A Technique for Controlling Matric Suction on Filter Papers Used in Seed Germination Tests, Imbibition, Root and Shoot Growth Studies

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Abstract

Moist filter papers are widely used for seed germination tests but their water content and matric suction are not usually controlled. A technique for controlling filter paper matric suction is described and used for germination studies involving fresh and aged sorghum seed **(Sorghum bicolor (L) Moench).** *Filter papers wetted to matric suctions of 0.1, 1 and 10 kPa were used to study germination, root and shoot growth rate of four cultivars (M35-1, ICSV-112, CSH-9 & CSH-11) of sorghum at 25°C. Time to germination, root and shoot growth were all affected by the increase in matric suction from either 0.1 to 1, or 1 to 10 kPa. Root and shoot emergence rate from seeds, decreased with increasing matric suction. The increase in matric suction resulted in significant reductions (P=0.05) in final root and shoot length. Final germination percentage was not affected by variations in matric suction. Ageing delayed shoot emergence. Seedlings with emerged shoots were significantly fewer at 10 kPa as compared to 1 kPa only for aged seed, and for all cultivars except ICSV-112. A link between matric suction, seed-liquid contact area and the rate of water uptake by seed is demonstrated. We conclude from this study: that changes in the matric suction on filter paper even at the wet end will cause variations in germination rate and therefore ought to be controlled during germination tests. Seed tests separated in time and space can not be compared if filter paper matric suction is not controlled.*

Keywords: Filter paper matric suction, Germination test, Seed-liquid contact area, Seed vigour, Sorghum

Introduction

The influence of matric suction on water absorption and germination of crop seeds has been a subject of much research (Collis-George and Hector, 1966). In the semi-arid tropics (SAT), soil moisture stress is perhaps the most important factor limiting crop establishment and production. Whilst establishment is only a part of the crop production system, it is the foundation on which all other factors affecting final crop yield are dependent (Hooke, 1987). Thus, unless the success of this early phase is assured, an entire planting may be doomed from the outset. Seed factors acting alone or in combination with soil and climatic factors frequently cause establishment problems. Screening seeds for germination and vigour in stress conditions in the field is complex because of the dynamic nature of the soil and above ground environment. Several laboratory methods have been tried, for example germination of seeds on soil-covered tension plate (Harper and Barton, 1966) or in soil pre-equilibrated to known potentials in pressure plate or membrane equipment (Hadas and Russo, 1974). The former method can only give matric suction values in the range 0 to 0.1 MPa and the latter is not really suitable for producing large quantities of equilibrated soil (Etherington and Evans, 1986). The use of osmotica such as polyethylene glycol (PEG) to control solution water po-

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ton and Evans, 1986). The use of osmotica such as polyethylene glycol (PEG) to control solution water potential is so far the commonest method of studying the effect of water stress to germinating seed under laboratory conditions. However, the method is not appropriate to ecological studies of seed germination in relation to soil water. In soil, seeds can absorb water only through a limited surface area which is in contact with the soil (Collis-George and Hector, 1966). In PEG, seeds are surrounded by the solution and thus, the seed-liquid contact area as a factor limiting water absorption is ruled out. There is evidence also that plants growing in PEG could suffer from oxygen deficiency in addition to toxic effects especially when high molecular weight PEGs are used (Maxel et al., 1975). Moist filter papers and other paper substrata are also widely used for seed germination tests but their water contents and matric suctions are not usually controlled. There is a need therefore to standardise the filter paper method of germination tests if (a) germination tests separated in time and space have to be compared, (b) rate of germination tests are to be performed, and rate of root or shoot growth studied. In this paper, a fast, simple method using filter paper to impose moisture stress on germinating seed is described and used to study germination, root and shoot growth of fresh and aged grain sorghum seed.

Methods

Background of the filter paper technique

Germinating seeds on calibrated filter papers is technically an off-shoot from the filter paper method for soil matric potential determination. Calibrated filter papers were first used by Gardner(1937) as a method for measuring the soil water release characteristic. Since then, the use of the technique for measuring soil matric potential has been thoroughly studied (Fawcett and Collis-George, 1967; Mullins, 1991) and a standard procedure for calibrating filter papers has recently been published (Deka et al; 1992).

The principle involved

The principle involved is the opposite of that used in determining soil matric potential in which a calibrated filter paper is placed in contact with a soil sample in a closed environment until equilibrium is reached. The gravimetric water content of the filter paper is then determined and converted to matric suction using a calibration curve or a regression equation of matric suction versus gravimetric water content. In this new technique, the amount of water ($g\ g^{-1}$) required to bring a unit mass of dry filter paper to a desired matric suction is obtained from a calibration curve or regression equation. A wide range of matric suctions from 1 kPa to 100 MPa can be measured with a filter paper (Fawcett and Collis-George, 1967) and hence can be imposed on filter papers for moisture stress studies. The requirements of the filter paper technique are:

1. A calibration curve: Matric suction versus filter paper gravimetric water content.
2. A supply of calibrated filter papers type Whatman No. 42 which should be from the same batch as those in (1).
3. Matric suction(s) to work at for example 0.1, 1.0 and 10 kPa in the reported study. By interpolation, obtain the corresponding gravimetric water content as 2.38, 1.75 and 1.15 from the calibration curve (1).
4. Calculate the amount of water required to bring weighed filter paper(s) to a chosen matric suction as: Mass of filter paper (g) x Gravimetric water content (w/w).

Worked example

To calculate the amount of water required to bring 5 discs of filter paper weighing 6.20 g to a matric suction of 10 kPa. The gravimetric water content corresponding to 10 kPa is 1.15 $g\ g^{-1}$ (from calibration curve) and thus, the amount of water required would be 6.20 x 1.15 = 7.2 ml.

Matric suctions used and how they were maintained

Three matric suctions (0.1, 1 and 10 kPa) were used in a range of experiments. Weighed Whatman no. 42, 12.5 cm filter papers were wetted to water contents of 2.38, 1.75 and 1.15

g g-1 calculated to bring them to matric suctions of 0.1, 1.0 and 10 kPa respectively. These calculations were based on filter paper calibrations made by Deka *et al.* (1992). Three replicates each containing 25 seeds of each cultivar were germinated in triple vent, 14 cm diameter Petri dishes each containing a stack of five filter papers which were maintained at a matric suction of 0.1, 1 or 10 kPa. Five filter paper discs were chosen because it was calculated that even if the 25 seeds (mean seed weight taken as 30 mg) imbibed to 50 % of their initial weight, filter paper water content wouldn't be reduced by 5 % in 24 h. The above strategy would prevent drastic increases in matric suction. After moistening them, the filter papers were left for 15 minutes to equilibrate before putting seeds on. At these small suctions water added to the filter paper equilibrates very fast (Greacen et al., 1987). The petri dishes were placed in a vented oven at 25 C for the study. To minimise water loss, the oven was lined with regularly moistened J-cloths. The matric suction of the filter papers was checked daily by weighing and any losses were corrected by adding more water. Meanwhile, the seeds were temporarily transferred to a stand-by petri dish maintained at the respective matric suction.

Seed

Sorghum seed cultivars M35-1, CSH-9, CSH-11 and ICSV-112 were obtained from the International Crop Research Institute for the Semi - Arid Tropics (ICRISAT). The seed samples have since been stored at 8° C. Mean seed weights (mg) were M35-1(35), CSH-9(39), CSH-11(31) and ICSV-112(26). Initial seed water content in all cultivars varied between 8.8 and 9.3 g per 100 g fresh weight. Fresh and aged seeds were germination tested. Ageing was by controlled deterioration (Mathews, 1980) at 40 C for 48 h following imbibition to a water content of 19 g per 100 g fresh weight. Aged seeds were not dried back before the germination test. The performance of aged seed is usually poor compared to that of fresh seed under moisture stress (Naylor, 1989; Gurmu, 1991). The inclusion of aged seed in this study was to investigate whether the differences in germination attributes between the two seed categories could be detected by this new technique.

Germination test on filter paper

Aged seeds from all cultivars were germinated at 1.0 and 10 kPa. Fresh seeds from cultivars M35-1 and ICSV-112 were germinated at 0.1 and 1.0 kPa, while those from cultivars CSH-9 and CSH-11 were only germinated at 1 kPa. To keep track of each individual seed during the experiment, a pencil grid was drawn and the cells numbered 1 to 25 on the top filter paper. Thus, each seed had a number. Dishes were inspected regularly to observe and record germinated seeds (i.e. seeds with radicle length of 4 mm or more). Whenever germination counts were made, radicle lengths were measured as well. Shoot emergence was also recorded and lengths measured once coleoptiles were longer than 4 mm. The experiment was discontinued after 117 h because seedlings were getting overcrowded with radicles becoming tangled. For each cultivar, an estimation of the time to reach 50% of final germination and coleoptile emergence was obtained by interpolation from graphs of cumulative germination against time.

Effect of filter paper matric suction on imbibition

Water uptake by seeds was assessed at matric suctions of 0.1 and 10 kPa. Two cultivars (ICSV-112 and CSH-9) were chosen for the experiment. Both cultivars had 100% laboratory germination. Seeds were selected for uniformity of size and mass achieved through sieving and weighing. All seeds from ICSV-112 weighed 25 mg each, those from CSH-9 weighed 38 mg. There were ten seeds per replication and five replicates for each cultivar. Seeds imbibed water from moist filter paper using the same procedure described in the preceding section. Water uptake was monitored by weighing after 1, 2, 3, 4, 5, 7, 9, 14, 25, 28, 31, 34, 39, 42, 46 and 50 h. Seeds were imbibed with the side opposite the depression facing downwards and were patted dry with a paper handkerchief before each weighing. Matric suction was maintained constant by transferring seeds onto freshly moistened filter papers hourly up to 9 h and after each weighing thereafter. The imbibition process lasted 50 h. Room temperature varied between 19 and 21 C during the experiment.

In a further experiment, seeds from all four cultivars were fully immersed in water to assess whether they differed in imbibition rate. The replicates and number of seeds were as described above. Only seeds whose mean weight fell within a range of 1s.d. were used in the experiment. Seeds were removed for weighing after 1, 2, 3, 4, 5, 6, 7, 18, 20, 22 and 50 h. Seed water content was calculated both on fresh and dry weight basis.

Effect of filter paper matric suction on seed-liquid contact area

Because it was expected that seed-liquid contact area could be a major factor controlling rate of imbibition, a procedure was developed to measure seed-liquid contact area and used to investigate the effect of matric suction on this parameter. Treatments were the four cultivars and three matric suctions (0.1, 1 and 10 kPa) on filter paper. At each matric suction, ten seeds per cultivar were placed in a Petri-dish containing 3 filter papers (Whatman No. 42, 12.5 cm) which had been moistened with deionized water to bring them to their respective matric suctions. Seeds were arranged so as to keep track of each seed during subsequent measurements. The side opposite the depression on sorghum seeds was placed in contact with moist filter paper. After 15 minutes, seeds were removed with tweezers and placed and pushed into a thick layer of fine powder of crystal violet with their wet surfaces down. The fine powder stuck and stained the wetted area on contact in all treatments except at 10 kPa on M35-1. This procedure, did not work well if seeds were placed on moist filter paper for a duration shorter than 15 minutes (e.g. 5 and 10 minutes). The seed coats for M35-1 and CSH-9 were water repellent, and apparently took some time to wet.

A travelling microscope was used to measure seed and wetted area diameters. Measurements were made along two main axes and an average calculated. The values so obtained were used to calculate total seed and wetted area according to Collins-George and Hector (1966).

Data analysis

The percentage of seeds in each cultivar which germinated at different matric suctions were tested for equality using the z-statistic. Similar tests were carried out on the percentage of emerged coleoptiles. Radicle and coleoptile lengths of seeds germinated at different matric suctions were compared using a t-test. Other computations were done using Statigraphics Version 5 (Statistical graphics Corporation, US).

Results

Radicle and coleoptile emergence of fresh seed at 0.1 and 1 kPa

Figure 1 shows cumulative radicle and coleoptile emergence for cultivars ICSV-112 and M35-1 as affected by different matric suctions. Germination was faster at the smaller matric suction. When radicle emergence was first observed after 35 h, and coleoptile emergence at 62 h, significantly(P=0.05) more radicles and coleoptiles had emerged at 0.1 kPa matric suction. Radicle emergence was 14 and 68 % at 0.1 kPa, compared to 0 and 20 % at 1 kPa respectively for M35-1 and ICSV-112. After 62 h, there were 56 and 80 % of emerged coleoptiles at 0.1 kPa compared to 12 and 22 % at 1 kPa. However, final germination percentage did not differ significantly between matric suctions for either cultivar.

Radicle and coleoptile emergence of aged seed at 1 and 10 kPa

Figure 2 shows cumulative emergence of radicles and coleoptiles versus time as affected by different matric suctions. As with fresh seeds, the rate of germination of all cultivars was significantly (P=0.05) greater at the smaller (1 kPa) matric suction. Radicle emergence was first observed after 35 h, but coleoptile emergence was delayed until 86 h. The percentages of emerged radicles recorded at 1 kPa after 35 h were x2 to x30 of those observed at

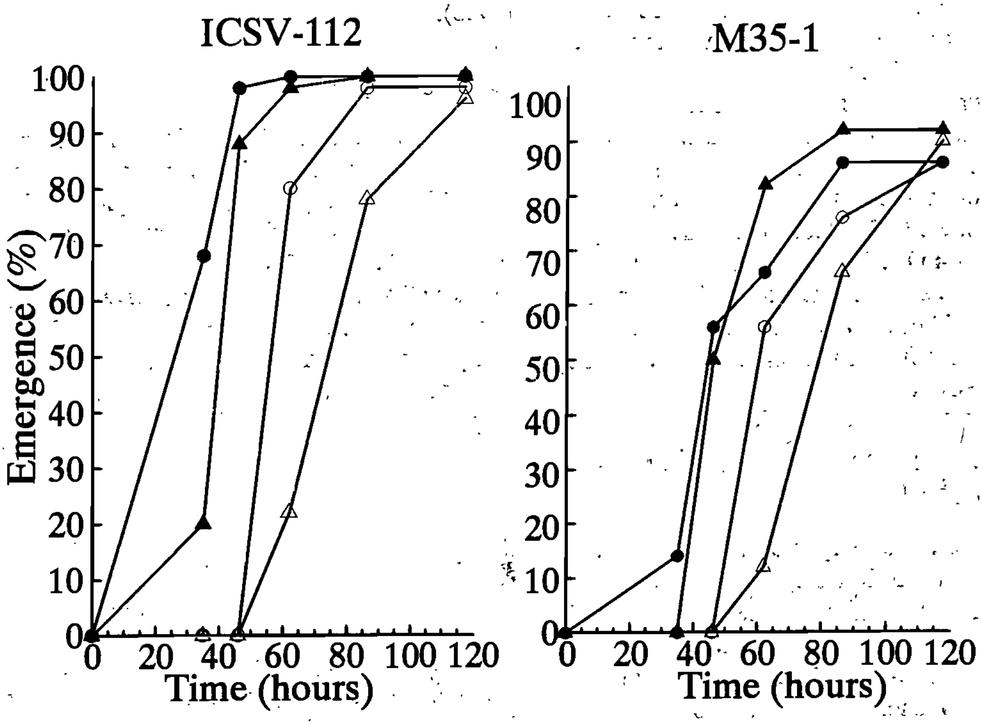

Figure 1: Radicle and coleoptile emergence - fresh seeds

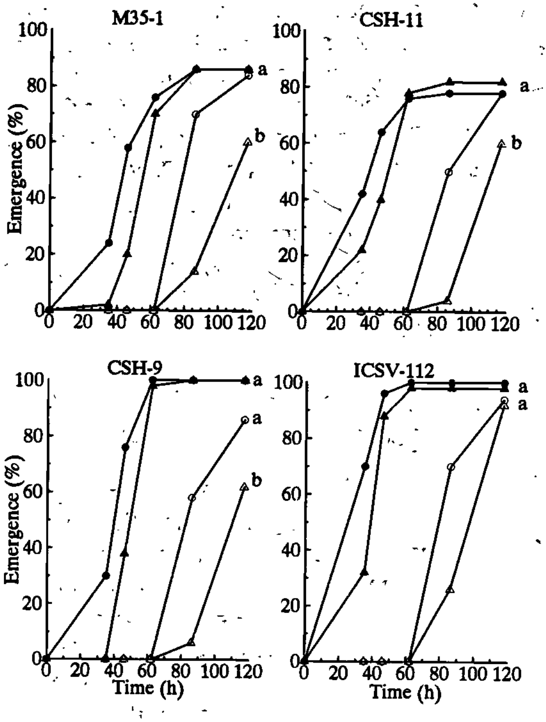

Figure 2: Radicle and coleoptile emergence - aged seeds

10 kPa. The final radicle emergence percentages did not differ significantly between the two matric suctions. Emerged coleoptiles after 86 h and at 1 kPa, were x3 to x13 of those observed at 10 kPa in the four cultivars. When the experiment was completed at 117 h, significantly (P=0.05) more seeds had their coleoptiles emerged at 1 than at 10 kPa for all cultivars except ICSV-112

Time to 50 % germination(T_{50})

Time to reach 50 % germination (T_{50}) based on final germination counts as was affected by matric suction is shown in Table 1. Less time was required to attain T_{50} at smaller matric suctions. T_{50} also varied between cultivars and with seed age. Only two cultivars were tested at 0.1 kPa. For fresh seeds, T_{50} was 35 h for ICSV-112 and 43 h for M35-1. At 1 kPa, T_{50} varied between 40 to 45 h for all four cultivars. In aged seeds, when germination was first observed at 35 h, T_{50} had already been exceeded in cvs CSH-11 and ICSV-112 at 1 kPa. T_{50} for cvs M35-1 and CSH-9 were 41.5 and 40 h respectively. For all four cvs, T_{50} was shorter in aged than in fresh seeds at the matric suction of 1 kPa. At 10 kPa, T_{50} values were 53.5, 50, 46 and 38.5 h for M35-1, CSH-9, CSH-11 and ICSV-112 respectively. T_{50} did not vary between experiments for similar treatments. At matric suctions of 0.1 and 10 kPa, T_{50} values were 31 and 42 h compared to 30.8 and 40 h in Table 1 and Table 4 respectively for cultivar ICSV-112. It is worthy noting, that the results presented in Table 1 and in Table 4 are from germination experiments separated by more than a year. The above is evidence therefore of the reproducibility of matric suction on filter paper.

Radicle and coleoptile lengths

Lengths of radicles and coleoptiles as affected by matric suction are given in Tables 2 and 3 respectively. Significantly (P=0.001) longer radicles and coleoptiles developed in seeds germinated at the lower matric suctions for all cultivars both for fresh and aged seeds. At higher matric suctions (1 and 10 relative to 0.1 and 1 kPa), radicles lengths were 68 to 73 % of their lengths at the lower suctions for all cultivars except ICSV-112 in which respective lengths were 82 to 86 %. Mean radicle lengths across cultivars and seed quality varied between 39.7 to 40.9 mm at 0.1 kPa; 27.9 to 39.2 mm at 1 kPa and 23.7 to 31.5 mm at 10 kPa.

Lengths of coleoptiles of aged seeds at 10 kPa were between 25 to 56 % of the lengths at 1 kPa. In fresh seeds, lengths at 1 kPa were 62 to 77 % of those at 0.1 kPa. Mean coleoptiles lengths varied between 5.3 to 6.3 mm at 10 kPa; 9.5 to 17.5 mm at 1 kPa and 20.7 to 21.9 mm at 0.1 kPa. A comparison between radicles and coleoptiles indicate that coleoptile growth was more sensitive to changes in matric suction than radicles.

The effect of matric suction on imbibition

Figure 3 shows water uptake rate by seeds imbibing fully immersed in water for the four cultivars. There were no significant differences (P=0.05) in water uptake by seeds between all cultivars in the first six hours, and throughout between M35-1, CSH-11 and ICSV-112. Cultivars CSH-9 and ICSV-112 persistently differed significantly (P=0.05) in water uptake after the first six hours. Seeds in all four cultivars had imbibed between 37 to 44 % (52 - 59 % dry wt.) of their initial seed weights by 50 hours. However, 50 % of the water was taken up within the first seven hours.

In cultivars CSH-9 and ICSV-112 which were also imbibed on filter paper at 0.1 and 10 kPa, water uptake was greater at the lower suction (Figure 4). However, the difference in imbibition rate between the two suctions was not significant (P=0.05). On the other hand, it took significantly (P=0.05) less time to reach 50 % germination at the lower suction (Table 4). Time to 50 % germination was 11 and 8 hours earlier at 0.1 than at 10 kPa respectively for ICSV-112 and CSH-9. However, seeds had imbibed to the same moisture content at T_{50} irrespective of the matric suction at which they imbibed. Between cultivars, ICSV-112 seeds germinated at a significantly (P=0.01) higher water content than CSH-9. Despite this, T_{50} was significantly (P=0.01) less for ICSV-112 than for CSH-9 at either suction.

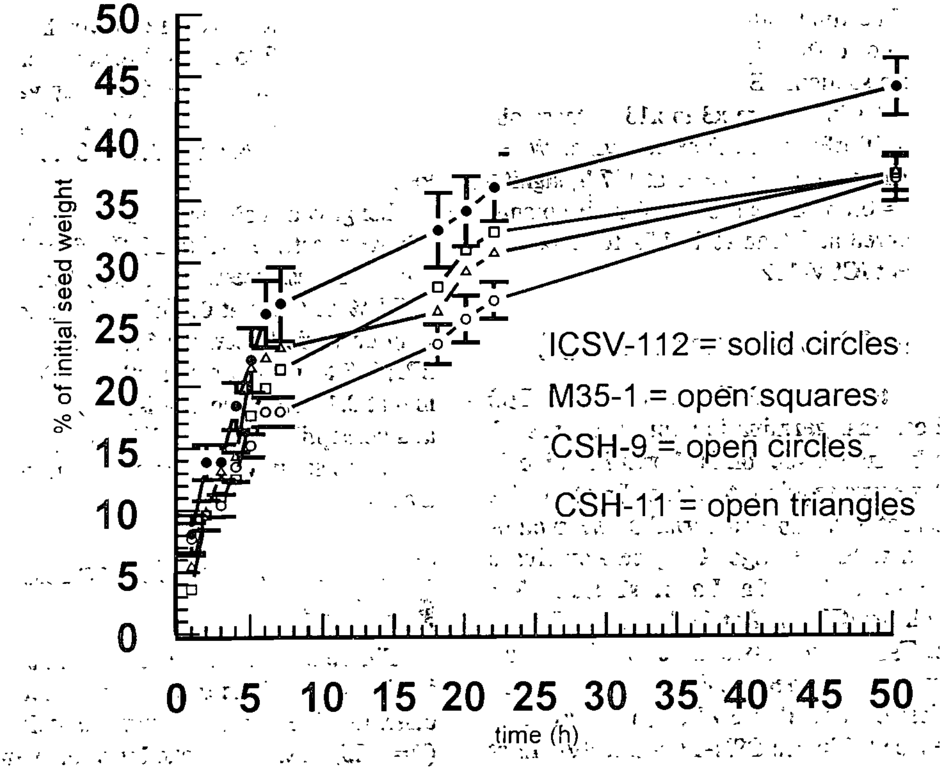

Figure 3: Water uptake curves - fully immersed in water

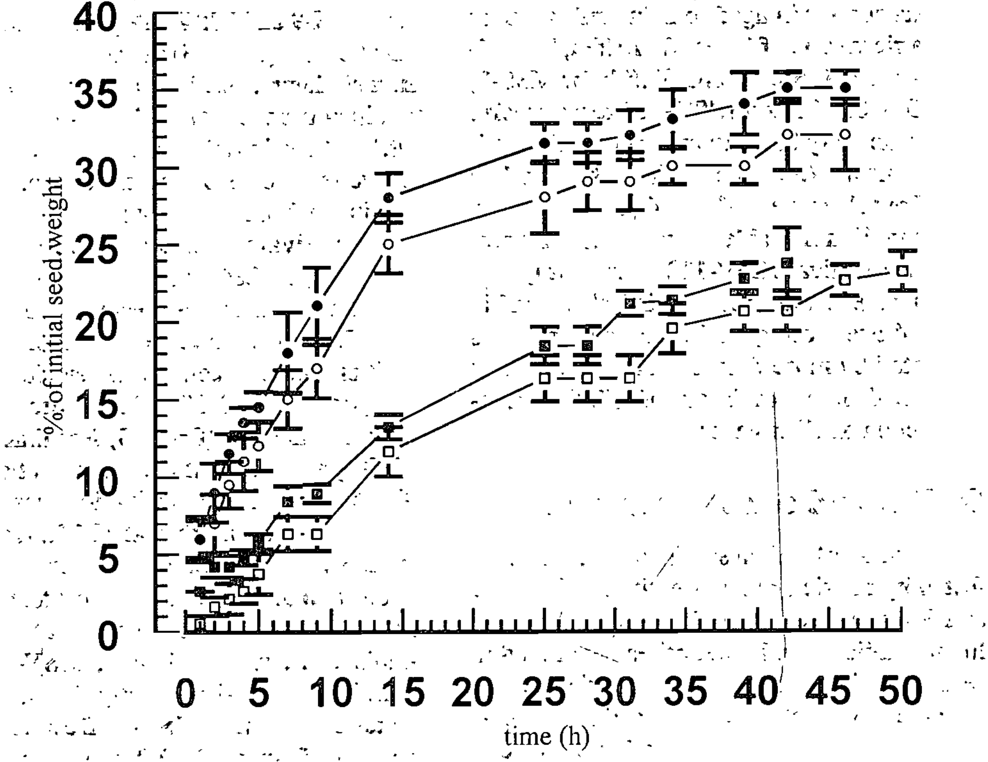

Figure 4: Water uptake curves - from moistened filter paper

Table 1: Time (h) to 50% germination and coleoptile emergence as affected by cultivar, seed age and matric suction

Cultivar	Seed age	Matric suction (kpa)	Final germination (radicles) (%)	T_{50} (radicles) (h)	Final germination (coleoptile) (%)	T_{50} (coleoptile) (h)
CSH-9	Fresh	1	100	42	94	75
	Aged	1	100	40	86	76
		10	100	50	62	100
CSH-11	Fresh	1	84	41	78	77
	Aged	1	78	32	78	81
		10	82	46	60	106
M35-1	Fresh	0.1	86	43	86	58
		1	92	45	90	77
	Aged	1	86	41.5	84	77
		10	86	53.5	60	97
ICSV-112	Fresh	0.1	100	26	100	40
		1	100	40	96	73
	Aged	1	100	25	94	78
		10	98	38.5	92	96

The experiment was terminated after 117 h because radicles were becoming tangled.

The effect of matric suction on seed-liquid contact area

The appearance of stained seed-liquid contact area (SLCA) as affected by matric suction is shown in Plate1. The circular shape of the stained area supports the assumption made that sorghum seeds are nearly spherical. The SLCA decreased as matric suction increased. Stain crystals/powder remained undissolved at the highest suction. At this suction (10 kPa), SLCA were undefined smudges on seeds of cultivar M35-1. Thus, the calculation of SLCA could not be undertaken for the cultivar.

Table 5 shows seed to seed-liquid contact area ratio as affected by matric suction for different cultivars. Significantly (P=0.05) higher ratios were obtained at a matric suction of 0.1 compared to 1 kPa. At 1 kPa, respective SLCA ratios for M35-1, CSH-9, CSH-11 and ICSV-112 had fallen to only 24, 25, 33 and 30% of the values obtained at 0.1 kPa. Between the matric suction of 1 and 10 kPa, significant differences (P=0.05) in the ratio were obtained only for cultivar CSH-11. Overall, the ratios varied from 12 to 23.1% at 0.1kPa; from 2.9 to 6.9% at 1 kPa, and between 2.2 to 4.2 % at 10 kPa. Pairs of cultivars, M35-1 and CSH-9, and CSH-11 and ICSV-112 differed significantly (P=0.05) in SLCA ratio at each suction. Cultivar CSH-11 and ICSV-112 had larger ratios than the other pair. The SLCA ratio for CSH-11 and ICSV-112 at 10 kPa were even larger than those for M35-1 and ICSV-112 at the lower suction of 1 kPa.

Water uptake after 7 h in cultivars CSH-9 and ICSV-112 which imbibed at the three matric suctions (0, 0.1 and 10 kPa), demonstrate the effect of seed-liquid contact area on imbibition (Figures 3 and 4). Water uptake was 17.9, 8.4 and 6.3 % for CSH-9; 26.6, 18 and 15 % for ICSV-112 respectively at a matric suction of 0, 0.1 and 10 kPa.

Discussion

The start of germination was significantly delayed by an increase in filter paper matric suction for all cultivars for both fresh and aged seed. The slower pace of germination at greater suctions was also evident from T_{50}. Smaller

Table 2: Mean radicle length (mm) after 86 h as affected by cultivar, seed age and matric suction

Cultivar	Seed age	Statistic	Matric suction (kpa) 0.1	1	10	Length ratio (high suction /low suction) (%)
CSH-9	Fresh	mean	-	35.8	-	
		se		1.1		
		CV(%)		20		
	Aged	mean	-	39.2	28.7	73
		se		1.2	0.8	
		CV(%)		20	20	
CSH-11	Fresh	mean	-	35.8	-	
		se		0.88		
		CV(%)		16		
	Aged	mean	-	35.1	24.9	71
		se		1.4	1	
		CV(%)		24	26	
M35-1	Fresh	mean	39.7	27.9	-	70
		se	1.3	1.5		
		CV(%)	21	32		
	Aged	mean	-	34.8	23.7	68
		se		1.3	1.1	
		CV(%)		23	29	
ICSV-112	Fresh	mean	42.9	37	-	86
		se	1	0.94		
		CV(%)	42	18		
	Aged	mean	-	38.4	31.5	82
		se		0.82	0.66	
		CV(%)		15	15	

All cultivars had greater radicle lengths ($p < 0.001$) at the smaller of each pair of matric suctions

T$_{50}$s were always associated with smaller matric suctions. Our results provide no evidence that total germination (radicle emergence) was reduced as matric suction increased but only that the rate of germination was significantly reduced. However, the final coleoptile emergence percentage was significantly reduced at 10 compared to 1 kPa for all cultivars except ICSV-112.

It is unlikely that the differences in matric suction were directly responsible for the differences in germination rate at such low suctions. Under field conditions, the matric suction values used (0.1, 1 and 10kPa) would correspond

Table 3: Mean coleoptile length (mm) after 117 h as affected by cultivar, seed age and matric suction

Cltiver	Seed age	Statistics	Matric suction (kpa) 0.1	1	10	Length ratio (high suction /low suction) (%)
CSH-9	Fresh	mean	-	11.9	-	
		se		0.52		
		CV(%)		29		
	Aged	mean	-	9.5	5.3	56
		se		0.34	0.26	
		CV(%)		21	27	
CSH-11	Fresh	mean	-	11.9	-	
		se		0.55		
		CV(%)		29		
	Aged	mean	-	12.1	5.4	45
		se		0.51	0.24	
		CV(%)		26	24	
M35-1	Fresh	mean	21.9	13.6	-	62
		se	0.84	0.50		
		CV(%)	25	23		
	Aged	mean	-	16.66	5.8	35
		se		0.77	0.23	
		CV(%)		26	22	
ICSV-112	Fresh	mean	20.7	16	-	77
		se	0.67	0.57		
		CV(%)	23	22		
	Aged	mean	-	17.5	6.3	36
		se		0.86	0.25	
		CV(%)		27	24	

All cultivars had greater radicle length ($p < 0.001$) at the smaller of each pair or matric suctions.

kPa) or wetter. Thus, the observed effects are almost certainly due to the effect of matric suction on the contact area of the water film between the seed and the filter paper. Similar effects are also likely to occur in the soil so that these results indicate the combined importance of seed-soil contact and matric suction in ensuring fast germination, root and shoot growth. The seed moisture content at 50 % germination was not affected by the matric suction at which the seeds imbibed. This shows that seeds must reach a specific water content at germination ir-

Table 4: Seed moisture content (% fresh wt.) at T50 and time to 50% germination as affected by matric suction and cultivar (Each value is a mean of five replicates)

Cultivar (kpa)	Matric suction	m.c at T_{50} mean ± se (CV) (%)	T_{50} (h)
ICSV-112	0.1	31.48±1.8 (7.3)	31±0.9 (6.8)
	10	31.49±2.5 (10)	42±0 (0)
CSH-9	0.1	27.48±0.8 (5.2)	42±0 (0)
10		27.17±1.3 (7.9)	50±0 (0)
LSD ($p<0.05$)		**6.1**	**1.52**

Seed moisture content at T_{50}, is based on all seeds (germinated and ungerminated) at 50% germination.

Cultivar Statistic Matric suction

Table 5: Effect of filter paper matric suction and sorghum cultivar on seed to seed-liquid contact area ratio (n = 10)

Cultivar	Statistic	Matric suction (kpa)		
		0.1	1	10
CSH-9	mean	$0.127^{b, a}$	$0.032^{a, a}$	$0.022^{a, a}$
	se	0.009	0.007	0.004
	CV(%)	21.6	40	46.4
CSH-11	mean	$0.207^{c, b}$	$0.068^{b, b}$	$0.037^{a, b}$
	se	0.014	0.005	0.006
	CV(%)	14.7	24.2	33.9
M35-1	mean	$0.12^{b, a}$	$0.029^{a, a}$	-
	se	0.009	0.003	
	CV(%)	24.8	27.5	
ICSV-112	mean	$0.231^{b, b}$	$0.069^{a, b}$	$0.042^{a, b}$
	se	0.011	0.006	0.001
	CV(%)	15.3	21.2	6.1

Multiple range test results (LSD) at $p < 0.05$ are given. The first letter compares between suctions for the same cultivar, the second letter compares between cultivars at the same suction. Means sharing the same letter are not significantly different.

respective of how fast they are allowed to imbibe. The above is in agreement with early work by Hunter and Erickson (1952) in which it was established that each crop species has to attain a specific moisture content to germinate. However, the current study has shown that cultivars from the same species (ICSV-112 and CSH-9) can have different moisture contents at germination.

This work has established a link between matric suction, seed-liquid contact area and rate of water uptake. Increased matric suction, reduced the water imbibing surface on the seed (SLCA) resulting in decreased water uptake rate. For example, fully immersed in water, ICSV-112 seeds imbibed to a moisture content of 27 % (fresh wt.) in 7 h. At 0.1kPa and on filter paper, about 13h were required and at 10 kPa 25 hours were needed to imbibe to the moisture content of 27 %. Therefore, this must be a contact area effect. Consequently, an increase in matric suction delays the initiation of

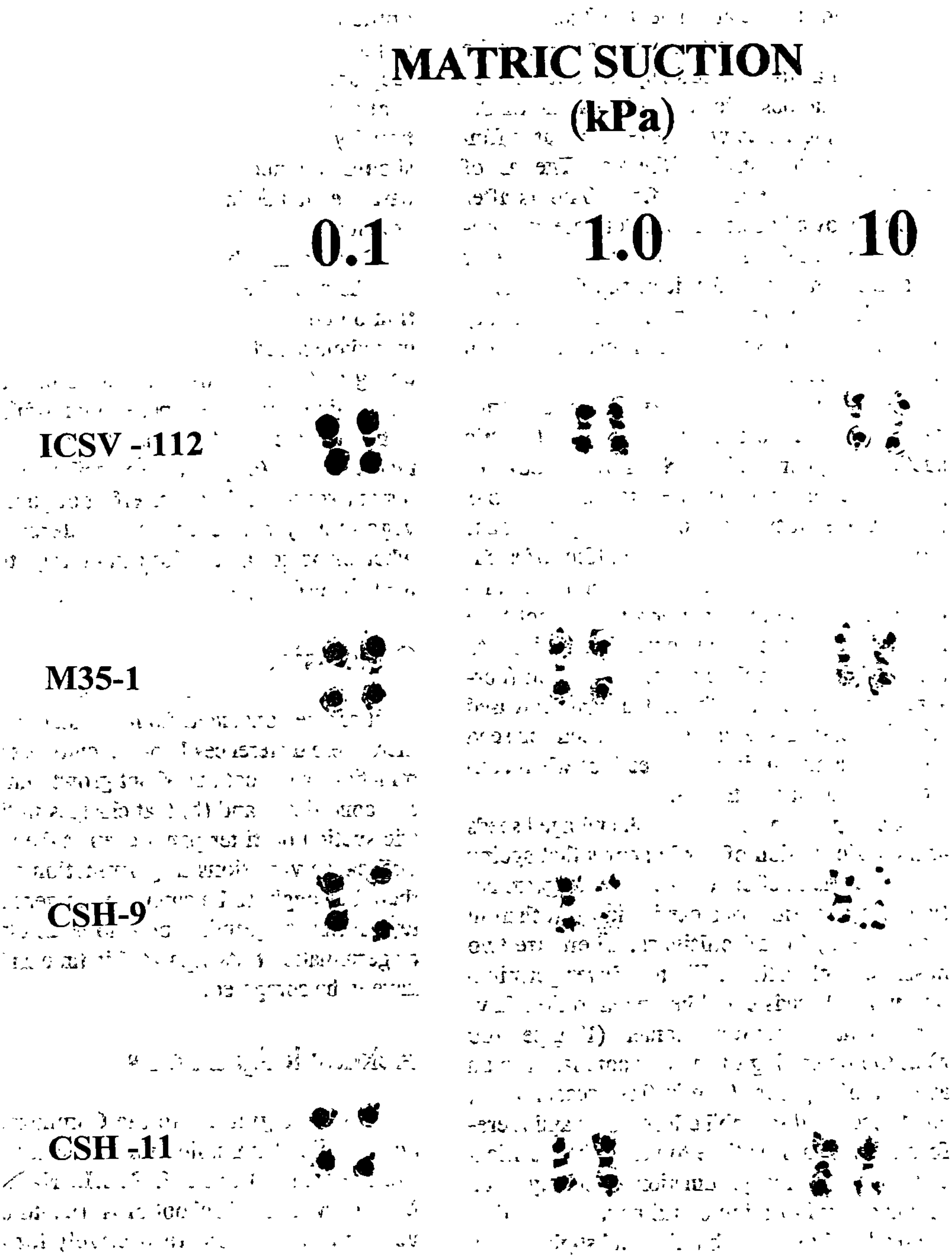

Plate 1:Stained seed-liquid contact area (SLCA)

germination and the attainment of T_{50}.

The rate of imbibition was not seen to be proportional to the seed liquid contact area (SLCA). One possible reason is that the SLCA gradually increases with time as a water film builds up on the outside of the seed. The rate of imbibition was high in the first 10 hours after which it slowed down. The water potential gradient between dry seeds and moist filter paper must have facilitated initial rapid uptake of water. The slowing down of uptake probably occurs when the wetting front passes through the whole seed.

Seed weight may also have affected germination rate. Between cultivars and at all matric suctions, lighter seeds, ICSV-112 and CSH-11 germinated earlier than heavier ones, M35-1 and CSH-9 both for fresh and aged seeds. However, this may reflect the effect of seed-soil contact area (smaller seeds having a relatively greater area of water film contact than larger seeds) rather than something intrinsic to the seed since Dunbabin and Aitcheson (personal communication) found that T_{50} decreased with increasing size of sorghum seed for seed from the same cultivar and seed lot when contact area was not restricted.

A comparison between fresh and aged seeds at a matric suction of 1 kPa shows that ageing had a beneficial effect on the onset of germination. Germination was earlier in aged than in fresh seeds, for all cultivars. There are two possible explanations. Firstly, faster germination in aged seeds could be linked to their having been at greater water content (19 g per 100 g) at the beginning of the test compared to an average of 9 g per 100 g in fresh seeds. Thus, aged seeds had to imbibe less water and therefore took a shorter time to reach their critical water contents for germination. Secondly, seed repair or invigoration could have occurred in the seeds following imbibition and slight ageing (Burgass and Powell, 1984).

There was a no clear influence of ageing on radicle or coleoptile length for seeds germinated at 1 kPa. For M35-1, ageing increased radicle and coleoptile length compared to fresh seed whereas for CSH-9 ageing increased radicle length but reduced coleoptile length. Since these results do not distinguish between the effects of ageing on time to germination or coleoptile emergence and the effects on the subsequent rates of root and coleoptile growth, they are inconclusive. Future work needs to deal separately with these two effects. Additionally, drying back of artificially aged seed should be a necessary procedure before comparative studies involving fresh and aged seed are undertaken.

Despite the above observations, it is clear that the overall effect of increasing matric suction on coleoptile was more pronounced than on radicle length. In circumstances where hardening of the soil surface due to crusting or hardsetting can prevent penetration of the coleoptile (Weaich, 1993), the effect of a greater matric suction (or poorer seed-soil contact) during emergence and / or the effect of poorer seed vigour may prove critical in determining whether or not a seed lot can emerge through the soil surface.

Conclusion

It can be concluded from this study: (a) that there were differences between cultivars in germination rate, root and shoot growth under the test conditions, and (b) that changes in the matric suction on filter paper even at the wet end will cause variations in germination rate and therefore ought to be controlled in germination tests if time to germination is to be determined, or germination tests separated in time and space have to be compared.

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References

Burgass, R.W. and Powell, A.A. 1984. Evidence for repair processes in the invigoration of seed by hydration. *Ann. Bot.* 53: 753-757.

Collis-George, N. and Hector, J.B. 1966. Germination of seeds as influenced by matric potential and by area of contact between seed and soil water. *Austr. J. Soil Res.*, 4: 145-164.

Deka, R.N., Wairu, M., Mtakwa, P.W. and Mullins, C.E. 1992. Use and accuracy of the filter paper technique for measurement of soil matric potential. *European J. Soil Sci.* 46: 233-238.

Etherington, J.R. and Evans, C.E. 1986. Technique for ecological studies of seed germination in relation to soil water potential. *Plant and Soil* 95: 285-288.

Fawcett, R.G. and Collis-George, N. 1967. A filter paper method for determining the moisture characteristic of soil. *Austr. J. Exp. Agric. Animal Husbandry* 7: 162-167.

Gardner, R. 1937. A method of measuring the capillary tension of soil moisture over a wide moisture range. *Soil Sc.* 43: 277-293.

Greacen, E.L. Walker, G.P. and Cook, P.G. 1987. Evaluation of the filter paper method for determining the moisture characteristic of soil. In: Proceedings of the International Conference on Measurement of soil and plant water status, Utah State University 1, pp.137-143.

Gurmu, M. 1991. The effect of seed vigour and moisture stress in germination and seedling establishment of wheat and sorghum. Ph.D. Thesis. Aberdeen University.

Harper, J.L. and Benton, R.A. 1966. A filter paper method for determining the moisture characteristic of soil. *J. Ecol.* 54: 151-166.

Hadas, A. and **Russo, D.** 1974. A filter paper method for determining the moisture characteristic of soil. *Agron. J.* 66: 643-647.

Hooke, M.H. 1987. How do we tackle crop establishment problems? In: Occasional Publication no. 34: 136-140, Crop Establishment Problems in Queensland: Recognition, Research and Resolution. Proceedings of a Workshop held at Queensland Agricultural College, Lawes, Australia.

Hunter, J.R. and Erickson, A.E. 1952. Relation of seed germination to soil moisture tension. *Agron. J.* 44 (3), 107-109.

Matthews, W. 1980. Controlled deterioration: a new vigour test for crop seeds. In: P.D. Hebblethwaite (Ed), Seed Production, Butterworths, London, pp. 647-660.

Mexal, J., Fisher, J.T., Osteryoung, J. and Reid, C.P.P. 1975. Oxygen availability in polyethlene glycol solutions and its implication in plant-water relations. *Plant Physiol.* 55: 20-24.

Mullins, C.E. 1990. Matric potential. In: Smith, K.A. and Mullins, C.E (Eds.). Soil Analysis: Physical methods. Marcel Dekker, New York.

Naylor, R.E.L. 1989. Analysis of the differences in germination of seedlots of perennial ryegrass in response to artificial ageing. *J. Agric. Sc. Cambr., 112, 351-357.*

Weaich, K. 1993. Pre-emergent shoot growth of maize (Zea mays L.) under high soil temperature and strength conditions. Ph.D. Thesis. University of New England, Australia.