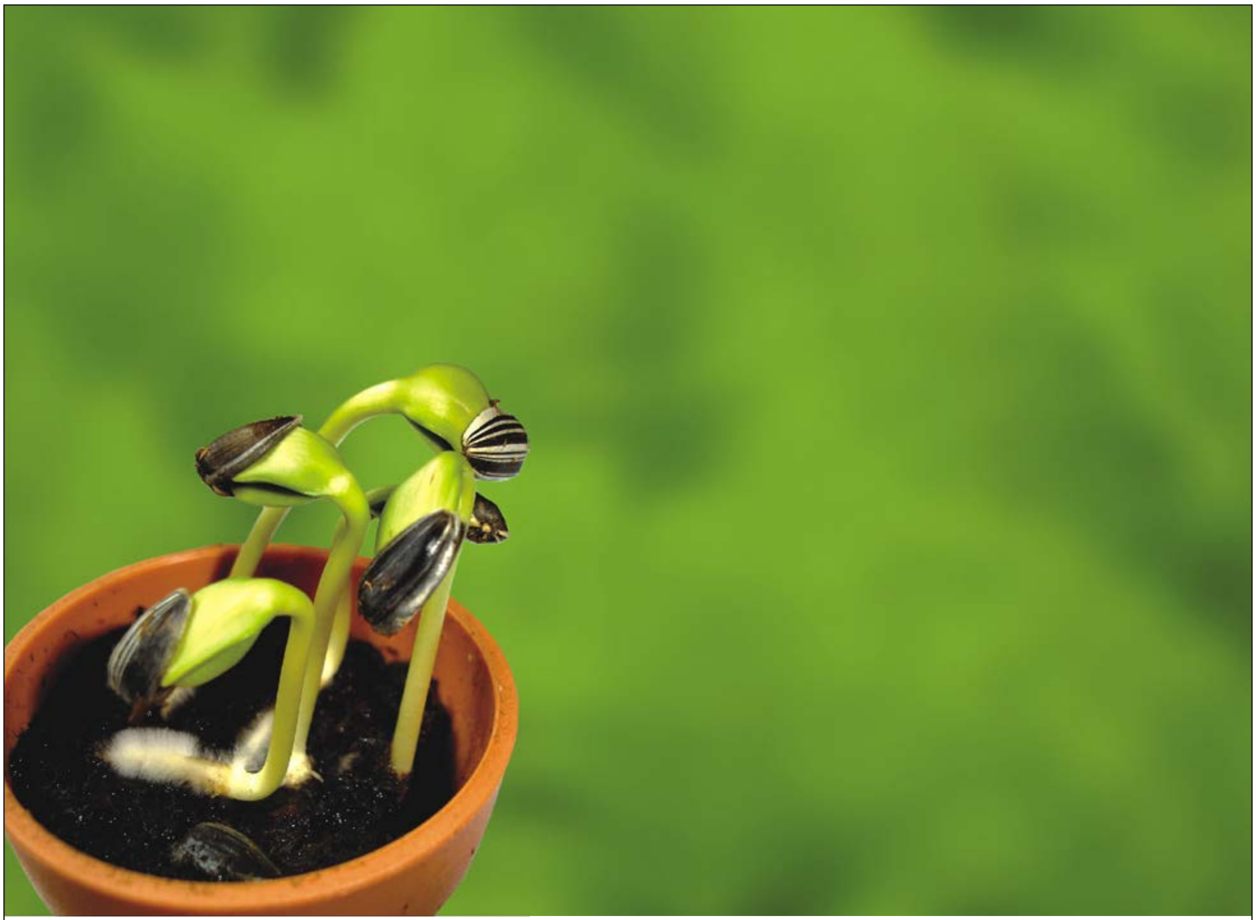

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Research Article
Evaluating the Efficacy of Synthetic Fibre Pollination Control Bags in Sorghum During the Rainy Season

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Abstract
Background and Objective: Sorghum breeders traditionally use paper pollination control bags for hybridisation and generation advance. However, these bags get torn off in the rainy season and by birds searching for food. High incidence of grain mold also occurs in these bags. The major objective of this study was to test novel nonwoven synthetic fabrics as replacement for paper bags. **Methodology:** Three newly developed pollination control bags from synthetic polyester and polypropylene nonwoven fibres were tested against the paper bags at Patancheru, ICRISAT, India on four sorghum genotypes for bag traits and many quantitative characters in the rainy seasons of 2014 and 2015 years that were diverse in total rainfall which was 231.9 mm more in 2015 compared with 2014 (77% higher) with 11 more rainy days than that in 2014 (299.3 mm in 42 rainy days). Data were analysed using the factorial analysis of variance and Pearson's correlations. **Results:** Bags were evaluated for three bag traits: Water resistance, intactness and ease of handling. The duraweb® SG1 and duraweb® SG2 were superior to other bag types for all these traits with duraweb® SG1 being significantly superior to duraweb® SG2. The duraweb® SG3 bag was similar to the standard paper bag for all bag traits. While bags did not show significant differences for grain yield they significantly differed for other six quantitative traits; duraweb® SG1 was the best performer followed very closely by duraweb® SG2 bag. The duraweb® SG1 bag was significantly superior to no bagging as well as all other types of bags for grain mold resistance. On average 12% bird damage on panicles was observed under paper bags compared with 36% on the uncovered panicles. Contrastingly, no bird damage was observed under bags made of all three novel materials. **Conclusion:** The new pollination bags made from nonwoven fabrics provide an effective alternative to traditional paper bags as they ensure near-ambient micro-environment within them for seed development and are strong enough to resist wind, rain and bird damage while allowing aeration to minimize fungal development in the rainy season.

Key words: Sorghum, pollination bags, nonwoven fabrics, bird damage, grain mold, quantitative traits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a self-pollinated diploid (2n = 2x = 20) C_4 grass with high photosynthetic efficiency. It is one among the few resilient crops that can adapt well to predicted climate change effects, particularly increased temperature and higher incidence of extreme rainfall events in addition to increasing soil salinity. It is the fifth most important cereal crop after wheat, maize, rice and barley in both area and production and is the dietary staple of more than 500 million people in 30 countries[1]. Sorghum is one of the cheapest sources of energy and micronutrients and it supplies more than 50% micronutrient needs in the low-income group populations[2,3]. The sorghum area in India (6 m ha) was about 13% of the world's area in 2014[4]. The crop is primarily grown in the Southern states of Maharashtra, Karnataka, Telangana and Andhra Pradesh which produce about 80% of all-India production. It is also grown in Madhya Pradesh, Gujarat and Rajasthan to a lesser extent. In India, sorghum is grown in both rainy (kharif) and post-rainy (rabi) seasons as grain and as stover for livestock, particularly in the dry season when feed resources are in short supply. Hence dual purpose types of sorghum are generally preferred[1,3]. The productivity is higher in the rainy season (1.2 t ha^{-1}) because of adoption of improved hybrids and the crop is grown in better moisture regimes compared to the post-rainy season crop which is grown entirely with residual soil moisture where Open Pollinated Varieties (OPVs) are the cultivars of choice resulting in lower yields (0.7 t ha^{-1}). However, in recent years, sorghum cultivation has shifted to the post-rainy season because of heavy incidence of grain mold in the rainy season resulting in poorer quality of the grain than the post-rainy season crop.

From a crop breeding point of view, rainy season is detrimental to crossing programmes and advancement of selfed progenies of breeding stocks because grain molds develop and bird damage can be considerable where fewer plants of a given genotype are grown in small plots[5]. Attempts to protect developing seeds from such damages have traditionally been accomplished by covering the panicles with pollination bags. The maintenance of thousands of germplasm accessions of sorghum collections in many countries and to preserve their genetic identity requires the use of a large number of pollination bags[6]. While the traditionally used paper bags can be torn off in the rainy season by wet and windy weather or by birds, attempts to replace them with more effective bags have been very scanty due to non-availability of synthetic fabric options. Recently, newly developed nonwoven polyester and polypropylene fabrics have been developed to ensure near-ambient micro-environment within them for seed development. The new materials are hardy enough to resist wind and rain damage while allowing aeration to minimize fungal development[7,8]. However the utility of these bags has been rarely tested in sorghum[9]. Therefore, this study explored hitherto rarely investigated area of improving the efficiency of plant breeding-hybridization and generation advance, by providing experimental evidence that the traditional paper pollination bags need replaced with novel nonwoven fabrics for better seed harvest. It lays foundation for new research area of developing, testing and using pollination bags made from novel nonwoven fabrics and how they provide more ambient micro-environment for healthy seed development within them than the paper bags.

The objectives of the present investigation were:

- Compare the effectiveness of novel pollination bags fabricated from newly developed nonwoven fabrics for healthy seed development in the rainy season sorghum
- Evaluate different bag types for traits that could influence seed development

MATERIALS AND METHODS

Trials were conducted during the rainy season of 2014 and 2015 at Patancheru campus of ICRISAT in India (altitude 545 m above mean sea level, latitude 17.53^0 N and longitude 78.27^0 E). Three newly developed pollination bags were tested for their comparative efficacy along with the standard paper bags used by sorghum breeders in India. The new pollination bags were made of nonwoven air-permeable polypropylene or polyester materials. All the new bags were designed to be light in weight but stronger than paper to resist damage from bird attack, high winds and rainy weather. Unlike the paper bags, new bags made from synthetic materials are reusable after cleaning by washing or autoclaving and have greater air permeability allowing exchange of temperature and humidity to create ambient micro-environment within them[10]. The five bag treatments were:

- **Duraweb® SG3 pollination bag:** A gusseted bag, 420 mm length × 140 mm width × 60 mm depth, made from carded, point-bonded, nonwoven polyester having 55 g m^{-2} mass. It has very open structure to maximize permeability
- **Duraweb® SG1 pollination bag:** 3D bag of size 420 mm length × 140 mm width × 60 mm depth, made of layers of point-bonded nonwoven polypropylene with the goal of maximizing air permeability while also creating strength and the ability to block pollen. It has 60 g m^{-2} mass

Table 1: Specification of new nonwoven fabrics used in the manufacture of pollination bags

Test	Duraweb® SG1	Duraweb® SG2	Duraweb® SG3
Polymers	Polypropylene	Polyester	Polyester
Mass per unit area (g m^{-2})	60	70	55
Thickness (mm)	0.36	0.11	0.6
Tensile strength (MD) (N/50 mm)	117	360	123
Tensile strength (CD) (N/50 mm)	95	190	108
Tear strength (MD) (N)	37*	7.0	9.3
Tear strength (CD) (N)	46*	8.0	8.7
Mean pore size (μm)	15	8.8	39
Air permeability (L m^{-2} sec^{-1})	192	67	2880

MD: Machine directional, CD: Cross directional, N: Newton, *Test done using trapezoidal test rather than the usual trouser test used for the other materials

Table 2: Major distinguishing traits of experimental genotypes of sorghum

Genotype	Type, season†	Panicle	Grain	Glume	Glume coverage	Grain mold (Score 1-9)	50% flowering (days)	Plant height (m)
ICSH 28001	Hybrid, K	Compact	White	Light red	25	Moderate (4)	67	2.3
Bulk Y	OPV, K	Semi-compact	White	Purple	25	Susceptible (8)	52	1.4
ICSR 14001	OPV, K and R	Compact	Cream	Cream	25	Moderate (4)	71	1.3
IS 14384	OPV, K	Loose	Red	Purple	75	Resistant (2)	66	3.2

†OPV: Open pollinated variety, K: Kharif (rainy season), R: Rabi (post-rainy or dry season)

Fig. 1: Loose and compact bagged panicles of different genotypes

- **Duraweb® SG2 pollination bag:** 3D bag of size 420 mm length×140 mm width×60 mm depth made from nonwoven polyester having 70 g m^{-2} mass, thermally bonded, with a smooth paper-like surface similar to that of traditional duraweb®
- Indian standard paper pollination bag
- No bagging

The mean pore sizes of the new fabrics were smaller than 40 μm to check the entry of unwanted sorghum pollen grains (Table 1). Duraweb® SG3 had greatest air permeability and duraweb® SG2 the least (Table 1).

All types of bags were tested on four sorghum genotypes, 1 = ICSR 14001, 2 = ICSH 28001, 3 = IS 14384 and 4 = Bulk Y that varied in different traits (Table 2, Fig. 1). ICSH 28001 is a grain mold resistant hybrid but all other genotypes are open pollinated varieties (OPVs). Of the OPVs, ICSR 14001 is being tested in on-farm trials for its release for cultivation in the rainy season. The IS 14384 was included as the grain mold resistant check and Bulk Y as a susceptible check. All genotypes have loose and sparse panicles except IS 14384 which has compact panicles with the highest glume coverage of 75% compared to 25% in all other varieties.

The experiment in 2014 was laid out in a split-plot design with bag types in the whole plots and genotypes in the sub-plots. The trial had three complete replicate blocks. However, the trial being of smaller size showed no advantage of using split-plot design over a factorial design. The statistical analysis of the design showed that mean squares for error (a) were not significantly different from that of error (b) for various traits. Therefore, the two errors were pooled to equate to the analysis of a factorial design. Based on experience of 2014 the trial in 2015 was laid out in a randomized complete block design with three replications as a two-factorial trial with 5 bag and 4 genotype treatments. There were five experimental plants in each experimental plot in both years.

Each treatment was allocated to a plot size of 1.5 m^2 having two rows of 2 m length with row-to-row spacing of 75 cm. Two seeds were planted per hill with a spacing of 10 cm between the hills and later thinned to a single seedling per hill to obtain a population stand of 20 plants per plot. The crop was supplied with 40 kg P_2O_5 ha^{-1} and one-third of

80 kg N ha^{-1} as basal dose. The remaining two-split doses of N were applied at tillering and flowering stages. Hand weeding and earthing up operations were practiced for weed control. Recommended cultural practices were adopted to raise a good crop.

Data were collected on five healthy plants per plot for agronomic and bag related traits. The agronomic traits were: DF = Days to flowering from sowing when 50% of each covered panicle of a plant flowered, GY = Yield of all dried grains per plant recorded in grams, SW = 100 seeds per treatment were counted and weighed in grams, GM = Grain mold score, BD = Bird damage scored as percent damage on panicles and grain Fe (iron) and Zn (zinc) concentrations measured in ppm in grains following harvest. Grain Mold (GM) occurrence on the inflorescence within the bag was recorded on a 1-5 scale, where 1 = Highly resistant (no disease), 2 = Very resistant (25% inflorescence diseased), 3 = Moderately resistant (50% inflorescence diseased), 4 = Low resistance (75% inflorescence diseased), 5 = No resistance (100% inflorescence diseased).

Among the bag traits water resistance (WR), intactness of bag (IB) and ease of handling of bag were measured on scale appropriate for each trait. Water resistance was measured as: (1) Fully resistant (no water or moisture in the bag), (2) Bag wetted by rain, (3) Moisture collected inside the bag. Intactness of bag was measured as: (1) Fully intact with no sagging, (2) Sagging onto the inflorescence, (3) Fully collapsed onto the inflorescence and or torn. Ease of handling of bag was compared with the existing practice of bagging with paper bags as: (1) More, (2) Same and (3) Less.

Data were analysed using factorial analysis of variance and Pearson's correlations[11]. Minitab 16 was used for statistical analysis and drawing graphs. The analysis of variance was restricted to two-factor interactions without determining the complex three-factor interactions. Of the two-factor interactions, bag types×genotypes and bag types×years interactions were important for discerning the efficacy of bag type and were interpreted. Genotypes×years interaction was not the subject of this study.

RESULTS

Yearly effect of rainfall: Rainfall pattern during the crop season from June-October was highly variable between 2014 and 2015 (Table 3). Total rainfall in 2015 was 231.9 mm higher than in 2014 (77% higher). There were 11 more rainy days in 2015 than in 2014. The distribution pattern showed that rainfall in 2015 was more during June, August, September and October the months that coincide with crucial stages of crop growth in the early stages and around flowering and grain filling. The increase of rainfall in 2015 was reflected in significantly delayed days to flowering and more bird damage, but significant decrease in 100-seed weight and grain Fe and Zn concentration (Table 4). The grain mold and grain yield were higher in 2015 but not significantly so (Table 4). Among the bag traits only ease of handling of bags had significantly lower score in 2015 by 31% which showed that compared with the standard paper bags, bagging was easier in 2015 than in 2014 (Table 4).

Analysis of bag traits: Analysis of variance for bag traits showed significant differences between bag types for water resistance, intactness and ease of handling (Table 5). Among genotypes there were significant differences for intactness and ease of handling but not for water resistance. The year 2015 was significantly better for ease of bagging than

Table 3: Rainfall distribution from June-October in 2014 and 2015 rainy seasons at ICRISAT-Patancheru, India

	2014		2015	
Month	Total rainfall (mm)	Rainy days (No.)	Total rainfall (mm)	Rainy days (No.)
June	42.2	5	109.4	13
July	60.3	14	45.8	6
August	101.8	10	139.4	17
September	47.6	9	173.0	13
October	47.4	4	63.6	4
Total	299.3	42	531.2	53

Table 4: Comparison of overall mean values for different traits† in 2014 and 2015 rainy seasons at ICRISAT-Patancheru, India

Year	GM	DF (day)	GY (g)	SW (g)	BD (%)	Fe (ppm)	Zn (ppm)	WR	IB	HB
2014	2.72	58.97	45.53	3.13	8.41	32.87	28.86	2.27	2.01	2.00
2015	2.80	66.31	67.52	2.88	10.80	23.24	22.75	2.25	2.03	1.53
SE mean	0.03	0.07	11.33	0.02	0.41	0.25	0.18	0.03	0.04	0.01
2015-2014	0.08	7.34**	21.99	-0.25**	2.39**	-9.63**	-6.11**	-0.02	0.02	-0.47**
Increase in 2015 (%)	3.00	12.00	48.00	-0.08	28.00	-29.00	-21.00	-0.90	1.00	-31.00

†GM: Grain mold, DF: Days to 50% flowering, GY: Grain yield per plant (g), SW: 100 seed weight (g), BD: Bird damage (%), Fe: Iron concentration (ppm), Zn: Zinc concentration (ppm), WR: Water resistance score, IB: Intactness of bag score, HB: Handling time of bag. **Significant at 1% level of probability

Table 5: Mean squares from analysis of variance for discrete traits measured on a scale

Source	df	Water resistance	Intactness of bag	Ease of handling
Rep/years	4	0.82**	2.15**	0.04**
Genotypes (G)	3	0.22	5.46**	0.05**
Bag types (B)	3	77.27**	35.51**	9.38**
Years (Y)	1	0.02	0.03	27.08**
G×B	9	0.07	0.73*	0.06**
G×Y	3	0.12	1.41**	0.05**
B×Y	3	10.56**	3.92**	48.71**
Error	453	0.16	0.32	0.01**

*Significant at 5% level of probability, **Significant at 1% level of probability

Table 6: Mean values and standard errors (±) for genotypes and bag types

Genotype/bag type	Water resistance	Intactness of bag	Ease of handling
Genotype	NS	**	**
Bulk Y	2.325	2.142	1.750
ICSH 28001	2.242	1.817	1.792
ICSR 14001	2.233	1.867	1.758
IS 14384	2.242	2.258	1.750
SE (±)	0.037	0.051	0.009
SE diff	0.052	0.073	0.013
LSD (5%)	0.102	0.143	0.026
LSD (1%)	0.135	0.188	0.034
Bag type	**	**	**
SG3	2.933	2.442	2.008
SG1	1.450	1.633	1.542
SG2	1.692	1.475	1.500
Standard paper	2.967	2.533	2.000
SE (±)	0.037	0.051	0.009
SE diff	0.052	0.073	0.013
LSD (5%)	0.102	0.143	0.026
LSD (1%)	0.135	0.188	0.034

NS: Not significant, **Significant at 1% level of probability

Table 7: Pearson's correlation coefficients between bag traits

Trait	Water resistance	Intactness of bag
Intactness of bag	0.587**	-
Handling ease	0.442**	0.251**

**Significant at 1% level of probability

2014 (Table 4). Interactions of bag types with years were significant for all traits and that of genotypes×bag types and genotypes×years for intactness and ease of handling (Table 5).

Genotypes ICSH 28001 and ICSR 14001 had an intactness score significantly less than 2, thereby showing that bags on them were more intact than the standard paper with a score of 2. All genotypes scored significantly less than 2 for ease of handling and showed more ease of handling of new bags than the standard paper bags with 2 score. However, ICSH 28001 was significantly less easy for handling than all other genotypes (Table 6).

The bag type duraweb® SG3 and standard paper bag were comparable for all traits. Duraweb® SG1 and duraweb® SG2 both performed better, showed wetting outside but not collecting water; duraweb® SG1 was significantly superior to duraweb® SG2 for water resistance. None of the bag types were was completely resistant to water as they were wetted from outside by rain but their structure, polymer composition and air permeability allowed them to dry off differentially; duraweb® SG1 appeared to dry the fastest. On the other hand, while both duraweb® SG1 and duraweb® SG2 showed significantly more intactness and greater ease of handling than the standard paper bag, the duraweb® SG1 bag was better than the duraweb® SG2 for these traits (Table 6).

Correlation coefficients between traits showed that water resistance was positively associated with intactness and ease of handling. Also there was significant and positive correlation between intactness and ease of handling. As the moisture content increased there was corresponding decrease in intactness because the higher score for intactness meant tendency to shrink or collapse over panicles. Similarly, the increased score for easiness of handling of bags meant lesser ease than the standard paper as with increase of moisture (Table 7). These correlations were based on pooled data over both years but data for years 2015 showed more easiness of handling (Table 4). This means that some degree of humidity in the air has a positive effect on the easiness of handling of bags.

Interactions between bag types and genotypes are given in Fig. 2. The interaction for water resistance was not significant and hence linear additive differences accounted for the effects of genotypes and bag types. Interaction for intactness was significant that mainly resulted due to differential performance of duraweb® SG3 and standard paper with ICSR 14001 and ICSH 28001 genotypes. The duraweb® SG3 showed better intactness on ICSH 28001 than the standard paper but this trend was reversed for ICSR14001. All genotypes showed better intactness with duraweb® SG1 and duraweb® SG2 bags but ICSR 14001 and ICSH 28001 showed more intactness than the other genotypes with these bags. The standard paper bag and duraweb® SG3 showed similar ease of handling on all genotypes. However, duraweb® SG1 and duraweb® SG2 bags were easier to handle on all genotypes as they showed consistently lower score than the standard paper bags. The duraweb® SG1 bag was significantly less easy to handle than duraweb® SG2 on ICSH 28001 genotype perhaps due to differences in the panicle type (Fig. 2).

Bag type and year interaction was significant for all traits. Duraweb® SG3 and standard paper bags showed similar and higher moisture over both years but duraweb® SG1 and duraweb® SG2, with lower scores, changed their ranks in the two years. Duraweb® SG1, which had more moisture in 2014, showed more moisture resistance in 2015. It means that it attracted less water in the higher rainfall year (Fig. 3). Reverse was true for duraweb® SG2.

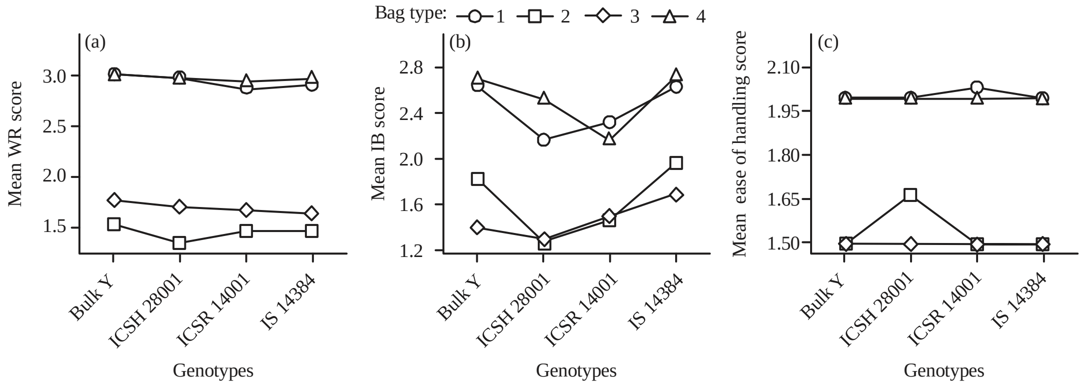

Fig. 2(a-c): Line plots of mean values for bag types×genotypes interactions for (a) Water resistance (WR), (b) Intactness of bag (IB) and (c) Handling ease of bag

Bag types: 1: Duraweb® SG3, 2: Duraweb® SG1, 3: Duraweb® SG2 and 4: Standard paper

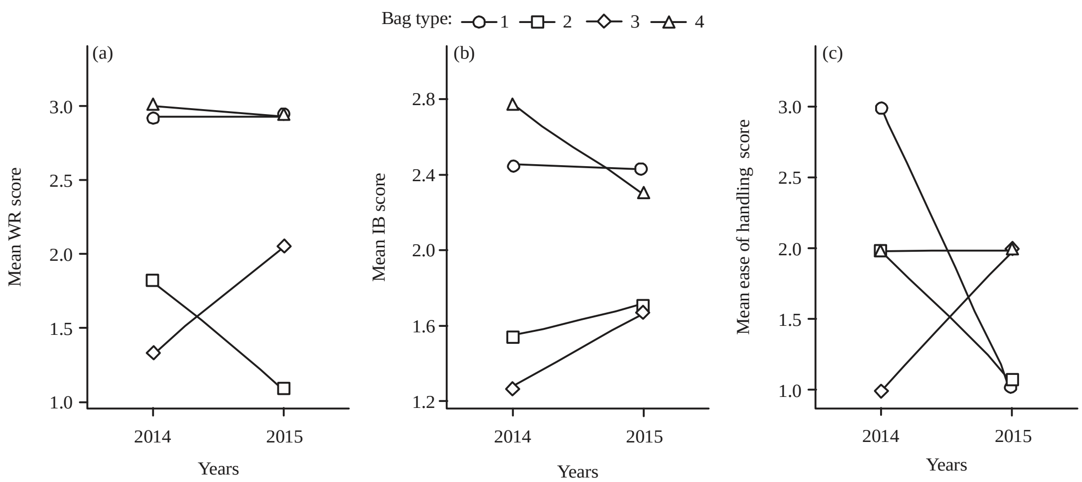

Fig. 3(a-c): Line plots of mean values for bag types×years interaction for (a) Water resistance (WR), (b) Intactness of bag (IB) and (c) Handling ease of bag

Bag types: 1: Duraweb® SG3, 2: Duraweb® SG1, 3: Duraweb® SG2 and 4: Standard paper

For intactness, the standard paper and duraweb® SG3 bags showed less intactness than the duraweb® SG1 and SG2 bags in both years. They also changed their ranks in the 2 years although the absolute change was small. Duraweb® SG1 and duraweb® SG2 showed more intactness than the other bags in both years but duraweb® SG2 had slightly better intactness than duraweb® SG1 in 2014 (Fig. 3).

Of all bag traits, ease of handling showed a very complex interaction between bag types and years. Compared with standard paper bags, duraweb® SG3 was the most difficult to handle in 2014 but it was the easiest to handle in 2015. A similar trend was shown by duraweb® SG1 which was similar in ease of handling to the standard paper bags in 2014 but very easy to handle in 2015. Duraweb® SG2 was reverse of duraweb® SG1. This means the fibres of duraweb® SG3 and duraweb® SG1 became soft and easier to handle when there was more moisture in the air due to higher rainfall. On the other hand, the fibre of duraweb® SG2 became harder and difficult to handle when there was more moisture in the air (Fig. 3).

Analysis of agronomic traits: Mean squares for grain yield per plant were non-significant for main effects of genotypes, bag types and years and their interactions. However, genotypes were close to be significantly different at $p = 0.065$. Perhaps there was high error for single plant grain yield measurement which required more sophisticated weighing balance for more precision.

For all other traits there were significant differences between genotypes and bag types. The year effect was also significant for all traits except grain mold and grain yield

Table 8: Mean squares from analysis of variance for quantitative traits

Source	df	DF	GY	SW	GM	BD	Fe	Zn
Rep/years	4	36.4**	31058	0.54**	1.09*	39.9	722.32**	288.24**
Genotypes (G)	3	8690.1**	93294+	19.90**	199.27**	2784.5**	213.89**	934.63**
Bag types (B)	4	7.0**	54099	0.40**	22.05**	28782.7**	142.11**	65.21**
Years (Y)	1	7527.6**	67578	8.49**	0.78	798.6**	12961.16**	5227.86**
G×B	12	3.7**	60777	1.00**	7.38**	1834.7**	259.85**	211.83**
G×Y	3	1517.6**	71663	1.50**	6.98**	144.1*	189.48**	20.29
B×Y	4	10.3**	69149	0.17	3.38**	4691.4**	357.03**	144.86**
Error	537	1.3	38496	0.08	0.36	49.3	19.22	9.83

*Significant at 5% level of probability, **Significant at 1% level of probability, +Significant at 10% level of probability. DF: Days to 50% flowering, GY: Grain yield per plant (g), SW: 100 seed weight (g), GM: Grain mold, BD: Bird damage (%), Fe: Iron concentration (ppm), Zn: Zinc concentration (ppm)

Table 9: Mean values and standard errors (±) for genotypes and bag types

Genotype/bag type	DF	GY	SW	GM	BD	Fe	Zn
Genotype	**	+	**	**	**	**	**
Bulk Y	51.9	33.6	2.94	4.08	15.90	26.7	22.5
ICSH 28001	62.5	50.8	3.25	2.68	8.54	29.5	26.3
ICSR 14001	67.4	94.1	3.33	2.99	7.96	27.5	25.8
IS 14384	68.9	47.5	2.50	1.28	6.03	28.5	28.5
SE±pooled	0.09	16.0	0.02	0.05	0.57	0.4	0.3
SE difference pooled	0.13	22.7	0.03	0.07	0.81	0.5	0.4
LSD (5%)	0.26	44.4	0.06	0.14	1.59	1.0	0.7
LSD (1%)	0.34	58.5	0.08	0.18	2.09	1.3	0.9
Bag type	**	NS	**	**	**	**	**
SG3	62.5	96.1	2.96	3.10	0.09	26.9	24.6
SG1	62.8	46.0	3.10	2.05	0.08	27.8	25.5
SG2	62.8	46.0	2.98	2.87	0.04	29.8	26.5
Standard paper	62.8	44.9	3.03	3.15	11.54	28.4	26.5
No bag	62.3	49.6	2.97	2.63	36.29	27.4	25.9
SE±pooled	0.10	17.9	0.03	0.05	0.64	0.4	0.3
SE difference pooled	0.15	25.3	0.04	0.08	0.91	0.6	0.4
LSD (5%)	0.29	49.6	0.07	0.15	1.78	1.1	0.8
LSD (1%)	0.38	65.4	0.09	0.20	2.34	1.5	1.0

**Significant at 1% level of probability, +Significant at 6.5% level of probability. DF: Days to 50% flowering, GY: Grain yield per plant (g), SW: 100 seed weight (g), GM: Grain mold, BD: Bird damage (%), Fe: Iron concentration (ppm), Zn: Zinc concentration (ppm)

(Table 8). Despite more rainfall in 2015 it did not reflect in higher mold incidence perhaps the ambient temperature, wind velocity and other conditions did not differ over years to result in differential occurrence of disease.

Interactions between main effects were very prominent (Table 8). Genotypes changed their ranks over the years resulting into a significant genotypes×years interaction for all traits except Zn concentration. Genotypes showed differential behaviour to bag types that resulted in significant genotypes×bag types interactions for all traits. Bag types×years interactions were significant for all traits except seed weight and grain yield.

Genotypes showed significant differences for days to flowering with Bulk Y the earliest and IS 14384 the latest to flower (Table 9). The earliest flowering Bulk Y genotype was significantly the lowest yielding and ICSR 14001 significantly the highest yielding genotype among all genotypes. The IS 14384 had the lowest seed weight and ICSR 14001 the highest seed weight. Bulk Y was the most susceptible and IS 14348 the most resistant for grain mold. Genotypes ICSH 28001 and ICSR 14001 were between "Very resistant" and "Moderately resistant" to grain mold. The maximum average bird damage of 16% was observed on Bulk Y and the least of 6% on IS 14384. Iron concentration was the highest for ICSH 28001 and least for Bulk Y. Genotypes ICSR 14001 and IS 14384 were similar in iron concentration. However, Zn concentration was the lowest for Bulk Y and highest for IS 14384 (Table 9).

Duraweb® SG3 seemed to mimic the natural (no bag) environment for days to 50% flowering as the no bag condition, whereas slightly later flowering was observed with duraweb® SG1, duraweb® SG2 and standard paper bags, all of which were on a par. Applying an unprotected LSD test for grain yield, duraweb® SG3 produced the highest yield but it was non-significantly different from no bagging, the treatment with the lowest grain yield. Duraweb® SG1, duraweb® SG2 and standard paper bags were on a par for their effect on grain yield. Duraweb® SG3, duraweb® SG2, standard paper and no bagging treatments all showed similar 100-seed weight, whereas, duraweb® SG1 showed significantly higher seed weight than all other bag types.

As regards grain mold, duraweb® SG1 showed a mean score significantly lower than all other bag types. Unexpectedly, it was even lower than non-bagging, which might be expected to produce the lowest disease infection in natural conditions. Duraweb® SG2 showed moderate resistance to mold, similar to no bagging. However, duraweb® SG3 showed moderate resistance like the standard paper bag (Table 9).

A mean bird damage of 36% occurred under no bagging. This was followed by 12% under the paper bags that got torn in the high winds and rains. Made weaker by water, paper bags were torn in the rainy season by the push of over-growing panicles and by birds who opened them in search of food within (Fig. 4, 5). As such paper bags were the weakest in the rainy season compared with all three duraweb® bags that did not show any significant bird damage or damage from the weather (Table 9).

The Fe concentration was lowest with duraweb® SG3 which was on par with duraweb® SG1 and no bagging. The Fe concentration was the highest with duraweb® SG2 which was similar to the standard paper bags. The Zn concentration was significantly lower for duraweb® SG3 than all other bag types. While the Zn content under duraweb® SG2 was equal to the standard paper bags the content of duraweb® SG1 was equal to no bagging (Table 9).

Correlation coefficients on mean values of five bag treatments in Table 9 between grain mold and Fe concentration ($r = 0.10$) and Zn concentration ($r = 0.10$) were non-significant. However, when correlations were computed on all observations, grain mold showed significantly negative correlation with Zn concentration and non-significant correlation with Fe concentration. While grain mold incidence increased with earlier days to flowering it was also associated with increased seed weight and bird damage. The earlier flowering might be falling in the best weather conditions for disease development and spread. Both bird damage and seed weight showed negative relationships with days to 50% flowering, which means that earlier produced bold grains might have been preferred by birds (Table 10). This study did not record the length of reproductive period but if earlier flowering plants had longer reproductive stage and still matured earlier than later flowering plants there would be a longer time for the grain to develop and produce higher seed weight that would be the obvious choice of birds for their earlier availability.

The iron and zinc concentrations were positively associated with each other but zinc was negatively associated

Fig. 4: Flying birds over panicles after being scared during photograph

Fig. 5: Damaged paper bags on compact panicles with grains exposed to bird damage

Table 10: Pearson's correlation coefficients between quantitative traits

Trait	GM	DF	GY	SW	BD	Fe
DF	-0.538**					
GY	0.019	0.058				
SW	0.267**	-0.247**	-0.080			
BD	0.200**	-0.128**	-0.021	0.004		
Fe	-0.001	-0.213**	-0.060	0.270**	-0.151**	
Zn	-0.212**	0.052	-0.027	0.170**	-0.090*	0.784**

*Significant at 5% level of probability, **Significant at 1% level of probability. DF: Days to 50% flowering, GY: Grain yield per plant (g), SW: 100 seed weight (g), GM: Grain mold, BD: Bird damage (%), Fe: Iron concentration (ppm), Zn: Zinc concentration (ppm)

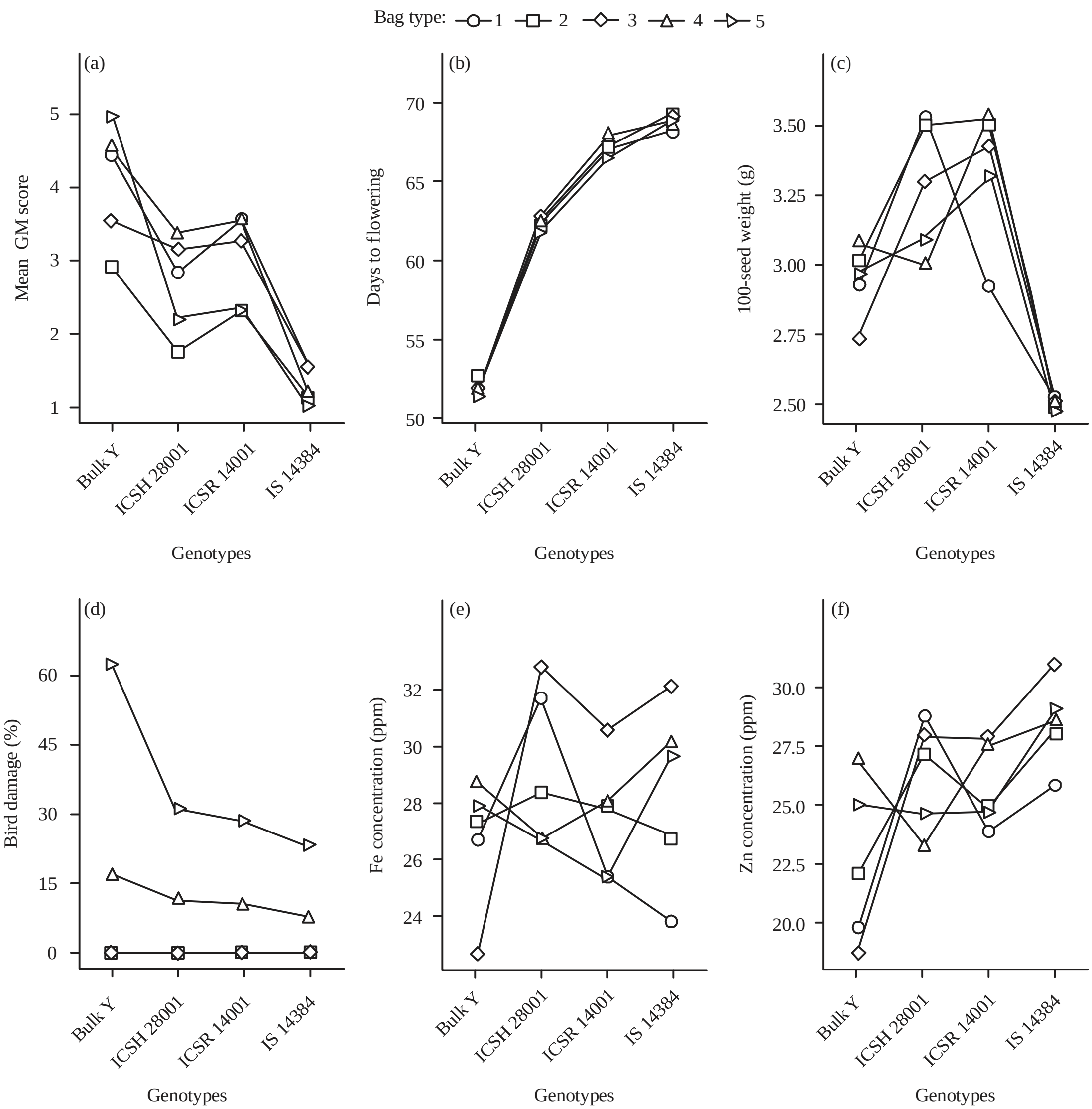

Fig. 6(a-f): Line plots of mean values for bag types×genotypes interaction for (a) Grain mold (GM), (b) Days to 50% flowering (DF), (c) 100-seed weight (g), (d) Bird damage (%), (e) Fe concentration (ppm) and (f) Zn concentration (ppm)
Bag types: 1: Duraweb® SG3, 2: Duraweb® SG1, 3: Duraweb® SG2, 4: Standard paper, 5: No bagging (control)

with grain mold increase. While higher incidence of grain mold can influence estimation of zinc by its under-estimation there seemed no such effect on estimation of iron concentration.

The bag types×genotypes interactions were quite interesting (Fig. 6). All bag types showed similar response on mold resistant genotype IS 14384. However, duraweb® SG1 showed moderate to very resistant response on all other genotypes. Duraweb® SG2 showed moderate response on susceptible Bulk Y and other genotypes. Duraweb® SG3 showed a high susceptibility response on Bulk Y but moderate to high disease on other genotypes. The interaction was not strong for days to flowering across any bag types. All three duraweb® bags were equally effective to control bird damage but genotypes responded differently to bird damage under standard paper bags and no bagging; the highest bird damage occurred on Bulk Y and the least on IS 14384 perhaps due to preference of grain of different genotypes by birds.

Bag types×genotype interactions were complex and strong for seed weight, Fe and Zn concentration (Fig. 6). Interactions between bag types and years were prominent (Fig. 7). Duraweb® SG2 showed moderate resistance response for grain mold in 2014 but very resistant response in 2015 when there was higher rainfall. On the other hand, duraweb® SG1 which showed very resistant response in 2014 had a slight increase in disease in 2015 but still at

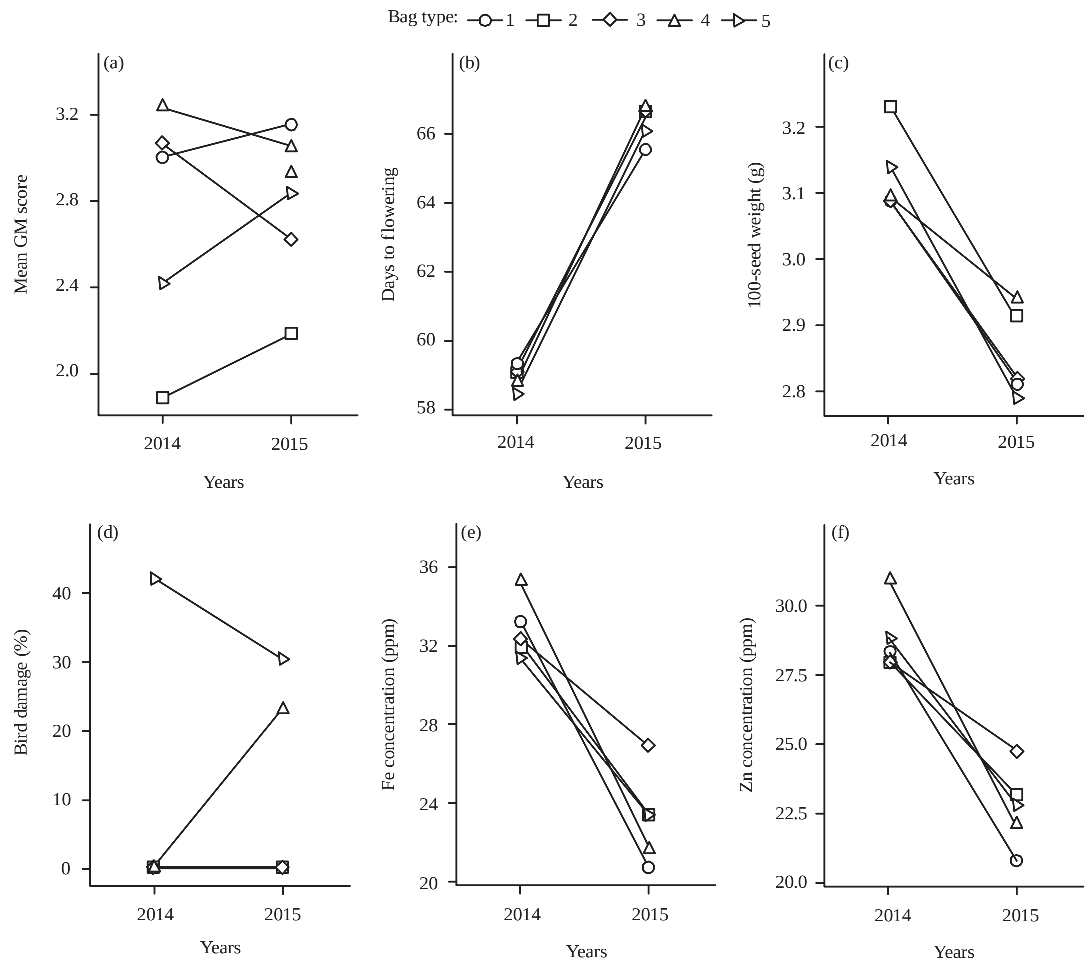

Fig. 7(a-f): Line plots of mean values for bag types × years interaction for (a) Grain mold (GM), (b) Days to 50% flowering (DF), (c) 100-seed weight (g), (d) Bird damage (%), (e) Fe concentration (ppm) and (f) Zn concentration (ppm)
Bag types: 1: Duraweb® SG3, 2: Duraweb® SG1, 3: Duraweb® SG2, 4: Standard paper, 5: No bagging (control)

moderate levels. The bird damage increased under the standard paper bags in 2015 but decreased with no bagging. Torn off paper bags with high rainfall might have increased the availability of food grains in 2015 compared with 2014. The bird pressure thus got distributed equally on no bagging and standard paper bag treatments resulting in lower effect with no bagging in 2015.

In general, there was delayed flowering and decreased expression for seed weight, Fe and Zn concentration in 2015. However, there were different changes in the ranking of bag types in the 2 years for different traits. Desirable interaction for early flowering was noticed under duraweb® SG3, high seed weight under duraweb® SG1 and high Fe and Zn concentration under duraweb® SG2. In general, duraweb® bags showed desirable interactions for one or the other traits.

DISCUSSION

The principle method used for improvement of sorghum is hybridisation between different types for generating new variability. This requires bagging of emasculated panicles to control unwanted pollen contamination. The selected female parents are emasculated first and the pollination is done on next day in the morning with pollen of chosen male plants whose panicles are also covered by a pollination bag for collecting the pollen[12]. The pollination bags create a fabric barrier between reproductive parts and outer conditions to control transmission of pollen by insects, wind or other agents. They are also used to collect the pollen from male panicles. Following pollination, these bags provide a micro-environment for the healthy growth of developing seeds and protect them from bird attack or climatic vagaries.

Evidently, pollination control bags play an important role in sorghum breeding; not only in facilitating the desired hybridisation but also in selfing of selected progeny, advancing of filial generations and maintenance of germplasm in small and isolated fields where bird damage could destroy the whole breeding nursery if not protected by pollination control bags.

Pollination bags traditionally used by sorghum breeders are made of brown paper that gets torn by rain and high wind and are damaged by birds searching for developing grains within them to eat. Despite this, sorghum breeders continue to use paper bags because of their easy availability, low cost and often due to a risk-averse mind-set. Recently, new synthetic fabrics that excel paper bags in their greater strength for bird or wind resistance, air permeability, lower moisture absorption and prevention of unwanted pollen have been developed[7,13,14,15]. Pollination bags made of such novel materials have been successfully experimented in species such as *Elaeis guineensis*, *Melaleuca alternifolia*, *Grevillea robusta, Phillyrea angustifolia* and *Miscanthus*[7]. More recently, Schaffert *et al.*[9] used pollination bags made of these new synthetic nonwoven fabrics in sorghum and reported them to offer more protection against bird damage and producing higher panicle weight, seed weight and average seed weight per panicle compared with paper bags. They suggested that the new bags provide an attractive alternative to paper bags in sorghum to improve seed quality, quantity and to protect against bird damage.

Bird damage can lead to major grain losses in the off-season such as rainy season when the crop does not occupy larger areas. This can cause problems not only in the outcome of hybridisation process but may also create difficulties in the maintenance of germplasms and generation advance of segregating stocks. The extent of damage, primarily by pigeons, in the present case was variety-dependent. The most preferred genotype by birds was Bulk Y (16% damage) and the least liked was the grain mold resistant genotype IS 14384 (6% damage). However, the two pre-release genotypes (ICSH 28001 and ICSR 14001) had similar damage in the range of 8-9%. Till bird resistant genotypes are available a sorghum breeder would need to deploy extra resources for the additional plots to ensure required amount of seed (e.g. for crosses with Bulk Y would require 16% over planting to compensate for bird losses) or protect the valuable materials with bird resistant pollination bags. These results are similar to those of Schaffert *et al.*[9] who also reported differential seed loss from birds over three varieties of sorghum. They observed high bird damage under no bagging and paper bags on white and red seeded varieties. However, no bird damage was observed on the brown seeded hybrid with tannin in the seed coat.

Gitz *et al.*[5] and Schaffert *et al.*[9] reported that paper bags offer minimal protection against birds as they offer weak protection in the rainy season when rains, high winds and over growing panicles within them can create holes that birds may open further in search of food. However, Hayes and Virk[10] reported that duraweb® polyester bags were strong and not forced open by the overgrowth of *Miscanthus* panicles within them. Schaffert *et al.*[9] reported no bird loss in sorghum with duraweb® bags made of nonwoven materials but a high damage occurred under paper bags. The present study confirms these results where nearly no bird damage occurred under any type of new bags while the damage under paper bags was about 12%, which was itself significantly lower than the non-bagged panicles (36%)[5,9]. Clearly, any sort of bag over panicles results in lower bird damage than no bagging; paper bags reduced losses by 24% despite their weak protection. Thus birds are able to differentiate between easy and slightly difficult access and prefer the former although they would go for the latter when easy access no longer exists. This is of significant consequence to sorghum breeders where generation advance of genetic materials is made difficult due to bird damage and inclement weather. The use of specifically designed pollination bags made of man-made fibre offers an advantage over the paper bags in such circumstances.

Two new synthetic bags-duraweb® SG1 and duraweb® SG2 of the three novel bag types, were superior to paper bags for water resistance, intactness and ease of handling. Duraweb® SG1 was significantly superior to duraweb® SG2. These results are in agreement with previous studies on oil palm[16], *Miscanthus*[10] and sorghum[9]. The bag types were significantly different for six quantitative traits-days to flowering, seed weight, grain mold resistance, bird damage and Fe and Zn concentrations; duraweb® SG1 being the best performer followed closely by duraweb® SG2 bag. The superior performance of duraweb® SG1 followed by duraweb® SG2 over paper bags is in line with the findings of Schaffert *et al.*[9] for seed weight. There also existed significant interactions of bag types with genotypes and years that implied a breeder should be careful in choosing a bag type in relation to genotypes and years. For instance, genotypes with compact, loose and semi-compact panicles may require pollination bags made of different fabrics. Similarly, bags made of different fabrics may be required in dry and wet years. Significant bags$\times$varieties interaction was also observed by Schaffert *et al.*[9] for panicle weight, seed weight and average seed weight per panicle.

Fig. 8: Moldy grains on white grained sorghum

One of the major purposes of conducting trials in the rainy season was to assess the effect of pollination bags on the occurrence of grain mold which is a major disease that affects grain quantity and quality in sorghum particularly the short duration modern varieties that mature in the rainy season[17]. The humid and warm conditions during flowering and grain development, in the rainy season, favour grain mold infection while dry conditions prevent it[17-19]. The use of pollination bags of any type creates a micro-environment around the panicle which has the potential to be more warm and humid than the ambient conditions, presenting a particular challenge for breeding activities. The disease is caused by a complex of fungal species interacting parasitically and or saprophytically with developing grains[17,19]. Some of the causal pathogens produce mycotoxins in the grain that are harmful to humans and animals (Fig. 8). Production losses due to sorghum grain mold may range from 30-100% depending on cultivar, time of flowering and prevailing weather conditions during flowering to harvesting[20,21]. Despite higher and frequent rainfall in 2015 mean grain mold score was not different from 2014. However, both genotypes and bags significantly interacted with years showing their differential influence.

It is interesting to find that one of the new bags duraweb® SG1 resulted in very resistant reaction to grain mold, in fact a score that was significantly lower than no bagging treatment. Although fabrics of all new bags have greater breathability than paper to improve micro-environmental conditions within, duraweb® SG1 minimized disease development compared with other types of bags and compared to the no-bag condition, perhaps because its air permeability allowed both a more favourable micro-environment inside and because its fabric structure protected the panicle from some rainfall, thus adjusting the temperature and humidity to levels that did not favour disease development. The paper bags had the highest disease score and seem to create micro-environmental conditions that favour disease development significantly more than no bagging natural conditions. It appears that paper bags are not appropriate type for avoiding grain mold and could be gainfully replaced by bags such as duraweb® SG1.

The choice of fabric for pollination bags is crucial since it determines the internal micro-environmental conditions within them. Hayes and Virk[10] observed a narrower range of temperature and humidity within polyester duraweb® bags compared to the Orchard Wholesale and Glassine bags in *Miscanthus*. The tighter control of temperature and humidity resulted in higher success of crosses and seed set rate. Gitz *et al.*[8] compared the micro-environments within hard form and soft form spun-bond polyethylene pollination bags with traditional brown paper bags in sorghum. Temperatures within brown bags in the morning were 10-15°C above the ambient temperature, with highest being 45°C. Generally, a considerable increase in temperature was measured within brown bags throughout the growing season compared to ambient temperature. Heating within soft and hard form polyethylene bags was 25 and 50% that of paper bags, respectively. The daily temperature differences within bags could have been affected by the air-permeability of the bag materials. The relative humidity in paper bags was associated with elevated bag temperatures during the day. Humidity was lower in soft polyethylene bags than the hard polyethylene and paper bags. They also observed mold on sorghum panicles under paper and soft polyethylene bags especially when the plants were irrigated during pollination and seed filling. Data on temperature and humidity were not collected in the present experiment since there was enough evidence from previous studies that duraweb® materials maintained ambient conditions within[10] that could result in better seed harvest in sorghum[8,9]. Evidently, a bird resistant bag that closely approximates ambient temperature and allowing moisture to freely pass might be useful for well irrigated fields due to rains in the rainy season and greenhouse conditions in the winter season.

For the agronomic traits, as expected, the two elite cultivars ICSR 14001 and ICSH 28001 showed significantly higher grain yield, larger grain size and grain mold resistance. In addition, they also showed higher grain Fe and Zn concentration. The ICSR 14001 genotype was developed for higher yield and higher grain Fe and Zn concentration. The ICSH 28001 genotype in its pedigree has male parent PVK 801 possessing grain mold resistance and high Fe and Zn. In this study, the correlation between Fe and Zn was found to be significantly positive as the genetic elements controlling Fe and Zn are co-localized[22,23]. The ICRISAT biofortification programme with harvest plus targets improving the micronutrient contents of sorghum for tackling the micronutrient deficiencies for Fe and Zn[22,23]. For many years now one of the major challenges in sorghum biofortification research has been the limited number of breeding cycles (one) per year as the crop grown in rainy season is often affected by grain mold rendering it unsuitable for Fe and Zn assessment. With identification of suitable bags in this study that permit healthy seed development in selfed panicles, it is feasible to hasten up the breeding program by having more breeding cycles (two) per year.

A proper economic analysis was not the objective of this research. However, we examined the effect of various factors determining the economic impacts of various types of pollination bags. This presents a very preliminary analysis following the approach of Schaffert *et al.*[9] based on extrapolated circumstantial evidence from the analyses provided by the available data.

While performing any economic analysis it should be remembered that a sorghum breeder is more interested in propagating his/her genetic stocks for speeding up breeding process, rather than growing a commercial crop. During early segregating generations the seed is always in small quantity as being collected from individual plants or plant progeny. Sorghum breeders also use off-season for generation advance and or increase seed for replicated trials in the advanced generations. Complete loss of any progeny from bird damage or weather conditions particularly in the early generations will be irretrievable which may misdirect the whole breeding programme. In addition the labour and effort used in the season are virtually wasted. Even when breeders keep the remnant seed of progeny a breeding season may be lost, time which may delay the release of a variety resulting in a huge economic loss to farmers who could have benefitted from the extra yield much earlier. Should the new variety be resistant to diseases the economic loss might be multiplicative if the year happened to be epidemic for the disease in question.

Analysis of factors considered in Table 11 shows that use of any type of pollination bag is better for control of bird damage than none, but the use of bags made of the novel materials provides the highest economic benefit. While covering with paper bags results in 24% less bird damage than no bagging there would be 36% less bird damage if new bags are used. Schaffert *et al.*[9] used plastic bags over paper bags to reduce bird damage which increased cost but the use of synthetic bags alone was more effective as in the present case.

Among other factors grain mold infection in the rainy season is important which was significantly reduced with duraweb® SG1 bags in comparison with no bagging or covering with other type of bags. Grain mold not only reduces the yield but also quality of grain and contributes to the spread of disease among progeny[21]. Factors such as re-usability, likelihood of loss of whole progeny and bag traits such as water resistance, intactness of bags over panicle and ease of handling favour the use of synthetic materials in pollination bags. Although the initial cost of synthetic bags would be higher than paper bags the multiple benefits accruing from them probably justify the replacement of paper bags with these specifically developed nonwoven fabric bags.

Table 11: Factors for comparing pollination bags for economic analysis

Treatment	Mean grain mold score (1 resistant to 5 susceptible) in rainy season	Mean bird damage (%)	Other factors with cost implication	Reusability	Risk of loss of genetic stocks	Relative cost
Duraweb® SG3	3.1, moderate	0.1*	WR = 2.9; IB = 2.4; EH = 2.0 Same as paper	#Yes Not tested here	Little	$$
Duraweb® SG1	2.1, very resistant	0.1*	WR = 1.5; IB = 1.6; EH = 1.5 Better in rains. No additional cost even in rainy season. Seed weight highest	#Yes Not tested here	Little	$$
Duraweb® SG2	2.9, moderate	0.0**	WR = 1.7; IB = 1.5; EH = 1.5 Good in rains. No additional cost even in rainy season	#Yes Not tested here	Little	$$
Paper	3.2, moderate	11.5*, more in rain damaged bags i.e., 20-25% going up to 100%	WR = 3.0; IB = 2.5; EH = 2.0 Worse in rains. Extra bags and labour to check/replace bags; additional planting to compensate loss	No	High under high pressure of birds, disease, rains etc.	$
No bagging	2.6, moderate	36.3, can be up to 100% in small isolated plots	Not applicable	Not applicable	Not applicable	Not applicable

WR: Water resistance (1 = no water, 2 = water wetting, 3 = water collection in bag), IB: Intactness of bag on panicle (1 = intact, 2 = shrinks, 3 = collapses), EH: Easiness of handling compared with standard paper bag (1 = more, 2 = same, 3 = less). $, $$ Indicate relative cost; single sign means low and double sign means high cost, *Schaffert *et al.*[9], **Gitz *et al.*[8], #Hayes and Virk[10]

The data also indicate that synthetic fabrics are not alike and choice of fabric may be fine-tuned for the crop plant under consideration. Preliminary results of this study need further confirmation with more robust experimentation on economic analysis and assessment of comparative micro-environmental climates within bags to better explain why their seed harvest outcomes are different.

This study explored relatively little investigated influence of type of pollination bags on healthy seed harvest following selfing for generation advance and hybridization in sorghum. It has been established that new synthetic pollination bags have multiple advantages over the standard paper bags in terms of protection against bird damage, high wind and rain, reduction in grain mold infection, increased seed weight, water resistance, intactness and ease of handling. The implications of using pollination bags made of nonwoven fabrics can be huge in breeding of all crops in assuring no contamination from foreign pollen and increasing seed quantity and quality following hybridisation, maintenance of genetic accessions, advancement of filial generations and seed increase of elite lines using more than one cycle per year.

CONCLUSION AND FUTURE RECOMMENDATION

Bird damage under high pressure can be reduced by covering with any type of bags but there was nearly no bird damage under nonwoven synthetic bags. Choice exists between nonwoven fabrics of which duraweb® SG1 provides the best option for sorghum for bird damage, grain mold resistance, higher seed weight, water resistance, intactness and ease of handling. This is closely followed by duraweb® SG2. The use of specifically developed nonwoven bags offers a number of economic benefits for replacing the traditionally used paper bags for germplasm maintenance, hybridisation processes and in increasing the number of breeding cycles for generation advance and speeding up breeding research in sorghum.

This study was, however, limited on parameters of ambient micro-environmental differentiation within new and old bag types because data on temperature and humidity were not collected. This will be addressed in more details in a separate study.

The major recommendation emerging from this study for plant breeders in general and sorghum breeders in particular is to consider new alternatives to the standard paper pollination bags for improving the outcome of plant breeding operations.

SIGNIFICANT STATEMENTS

This study discovered the possible replacement of problematic paper pollination bags in crop breeding with more hardy, weather and pollen proof pollination bags made from novel nonwoven fabrics. Hitherto, using paper bags was prone to losing a season from loss of seed of crucial genetic stocks due to bird attack or inclement weather. This study uncovers the critical area of research of pollination control bags that many researchers were unable to explore previously due to non-availability of alternative fabrics. The recently developed novel nonwoven fabrics and their superior performance over the paper bags as found in this study will hugely benefit researchers to explore them further for increasing the efficiency of plant breeding operations. For sorghum breeders the duraweb® SG1 bags followed by duraweb® SG2 provide the best option for no bird damage, high resistance to grain mold and higher seed weight, water resistance, intactness and ease of handling. The use of novel bags is expected to reflect in assured and rapid turnover of more than one crop cycle per year and hence benefit the society by providing the seed of new varieties earlier.

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