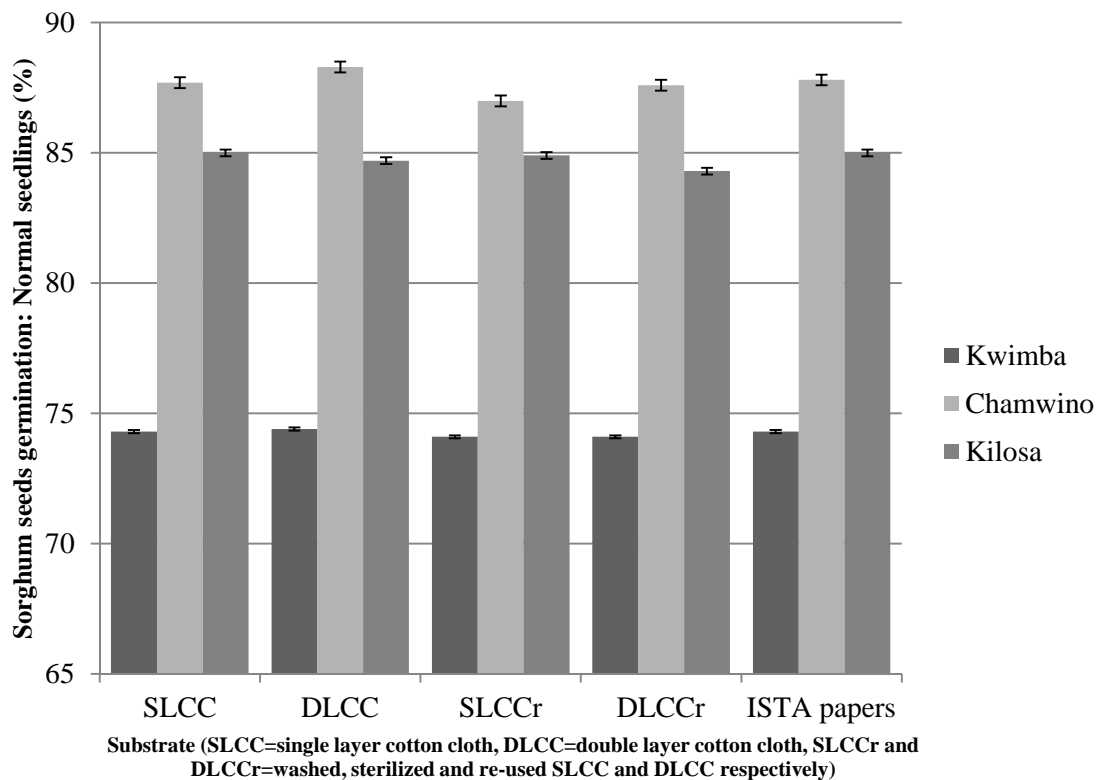


Figure 1: Sorghum normal seedlings from seeds sourced from Kwimba, Chamwino and Kilosa locations tested for germination on different types of substrates
Error bars are standard errors

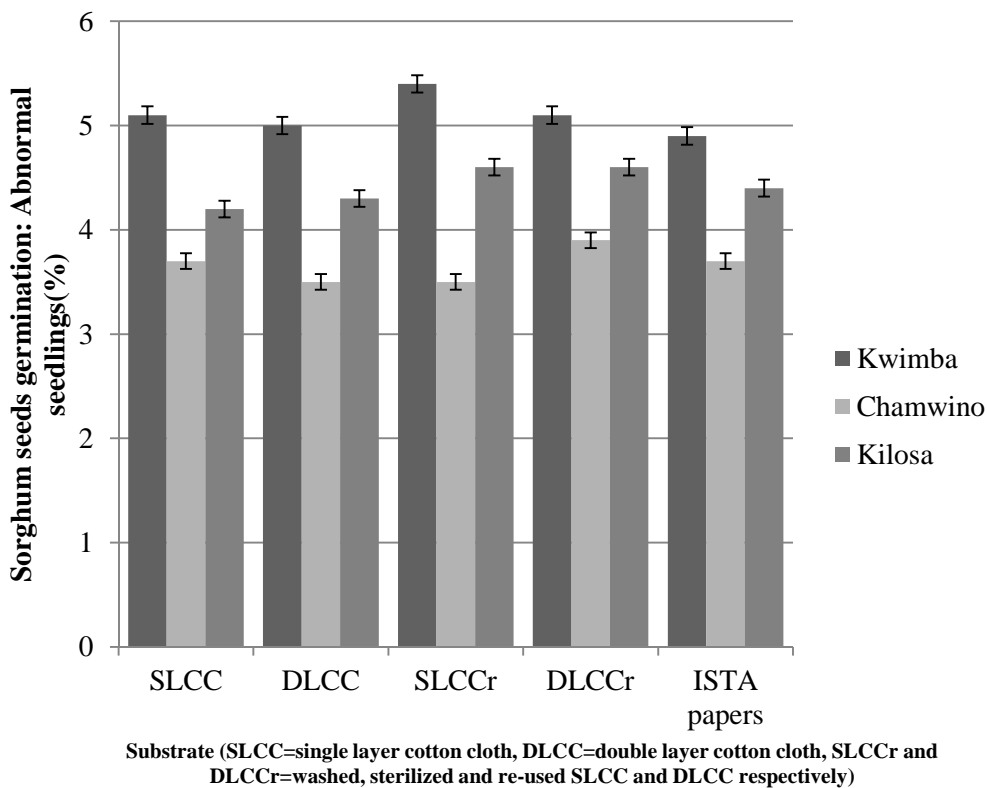


Figure 2: Sorghum abnormal seedlings from seeds sourced from Kwimba, Chamwino and Kilosa locations tested for germination on different types of substrates
Error bars are standard errors

The dead sorghum seeds obtained from the three locations of Kwimba were SLCCS 5.1%, DLCCS 5.0%, SLCCSr 5.4%, DLCCSr 5.1% and ISTA papers 4.9% and were not significantly different ($P < 0.05$) different. The similar trend of the results was obtained from other locations of Chamwino and Kilosa (Fig. 3). The Chamwino had dead seeds from SLCCS 3.7%, DLCCS 3.5%, SLCCSr 3.5%, DLCCSr 3.9% and ISTA papers 3.7%; while Kilosadead seeds were SLCCS 4.2%, DLCCS 4.3%, SLCCSr 4.6%, DLCCSr 4.6% and ISTA papers 4.4% (Fig. 3).

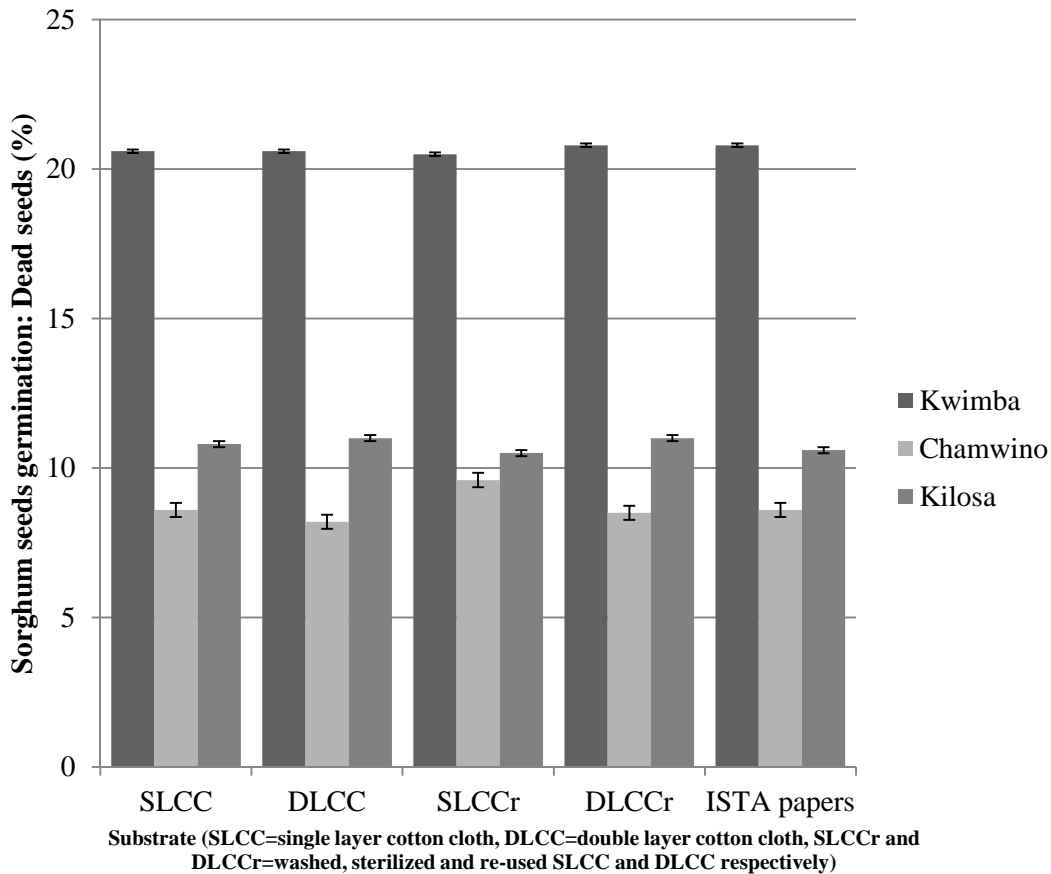


Figure 3: Sorghum dead seeds from seeds sourced from Kwimba, Chamwino and Kilosa locations tested for germination on different types of substrates
 Error bars are standard errors.



Figure 4: Sorghum seedlings during laboratory germination tests: Substrates A is a double cloth cotton cloths and B is ISTA papers

The efficiency of cotton cloth substrates and ISTA paper in conducting sorghum seed germination tests from various locations did not differ significantly ($P < 0.01$) between all treatments (Fig. 4).

DISCUSSION

In assessing germination percentage, normal seedlings were used as an assessment criterion, for finding the percentages of seed germinations. Therefore, all seedlings presenting normal structures such as primary root, hypocotyl and cotyledons were considered as normal seedlings, besides the percentage of dead seeds, abnormal seedlings, fresh seeds and hard seeds according to rules for seed testing [4]. The necessary qualities of substrate for conducting germination tests include; ability to show or expose the growth and development of seed-borne fungi and other microbes associated with seeds, inability to induce toxicity to developing seedlings, incapable of supplying abundant nutrients to seedlings, water retention ability and surface which are incapable for roots and plumules of seedling to penetrate through [10]. The cotton cloth substrates of both single and double layer were suitable for conducting laboratory sorghum seed germination tests, as they provided comparable results to ISTA paper. The DLCCS, ISTA paper and SLCCS performed better while the remaining substrates provided much reduced germination percentages although not significant different between each other. These results were in accordance with the previously conducted studies [5, 6, 8,16] that seeds of different plant species gave discriminated performance when subjected to different substrates depending on the basic components of the test.

According to Brasil[4], choosing substrates need consideration of size and its sensitivity to light, besides of their capacity of water retention, easiness of handling, and assessment of results. In re-used cotton cloth substrates several seedlings were observed deformed with smallest seedling length as compared to remaining substrates. This might probably be due to consequence of configuration of the rolling that presses the tender seedling tissues in "folds". Not only substrates used in sorghum seeds germination test affects the performance of seed lot but also the location where production was conducted, storage conditions and storage containers [5, 6, 11, 14]. Therefore, while seeds are produced all factors which will affect the seed lot germination performance should be taken care of to avoid rejection.

According to the results obtained in this study, it is not necessary to use double layer cotton cloth substrates to minimize the costs since single cotton substrates provided the same results as double layer cotton cloth substrates. Also it is desirable to re-use cotton cloth substrates after thorough washing with sodium hypochlorite plus soap and sterilization by autoclaving. However, re-used cotton cloth substrates should be thoroughly and carefully washed to remove sorghum seedling root exudates stains, otherwise they may not be suitable for determination of fungal infection in the next sample due to masking of fungal propagules by stain.

CONCLUSION

Cotton cloth substrates are suitable for conducting laboratory sorghum germination test, especially in developing countries to minimize cost of importing ISTA papers. Cotton cloth substrates are locally available and are of low price compared to ISTA papers. Generally seed dealers (producers, certification institutes, stockists) will have a direct benefit when they use these novel substrates due to its local availability and cost effectiveness.

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