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Studies on germinability and some aspects of pre-harvest physiology of sorghum grain

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Summary

Germinability water absorption and electro-conductivity of seed leachates were determined during grain development in a set of sorghum genotypes There was a steady increase in the mean percentage of germination throughout the course of grain filling despite significant genotypic differences Some genotypes were capable of germination as early as 10–15 days after anthesis (milk stage) while others would germinate only after 30–35 days (approximately at black layer formation) There was an increase in water absorption and electro-conductivity of seed leachates with the maturation of the grain

Résumé

Etudes sur la faculté germinative et quelques aspects de la physiologie du grain de sorgho avant récolte

La faculte germinative l absorption d eau et la conductivite electrique des exsudats de semences ont ete determinees pendant le developpement du grain dans un ensemble de genotypes de sorgho Il y avait une augmentation constante du pourcentage moyen de germination pendant tout la duree du remplissage du grain malgre des differences entre genotypes significatives Quelques genotypes etaient deja aptes à la germination 10 a 15 jours apres l anthese (stade laiteux) alors que d autres germaient soulement 30 à 35 jours après (approximativement au moment de la formation de la couche noire) Il y avait une augmentation de l'absorption d'eau et de la conductivite electrique des exsudats de semences avec la maturation du grain

Zusammenfassung

Untersuchungen über die Keimfähigkeit und einige Aspekte der Vorerntephysiologie beim Sorghum-*Korn*

Die Keimfähigkeit, die Wasseraufnahme und die elektrische Leitfähigkeit des Quellungswassers wurde bei einem Satz *Sorghum*-Genotypen im Verlauf der Kornentwicklung bestimmt Ungeachtet signifikanter genotypischer Unterschiede fand sich während der gesamten Kornfüllungsphase eine stetige Zunahme im mittleren Prozentsatz der Keimfähigkeit Einige Genotypen waren imstande, schon 10–15 Tage nach der Blüte (Milchreife) zu keimen, während andere erst nach 30–35 Tagen (etwa bei der Blacklayer-Bildung) keimten Im Verlauf der Kornreifung nahm die Wasseraufnahme und die elektrische Leitfähigkeit des Quellungswassers der Samen zu

Introduction

Pre-harvest sprouting is one of the major problems in early maturing sorghum varieties when grain maturation occurs in rainy weather. This leads to loss of seed viability and enhances the development of grain moulds. Enzymes activated or synthesised during germination initiate hydrolysis of starch in the endosperm, causing chalkiness of the grain and loss of weight, and provide a favourable environment for saprophytic fungi (Castor and Frederiksen, 1977, 1978). Seed dormancy during and after maturation could aid in reducing grain weathering and improve crop quality, in addition to protecting the viability of the seed.

There are several reports of pre-harvest germination in sorghum (Kersting, Stickler and Pauli, 1961; Harris, Johnson and Stacy, 1962; Clark, Collier and Langston, 1967). The tannin content of the testa has been associated with both reduced pre-harvest germination (Harris and Burns, 1970), and reduced pre-harvest grain moulding (Harris and Burns, 1973). Rate of water uptake by the grain and electrical conductivity of seed leachates have both been found to be associated with post-maturation grain deterioration (Glueck and Rooney, 1978). The present study was undertaken to determine the magnitude of genotype differences in sorghum for these parameters and to identify materials for a study of the relationship of these parameters to grain weathering.

Materials and methods

Experiment I

Forty-seven germplasm lines selected at random and three standard cultivars were sown in the rainy season on July 3, 1978 at ICRISAT Centre, Patancheru. The single row plots of 2 m length were spaced at 75 cm. Plant to plant spacing within a row was 10 cm, with a total of 20 plants per entry.

Six panicles which flowered on the same day were tagged at flowering and divided into two subsamples of three panicles each. At five day intervals, beginning five days after flowering and continuing until 40 days after flowering (approximate harvest maturity, about 10% moisture) five grains from the top, middle and base of each panicle were removed from the tagged heads and mixed by subsample to form two samples of 45 grains each. Fresh weights of the grain samples were determined; the samples were washed for 15 s in distilled water and then transferred to test tubes containing 25 ml distilled water. After 15 minutes the test tubes were thoroughly shaken and the seeds removed. The electro-conductivity of the seed leachates was determined with a commercial conductivity bridge and the specific conductance in micromhos/cm calculated by multiplying the measured conductance by the cell constant (0.1/cm) (procedure through courtesy of R. W. Rooney, unpublished). After the electro-conductivity measurement, the seeds were transferred to a new set of tubes containing 25 ml of distilled water. After four hours of soaking, the seeds were blotted to remove the adhering water, weighed, and the water uptake was determined as a percentage of the fresh weight The seeds were then transferred to petri dishes lined with moist filter paper and kept in a seed germinator at 35 C The germination count (with radicle emerged) was made after three days

Percentage moisture in the grain and accumulation of dry matter at different stages of grain filling were determined by taking fresh and dry weights at all sampling times in a parallel set of samples Dry weights were taken after putting the seeds in an oven at 80 C for two days

Additional grain samples were collected at 40 days after anthesis and sun dried for two days for observations of grain hardness and endosperm thickness Grain hardness was tested with a hardness tester (Kiya Feisakusho Ltd Tokyo) which records breaking force in kg Longitudinal sections of the grain were cut and the thickness of corneous layer in the endosperm was scored on a 1 to 5 scale 1 for minimum thickness of the corneous layer and 5 for maximum

Grain samples were not evaluated for weathering at the time of sampling Slight to moderate amounts of weathering (spotting and discolouration) were present but no visible mould growth or sprouting was noticed on any of the samples Data on germination percentage moisture percentage weight electro-conductivity and water absorption of the seeds were analysed for main effects (genotype and time of sampling) and their interactions

Experiment 2

A wide range of lines (193) were grown under irrigation in the post rainy season of 1978 in an experimental design similar to that in Experiment 1 As grain weathering is not normally a problem in this season (due to the absence of rains) the lines were sampled for germinability only Thirty seven of the lines grown in the rainy season were included to test seasonal effects and interactions of season genotype and time of sampling on germination

Results

Grain growth

Grain fresh weight increased linearly from anthesis to approximately 25 days after anthesis Thereafter it decreased gradually as grain moisture percentage continued to decline (figure 1) The grain dry weight increased linearly slowly initially and more rapidly from approximately 10 to 25 days after anthesis Thereafter there was practically no further increase (figure 1) Black layer formation (Eastin Hultquist and Sullivan 1973) in general occurred at 32 days after anthesis At any given time of sampling there was a wide range in the individual genotype values for grain fresh and dry weights and moisture content (table 1)

Water uptake

Percentage water uptake was high in very young seeds (five days after anthesis), fell to a minimum between 15-30 days and then increased sharply after physiological matu-

Table 1 Means and ranges of seed characters at different stages of grain development (Experiment 1)

Days after flowering	Seed dry weight (g/100 seed)		Seed moisture percentage		Germination percentage		Conductivity (μ mhos cm)		Water absorption percentage of fresh seed weight)	
	X̄	Range	X̄	Range	X̄	Range	X̄	Range	X̄	Range
5	0 22	0 08–0 60	74 2	53 7–88 2	0	0– 0	16 6	7 7 24 5	12 4	3 2–26 0
10	0 56	0 12–0 98	68 4	55 7 85 1	0	0– 0	13 6	5 4–24 9	5 2	1 3–12 9
15	1 13	0 68–1 70	55 8	47 2–66 8	0 3	0– 7 8	14 1	5 1 24 0	3 3	0 7 8 1
20	1 73	0 93–2 85	45 6	32 0–57 0	1 5	0– 25 6	14 1	5 1 25 0	2 5	0 4– 6 6
25	2 26	1 25–3 10	37 9	18 7–48 8	12 9	0– 92 2	14 9	6 4–25 5	2 7	0 8 6 6
30	2 40	1 18–3 80	31 0	20 7–43 2	45 9	0–100	16 3	5 2 31 0	3 7	1 1 9 8
35	2 38	1 02 3 82	23 1	5 3–37 6	74 5	13 3–100	20 7	7 5–45 2	5 9	1 6–13 0
40	2 32	0 98 3 95	9 3	4 8–15 1	92 0	75 6–100	30 3	6 5–52 5	9 9	3 3–37 0

Table 2 Analyses of variance of different seed characters (Experiment 1)

Source of variation	Days after flowering	F values				
		Seed weight	Seed moisture percentage	Germination percentage	Conductivity	Water absorption (percentage of fresh seed weight)
Genotype	49	26 7**	13 8**	92 5**	82 7**	19 1**
Time of sampling	7	1884**	4631**	10259**	859**	700**
Genotype × time of sampling	343	6 24**	4 77**	37 7**	17 5**	10 0**
Sampling error	400					

**P < 0 01

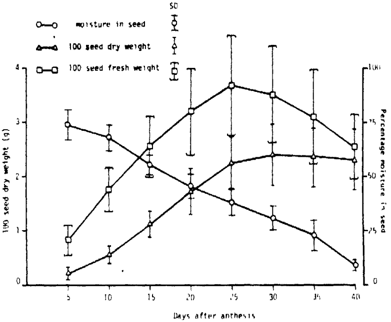

Figure 1. Changes in seed fresh weight, dry weight and moisture percentage from five to 40 days after anthesis (Experiment 1). Data are means of all genotypes.

rity (figure 2). The range within genotypes for percentage water uptake was small during the period when mean rates were low, but increased significantly by 40 days after anthesis (table 1). Genotypic differences at 40 days (3%–37%) could be of particular significance for resistance to weathering and prevention of germination on the panicle in periods of light rains (Castor and Frederiksen, 1977).

Electroconductivity

There was no change in the electro-conductivity of the seed leachates between five days and 30 days after anthesis (approximately black layer stage). Thereafter there was a marked increase in electro-conductivity together with water uptake (figure 2). Variation among genotypes was considerably greater for conductivity than for water uptake over the whole sampling period (table 1).

Germination, Experiment I

Most genotypes would germinate between 20 and 30 days after anthesis. However, the actual percentage of the seeds which germinated in this period varied considerably among lines (figure 3). At 30 days after anthesis, when all but two lines would germinate, the actual percentage germination in the rest of the genotypes ranged from three to 100. By 40 days after anthesis, the minimum germination was 76%, indicating that at the time of harvest maturity (about 10% moisture – figure 1) there was no significant dormancy in any of the lines examined. There were a number of genotypes with less than 50% germination at 35 days after anthesis (e.g., IS 6127, IS 6205, IS 6204, IS

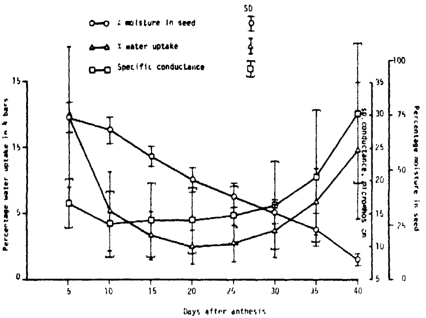

Figure 2. Changes in seed moisture percentage, percentage water uptake and conductance of seed leachates from five to 40 days after anthesis (Experiment 1). Data are means of all genotypes.

9374, IS 3921 and IS 165). Whether this character has any advantage or not during rainy weather under field conditions is not yet known.

Germination, Experiment 2
The general trend of ability to germinate among the 193 lines tested in the post-rainy season was similar to that in the rainy season (figure 4). Germination, however, began earlier as 5% of the lines could germinate 10 days after anthesis and 92% at 25 days. At physiological maturity (around 35 days) all lines could germinate but 100 lines showed less than 50% germination, again in contrast to the rainy season. There were nine lines showing less than 15% germination (IS 2074, IS 4310, IS 6074, IS 6131, IS 9333, IS 15021, IS 15709, IS 16201 and IS 16657).

Analysis of results
Time of sampling, genotype and genotype × time of sampling effects were significant for all measured parameters in Experiment 1 (table 2) and percentage germination in Experiment 2. An analysis across season, for the 37 genotypes which were common to both seasons showed significant interactions of season with both genotype and genotype × time of sampling for percentage germination (table 3).

Correlations among the measured traits were examined for 30 days after anthesis (when variation in germinability was the greatest) and for the final sampling (40 days after anthesis). Precentage germination was not correlated to any of the measured variables at 30 days and correlated only to specific conductance at 40 days (r = −0.38,

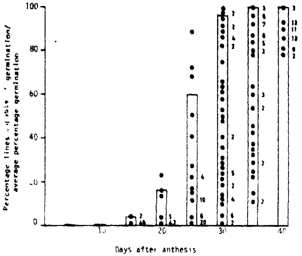

Figure 3. Changes in percentage of lines capable of germination (bars) and mean percentage germination of each line (●) from five to 40 days after anthesis (Experiment 1).

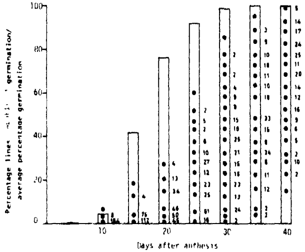

Figure 4. Changes in percentage of lines capable of germination (bars) and mean percentage germination of each line (●) from five to 40 days after anthesis (Experiment 2).

$P < 0.01$). This correlation may simply reflect weathered seeds which would be expected to have a higher leachate conductivity and lower germination (although data on degree of weathering was not recorded).

Water uptake was weakly and negatively correlated to seed dry weight ($r = -0.43$ and -0.41, at 30 and 40 days, respectively, $P < 0.01$) indicating greater relative (as a percent of dry weight) uptake by smaller seeds. Water uptake and conductivity of seeds were unrelated, but conductivity of the mature seed leachates was correlated negatively ($r = -0.36$, $P < 0.05$) to grain hardness, indicating greater electrolyte leakage in the soft grain types.

Table 3. Analysis of variance of germination percentage.

Source of variation	Days after flowering	F value
Genotype	36	93.86**
Time of sampling	7	8935.68**
Season	1	735.69**
Genotype × time of sampling	252	23.31**
Genotype × season	36	57.15**
Time of sampling × season	7	302.6**
Genotype × time of sampling × season	252	18.36**
Sampling error	592	

$^{**}P < 0.01$

Discussion

Castor and Frederiksen (1977, 1978) have shown that germinability during grain filling in the rainy season promotes the growth of saprophytic fungi and grain deterioration. In the present study a significant range of variability in germinability of grain, prior to physiological maturity of the seed, was observed. At harvest maturity stage (about 40 days after flowering) most of the genotypes were capable of germination. These results are similar to those reported by Brown *et al.* (1948) (quoted by Gritton and Atkins 1963) in which only five out of 147 varieties of sorghum were found to possess even partial dormancy at harvest.

There was an increase in electro-conductivity and water uptake in the later stages of grain development which may be associated with an increase in permeability of the membrane, either naturally or due to weathering. Entry of water into the seed and leaching of materials out of the seed at harvest maturity were not correlated, however. In addition, conductivity was less in seed with a corneous endosperm ($r = -0.50$, $P < 0.01$) whereas water uptake appeared to be independent of the corneous rating of the endosperm. Thus, water uptake and conductivity appear to be governed by different factors.

Rate of water uptake (which is important in germination) was correlated only to seed size which may reflect the greater surface/volume ratio in small seed (although no information on this point could be found in the literature). Specific conductivity at maturity was negatively related to percentage moisture in the seed ($r = -0{,}42$, $P < 0.01$), as it was during the grain development period. This relationships probably reflects differences in maturity rather than a 'physiological' effect (figure 2). Combining both factors (40 day data) which were simply correlated to conductivity of seed leachates (hardness and percentage moisture in the seed) gave a multiple regression coefficient slightly higher than either individual coefficient ($r = -0.61$, $P < 0.01$, compared to $r = -0.50$ and -0.42 for hardness and percentage moisture respectively). The predictive value of this relationship, however, is not high enough to be of practical value.

Correlations between these same parameters at maturity and during grain development were not significant i e , mature grain values did not predict values during grain filling In addition, the combined analysis of the common entries in the two experiments gave evidence of a seasonal difference in the dormancy of certain entries Thus, sampling procedures for germinability, water uptake, etc need to take into account the effects of both environment and stage of development and maturity of the seed

Despite these effects, however, some genotypes were identified which showed some level of dormancy at physiological maturity (30-35 days after anthesis) in both the seasons e g , IS 83, IS 188, IS 219 IS 1235, IS 1352, IS 2468, IS 6117 and IS 6204 It is not known if this delayed germinability has a measurable effect on grain weathering and sprouting of the seeds during the rainy season As there was no dormancy following physiological maturity these lines will be affected by rains occurring following maturity Therefore, instead of looking into these lines in more detail, a large number of germplasm lines should be screened for entries which have dormancy at late stages of maturity (at about 40 days after anthesis), and which would be much more useful for breeding for weathering resistance Therefore more concerted effort should be made on this line of research

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