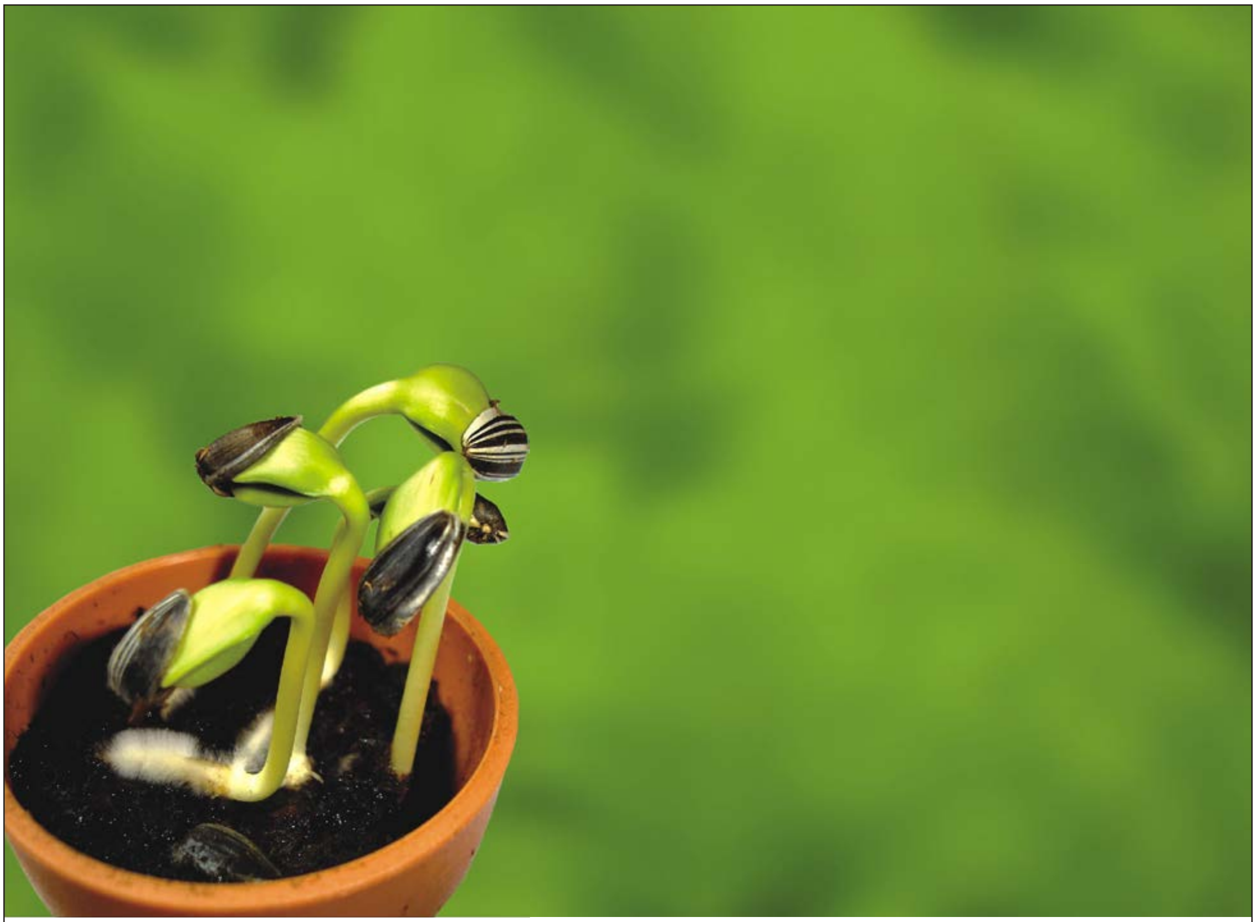

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Research Article

Assessing the Relative Efficacy of Polyester Pollination Bags and Crossing Tents and Isolation Chambers for Seed Harvest in *Miscanthus* Crosses

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Abstract

Background and Objective: Pollination bags and tents play a crucial role in artificial hybridisation in plant breeding. They offer protection against unwanted foreign pollen and bird damage and provide ambient conditions for hybrid seed development. **Methodology:** Five hundred and sixty four inter- and intra-specific crosses were made in *Miscanthus* using three types of pollination bags in glasshouse and isolation chamber conditions in experiment 1. Another 16 crosses were made in a duraweb® tent and isolation chamber under external and glasshouse conditions in experiment 2. Data on quantitative traits were analysed using Z-test and analysis of variance techniques. **Results:** The success rate of crosses with duraweb® bags was the highest (45%) which exceeded the glassine bags by 15% and orchard type bags by 7%. The success rate for the intra-specific crosses was 47% that was 13% higher than the inter-specific crosses. The seed set rate with duraweb® crossing tents was much higher than in isolation chambers. The average seed number in tents was 82% higher than in isolation chambers in glasshouse conditions. Better seed set rate in duraweb® bags and tents resulted from a better control on the temperature and humidity within them. The isolation chambers had higher temperature and humidity than the duraweb® tents and hence showed a lower seed number. Both duraweb® bags and tents were reusable following an autoclave cycle and were resistant to slug damage. **Conclusion:** Both duraweb® bags and crossing tents showed greater efficacy in hybrid seed production by providing an ambient environment for seed development.

Key words: *Miscanthus* species, pollination bags, hybrid seed-set, isolation chambers, crossing tents

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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

High biomass potential with high output: input energy ratios of *Miscanthus*, a genus of rhizomatous, perennial C_4 grasses with origins in E. Asia, has been a source of attention of grass breeders for its genetic improvement[1,2]. A sterile triploid inter-specific hybrid clone (*Miscanthus*×*giganteus*) has been greatly exploited in Europe including the UK for many years for its high harvestable yield[3-5] above 12 t dry matter ha^{-1} $year^{-1}$ and mitigating up to 7 t carbon ha^{-1} $year^{-1}$. It has been calculated that energy production could increase from present levels by 88% (to 2360 PJ) and mitigate 42 Tg of CO_2-C equivalent using 10% arable land through plant breeding endeavours[6]. Breeding superior *Miscanthus* clones in the UK is underway at IBERS, Aberystwyth, Wales as a substitute for fossil fuel[7,8].

As *Miscanthus* is not a domesticated species there have been many obstacles to obtain successful crosses such as flowering asynchrony and height differences between species[9]. To improve the crossing success rate of the *Miscanthus* breeding programme the climatic conditions inside the crossing bag/chamber should be as close to ideal as possible. If a cross is subsequently unsuccessful the reasons for this could then be put down to genetic incompatibility of the parents.

Like other grasses, pollination in *Miscanthus* takes place by wind. The distance pollen can travel for successful pollination depends on wind velocity and pollen longevity and viability. Artificial hybridisation, to exploit heterosis, requires enclosing of flower heads within a pollination bag or whole plants in pollination tents, to exclude extraneous pollen from contaminating the cross[10-12]. Pollination bags and tents have a very crucial role in the success of a hybridisation programme; they must be impermeable to pollen of any species capable of fertilizing the species concerned and provide ambient environmental conditions within them for healthy seed development following pollination and fertilization by the desired pollen.

Great strides have been made in developing new fabrics for pollination bags but only limited studies have compared their efficacy in grasses, e.g., non-woven polyester materials were found to be effective for pollen screen and increasing seed production over the traditional glassine and paper bags[13-15]. Of the various materials with woven and pressed fibres, some are capable of producing an ideal environment within them for seed development. In addition, how these bags interact with the outer environment or climatic conditions may determine their strength. The fibre of some bags is such that they can be re-used after treatment compared with others. Further, in the event of scanty information on the efficacy of these fabrics as crossing tents for polycross nurseries, it would be interesting to investigate them under natural and glasshouse conditions.

The objectives of this study were to compare the efficacy of pollination bags made from synthetic material, duraweb®, specifically developed for plant breeding in comparison with the most commonly used bag types by grass breeders under glasshouse and isolation chamber conditions and to compare the seed harvest from tents and isolation chambers.

MATERIALS AND METHODS

Two experiments were conducted to investigate the efficacy of pollination bags and climatic conditions for hybrid seed set in *Miscanthus* during 2013 at IBERS, Aberystwyth, Wales, UK.

Experiment 1

Relative efficacy of bag types and climatic conditions on seed set: A total of 564 pair-wise inter-specific and intra-specific crosses were made in *Miscanthus* during the 2013 crossing season. The *Miscanthus* species used were: *Miscanthus sinensis*, *M. sacchariflorus*, *M. transmorrisonensis*, *M. floridulus*, *M. condensatus* and inter-specific hybrids (these included naturally occurring hybrids and ones which were made during the course of the breeding programme). These five species and one inter-specific hybrid resulted in 39 paired combinations of female and male parents in crossing; 27 were inter-specific pairs and 12 were intra-specific pairs. Within each parental combination, the number of crosses attempted varied from 2-98 resulting in a total of 564 crosses across all 39 paired combinations. The experience at IBERS had shown that species used were cross-compatible that set seed on hybridization.

The paired crosses were made using three types of pollination bags. These were: Glassine bags made of glazed paper, orchard whole sale crossing bags made from wet strength kraft paper and duraweb® bags made from nonwoven air-permeable polyester. The glassine bags were disposed off after a single use, as they were not reusable. However, duraweb® and orchard bags were autoclaved after use for re-using in the crossing programme. This worked well for the duraweb® bags but the orchard whole sale bags came apart after this treatment and thus were only suitable for single use.

Crosses were made under two climatic conditions; glasshouse and isolation chamber. Four modified compartments of a Venlo glasshouse were used for making paired crosses. These compartments had temperature control through automatic roof vents, reflective screens and heating pipes, supplementary lighting and irrigation via capillary matting, drip feeders and overhead spray nozzles. These compartments were controlled with a Campbell logger in order to set the temperature and photoperiod climate required to induce flowering in a sub-tropical species under temperate conditions. The isolation chambers were small pollen-proof compartments, which were used to cross groups of plants in isolation from any external pollen. The air was filtered before being blown into all compartments which aided the distribution of pollen in the chamber and also maintained a positive air pressure in the compartment to prevent the entry of external pollen. The chambers were irrigated by capillary matting that was kept wet by means of a header tank (Fig. 1).

During the course of the crossing season, bags were also monitored for temperature and humidity within them and compared with the ambient conditions. Temperature (°C) and percent (%) humidity were recorded inside and outside the bags daily from the first week of May to July, by the use of a Tinytag Extra TGX-3580.

Data were collected on percentage success of crosses. Paired combinations of genotypes that showed seed set in the bag following pollination were taken as successful cross. Percentages of successful crosses with seed set as proportion of the total number of crosses attempted were computed for further analysis. Data on percent successful crosses were analysed by performing an analysis of variance on percentages and their angular transformations since there occurred a number of crosses with 0 and 100% of success. The percentages were transformed to arc sin or angular transformation for proportions where, angle = arc sin√percentage. In the angular transformation, proportions near zero are spread out to increase their variance. With n<50, a zero proportion is counted as 1/4n before transforming to angles and a 100% proportion as (n-1/4)/n. In the present case, number of crosses per cross combination was always <50. However, the angular transformation does not remove inequalities[16] in variance arising from different values of n.

The analysis of variance allowed for the partitioning of sum of squares due to bag types for 2 df into the following orthogonal two contrasts with 1 df for each:

	Coefficient		
Comparison	Duraweb®	Orchard	Glassine
Duraweb® vs others	2	-1	-1
Glassine vs orchard	0	1	-1

A Z-test was performed for comparing mean percent success of crosses for bag types and cross types to test the significance of their differences[16].

Experiment 2

Effect of climatic conditions on hybrid seed set: Sixteen paired and synthetic crosses were attempted in *Miscanthus* during the 2013 crossing season at IBERS, Aberystwyth, Wales, UK. The different *Miscanthus* species used were: *Miscanthus sinensis*, *M. sacchariflorus*, *M. floridulus*, *M. condensatus* and inter-specific hybrids (these include naturally occurring hybrids and ones which were made during the course of the breeding programme).

Since, the purpose of the experiment was to assess the effect of climatic conditions on quantity of seed set following crosses, four micro-climatic environments were created by providing tent and isolation chamber in the glasshouse and external climatic conditions. The tent was made of duraweb® material. This material is created by bonding randomly laid polyester fibres without the addition of other chemicals. The

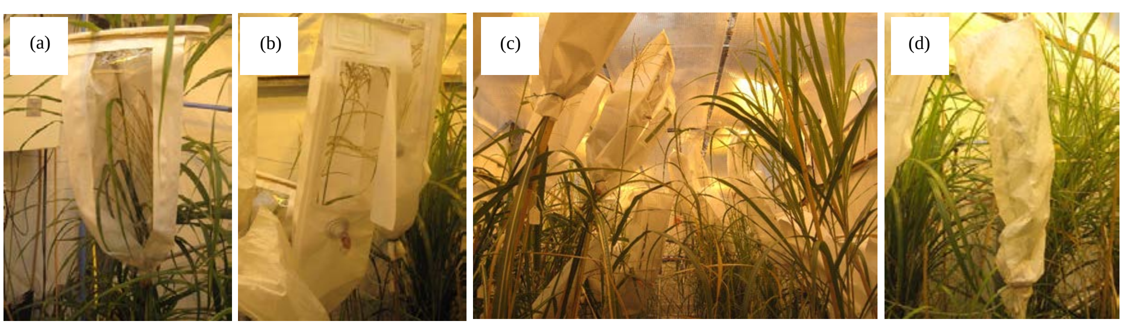

Fig. 1(a-d): (a) Cross in an orchard wholesale bag, (b) Cross in a duraweb® bag, (c) Overview of crosses in a Venlo compartment and (d) Cross in a glassine bag

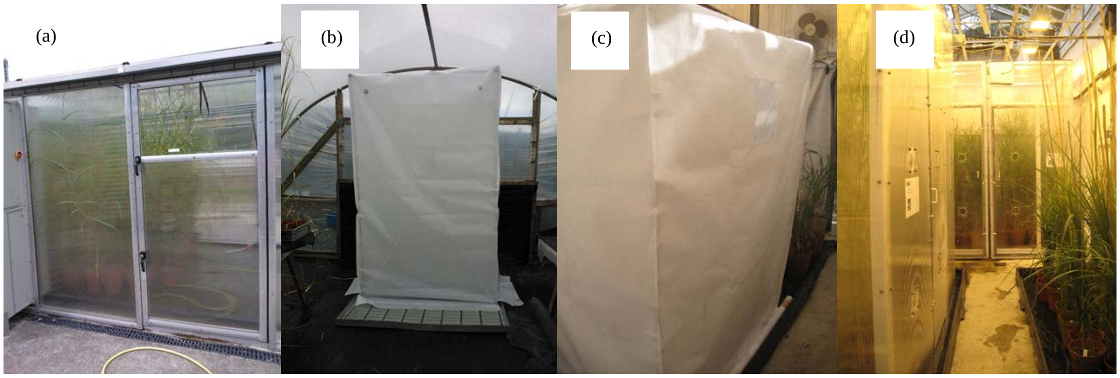

Fig. 2(a-d): (a) External isolation chamber, (b) External crossing tent, (c) Internal crossing tent and (d) Internal isolation chamber

architecture of this material creates its unique properties. Although, breathable, allowing air and moisture to pass through, duraweb® halts unwanted pollen. It has an average pore size smaller than *Miscanthus* pollen grains.

Four modified compartments of a Venlo glasshouse were used for the tent crosses. These compartments had photoperiod, temperature and humidity control as described in experiment 1 (Fig. 2).

For each of the crosses, between five and ten plants of each parent were placed in the crossing tent and/or the isolation chamber.

Data were collected on total number of seeds and number of heads per cross. These data were used to compute the average number of seeds per head for a cross combination.

Three crosses had zero seed set despite having a number of heads, which could have been due to lack of synchronisation of the flowering stages or due to chromosomal rearrangements after inter-specific crosses, making the plants female sterile. Such crosses were excluded from the analysis as failed. These were: One each in glasshouse chamber (*M. sacchariflorus* (2x)×*M. sinensis*), external chamber (multiple *Miscanthus* species synthetic) and external tent (*M. sacchariflorus/sinensis*× *M. condensatus*). Data from the remaining 13 crosses were subjected to analysis of variance.

RESULTS

Experiment 1

Analysis of seed set: The two analyses, the one on original percentage of success of crosses and the second on angular transformation did not reveal any discrepancies in results. Therefore, we describe the results of analysis of the original data only. The detailed partitioning in the ANOVA, apart from main factors-climatic conditions, cross type and bag type, also included interactions such as: Climatic conditions×cross type, climatic conditions×bag type, cross type×bag type. However, none of these interactions were significant. Therefore, we pooled the variance of all interactions with that of error variance. The reduced ANOVA without interactions (Table 1) revealed non-significant effect of climatic conditions on seed set and success of cross. Cross types and bag types, however, had significant effect on seed setting upon pollination.

Table 1: Analysis of variance for percent success rate of crosses

Source	df	MS	F	p-value
Climatic conditions	1	70.73	0.08	0.78
Cross type	1	4593.31	5.33*	0.03
Bag type	2	3180.11	3.69*	0.04
Duraweb® vs other types	1	6032.17	7.00**	0.01
Glassine vs orchard	1	328.04	0.38	0.54
Error	34	861.70		
Total	38			

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

Partitioning of bag types into orthogonal comparison of duraweb® vs. both glassine and orchard type and glassine vs orchard type showed the significance of the former comparison. While, the glassine and orchard types did not differ significantly, the duraweb® bags were superior to the combined effect of other two types of bags by 12% more success of crosses (Table 2 and 3).

Mean values for percent success of 45% was the highest with duraweb® bags which exceeded by 15% over glassine and 7% over orchard type (Table 2). The Z-test showed a non-significant difference between glassine and orchard types. However, the success rate for the duraweb® bags was 12% higher than both glassine and orchard types together which was highly significant on a Z-test (Table 3).

The success rate of 47% for the intra-specific crosses was 13% higher than the inter-specific crosses (Table 2) and this difference was highly significant indicating that seed set will be higher when crosses are made between genotypes of *Miscanthus* that belong to the same species (Table 3). Rounsaville *et al*.[17] found that seed set varied significantly for different diploid cultivars and this may account for the differences in seed set shown within the crosses discussed here. A representative set of crosses were carried out using each of the different crossing bags tested and so any difference can be attributed to the material used rather than to the cross itself.

Analysis of climatic factors: The general trends for temperature and humidity were the same both inside and outside of the bag (Fig. 3). The range of temperature and humidity exhibited by the different bag types were analysed (Fig. 4). The range of temperature and humidity found within the duraweb® bags compared to the orchard and glassine bags was much smaller (Fig. 4). The tighter control of temperature and humidity demonstrated by the duraweb® bags may have had an impact on crossing success and seed set rate.

Experiment 2: The analysis of variance showed a non-significant effect of climatic conditions on total number of seeds and total number of heads of crosses. However, the effect of climatic conditions was significant on the average number of seeds per head (Table 4). Apparently inconsistencies for seed traits among crosses within climatic conditions were high to produce higher error variance.

The mean values for total number of seeds and average number of seeds per head were consistently higher for tents whether in the external or glasshouse conditions (Table 5). However, the total number of heads did not follow this trend; the highest number of heads being produced in external tent (48.00) followed by isolation chamber in glasshouse (41.80).

We did not measure the size (length and breadth) of heads for crosses. Fewer and longer heads with densely packed spikelets would have more seeds per head and vice versa. Despite this limitation our estimates of mean values for number of seeds per head were consistent with those of total number of seeds under all environmental conditions. The mean number of seeds per head was higher when crosses were made in tents than in chambers in both external and glasshouse conditions (Table 5).

However, the comparison of tent with chamber in external conditions for seed number per head was not significant as the standard errors were larger (Table 6). The most of the significance in mean squares for mean number of

Table 2: Mean values of percent success of crosses using different type of bags and crosses

Bag type/ cross type	No. of pairs	Total number crosses attempted in all pairs	No. of successful crosses	Success of crosses (%)	SE (%)
Bag type					
Duraweb®	18	266	119	45	3.1
Glassine	12	180	54	30	3.4
Orchard	9	118	45	38	4.5
Total	39	564	218	39	2.1
Cross type					
Inter-specific	27	362	123	34	2.5
Intra-specific	12	202	95	47	3.5
Total		564	218		

Table 3: Z-test for testing the difference between bag types and types of crosses

Test	Attempted	Successful	Success (%)	Difference (%)	SE difference (%)	Z-value
Duraweb®	266	119	45			
Others	298	99	33			
Duraweb®vs others				12	4.1	2.81**
Glassine	180	54	30			
Orchard	118	45	38			
Glassine vs orchard				8	5.6	1.45^{NS}
Inter-specific	362	123	34			
Intra-specific	202	95	47			
Intra- vs inter-specific crosses				13	4.3	3.03**

NS: Non-significant, **Significant at 1% probability level

Table 4: Means squares from analysis of variance for total number of seeds, total number of heads and average number of seeds per head for 13 *Miscanthus* crosses

Source	df	Mean squares for total No. of seed	Mean squares for total No. of heads	Mean squares for average seed numbers per head
Climatic conditions	3	167039^{NS}	600.5^{NS}	4545*
Error	9	1173492	739.1	1089
Total	12			

NS: Non-significant, *Significant at 5% probability level

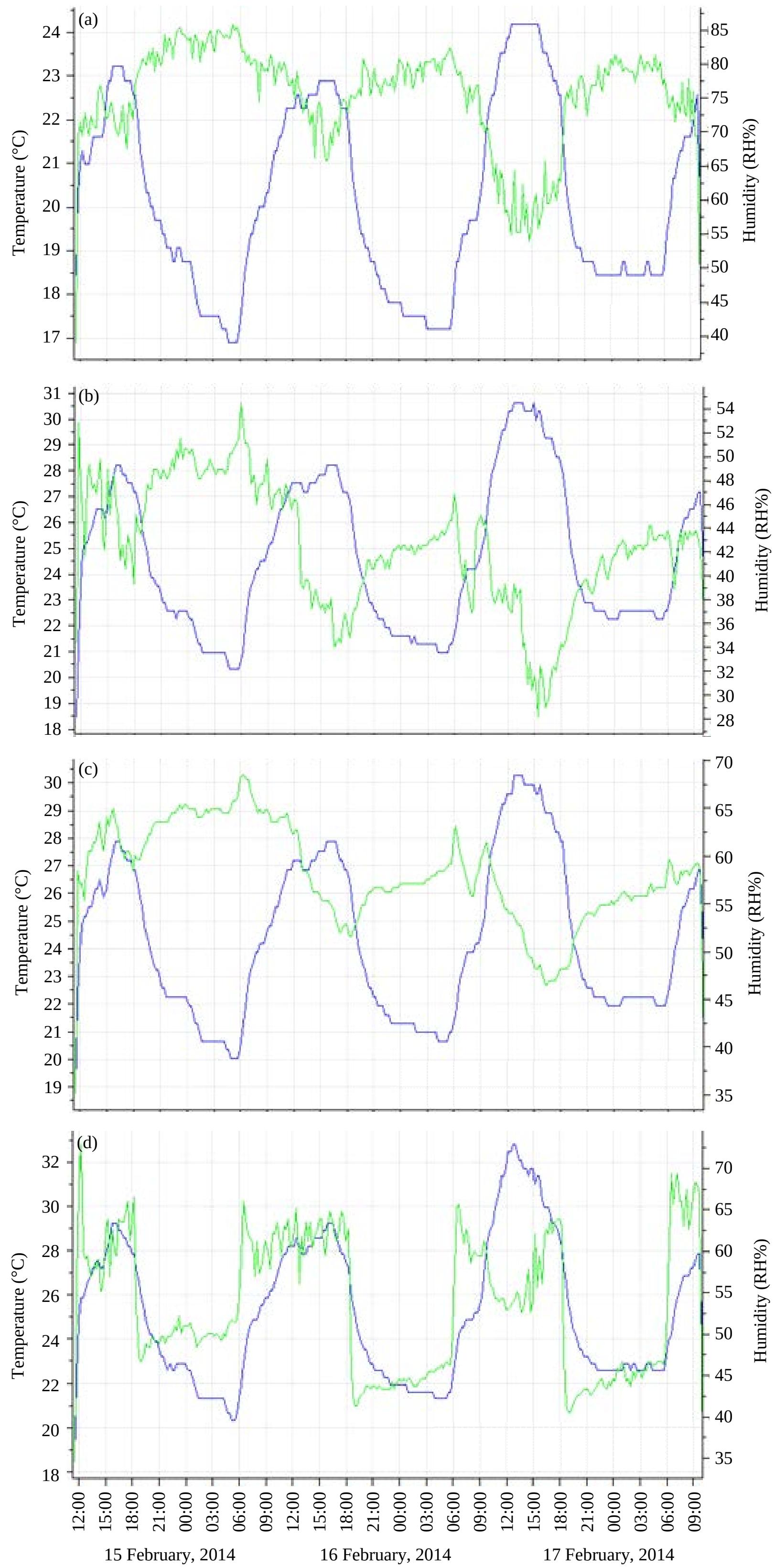

Fig. 3(a-d): Charts show the humidity and temperature inside and outside a duraweb crossing bag, an orchard whole sale crossing bag and a glassine crossing bag, (a) Venlo chamber, (b) Duraweb® bag, (c) Orchard whole sale bag and (d) Glassine bag

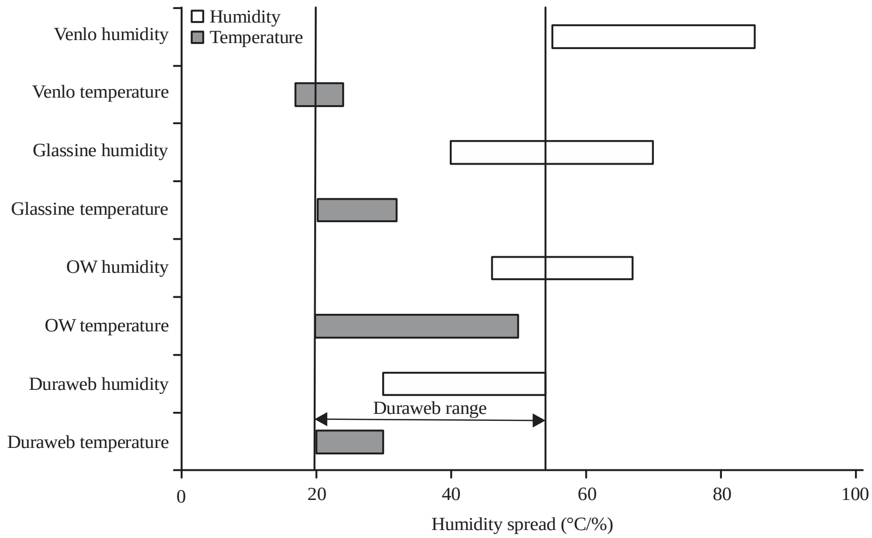

Fig. 4: Chart to show the range of temperature and humidity found within the different crossing bags and within the glasshouse

Table 5: Least square mean values for total number of seeds and total number of heads and number of seeds per head produced following crossing in four environmental conditions

	Total No. of seeds		Total No. of heads		No. of seeds per head	
Climatic condition	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)
External chamber	10.50	765.99	19.50	19.22	0.41	23.33
External tent	1504.00	765.99	48.00	19.22	31.93	23.33
Glasshouse chamber	579.20	484.46	41.80	12.16	15.26	14.76
Glasshouse tent	1647.75	541.64	20.75	13.59	83.23	16.50

Table 6: Comparing mean values for number of seeds per head

Climatic condition	Mean seed number per head	SE	SE diff for comparing chamber with tent	t-value
External chamber	0.41	23.33		
External tent	31.93	23.33	1 vs 2 = 32.99	1 vs 2 = 0.95^{NS}
Glasshouse chamber	15.26	14.76		
Glasshouse tent	83.23	16.50	3 vs 4 =22.14	3 vs 4 = 3.07**

NS: Non-significant, **Significant at 1% probability level

heads thus lies in the larger difference between number of heads per cross in the comparison of tent versus isolation chamber in glasshouse conditions. The glasshouse tent produced 83 seeds per head which was significantly higher by 82% than the isolation chamber in glasshouse conditions (Table 6).

The temperature and humidity (measured using a tiny # tag) inside the crossing tent followed the same pattern as shown by the ambient conditions in the Venlo glasshouse (Fig. 5). The temperature and humidity shown by the glasshouse isolation chamber was lower than that shown by both the crossing tent and the ambient conditions of the Venlo glasshouse. The difference in humidity and temperature shown by the different crossing environments was probably the reason why there was reduced seed set, on average, between the isolation chambers when compared with the results from the crossing tents.

The results given in Fig. 6 show that the temperature inside the crossing tent (7-27°C) was lower and more controlled than in the isolation chamber (5-40°C). The humidity cycled continuosly in the crossing tent, whereas in the isolation chamber it stayed high for a period of 20 days which might also have affected pollen viability.

DISCUSSION

Pollination bags provide a fabric barrier between reproductive parts of flowers and environment. Bag materials

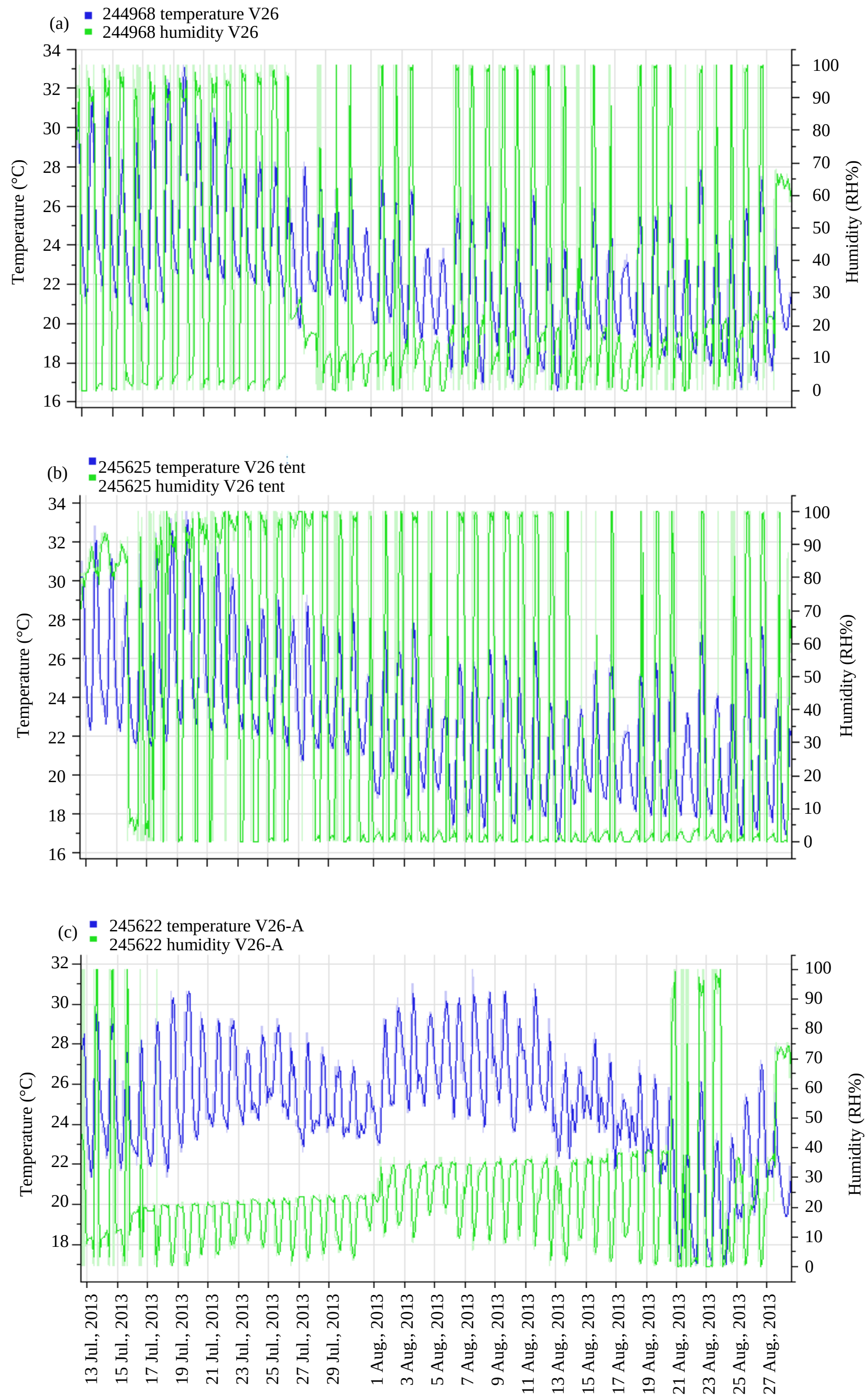

Fig. 5(a-c): Chart to show the humidity and temperature, (a) Outside in the Venlo compartment (V26), (b) Inside a crossing tent (V26 tent) and (c) Inside an isolation chamber (V26-A)

made from muslin, micromesh, polyethylene, cellulose acetate, micropore acetate bread bags and paper have been studied for decades[18-23]. Each of these materials has positive and negative attributes. While new fabrics are being

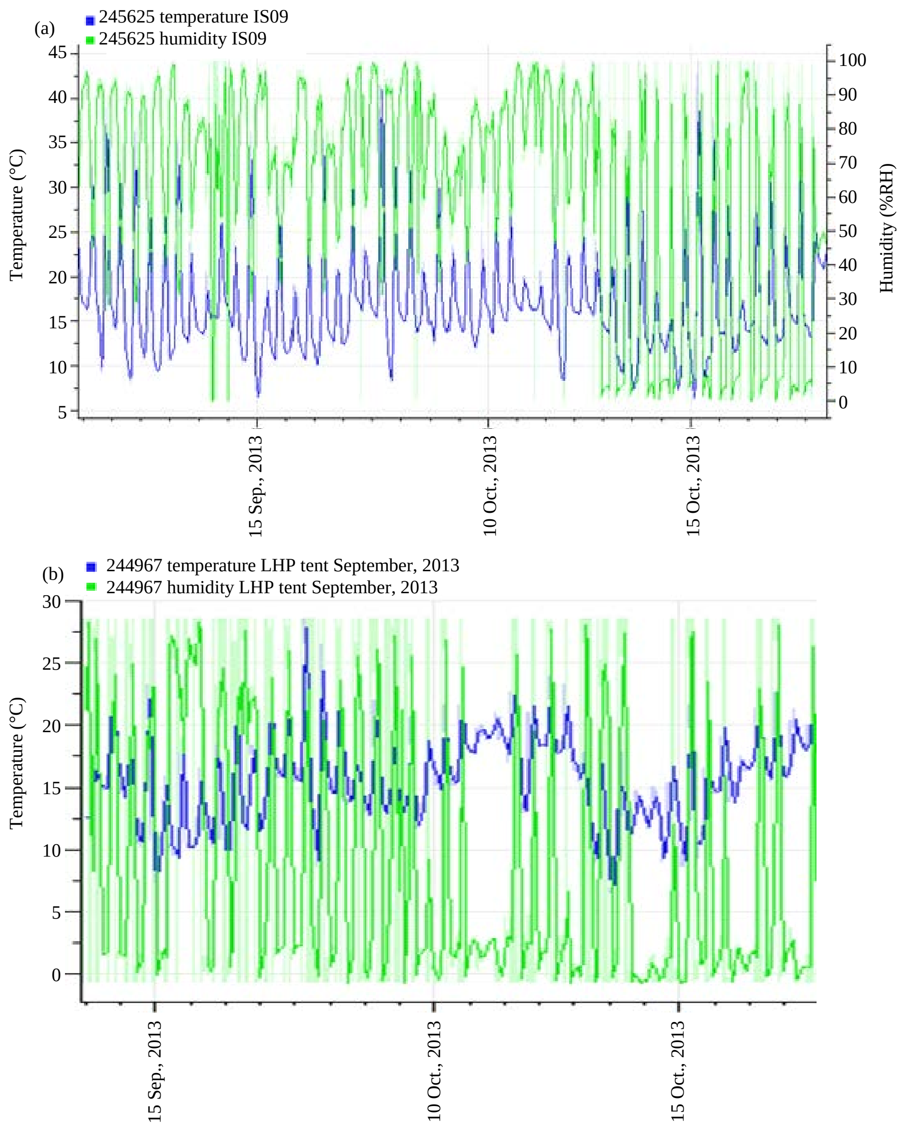

Fig. 6(a-b): Chart to show humidity and temperature measurements in a crossing tent in (a) An external isolation chamber (IS09) and (b) A polytunnel (LHP tent)

continuously developed plant breeders tend to use paper or glassine bags primarily due to low cost, ready availability and adherence to standard practice.

In order that plant breeders have confidence in the newly manufactured pollination bags a comparison of the new fabrics is required with the paper and glassine options. It is highly desirable that the new options are tested for their suitability in field and glasshouse conditions. The results from our pollination bag experiment showed a significantly higher rate of success with the duraweb® bags when compared to the glassine and orchard types. This is of great benefit to plant breeders as a greater number of progeny enables a greater number of trials to be carried out for assessment. Vogel *et al*.[10] obtained four to tenfold increase in seed produced per cross in micro-mesh fabric pollination bags in switchgrass that allowed larger progeny for evaluation in replicated trials. McAdam *et al*.[15] and Adhikari *et al*.[12] both showed that polyester bags were more reliable than traditionally used bags in controlling contamination by foreign pollen, thereby giving greater confidence in the crosses carried out.

It needs hardly be emphasised that actual seed set is greatly influenced by environmental conditions within the bag[14]. Gitz *et al*.[24] studied micro-environment within novel spunbond polyethylene and brown paper bags in sorghum and reported that bags induce micro-environmental changes

Table 7: Comparative costing of a successful cross from different types of bags

Bag type	No. of crosses attempted	Total cost of crosses (£)	No. of successful crosses	Cost per successful cross (£)	Price compared to glassine (£)	Relative saving (%) from duraweb®
Glassine	180	9000	59	153	0	28
Orchard	118	5900	47	126	-27	13
Duraweb®	266	13300	121	110	-43	-

that should be considered when designing experiments. Hot pin perforated polyolefin bags may be suitable for greenhouse applications where mold is a concern and where ambient air velocities are low or in field settings where pollen production by panicles being covered is high. A considerable increase in temperature was measured within brown bags throughout the season as compared to ambient temperatures. However, temperatures within polyethylene bags were lower than paper bags because of air permeability. Humidity was lower in soft polyethylene bags than hard polyethylene and paper bags that resulted in mold especially in the recently irrigated plants. In this study, the duraweb® bags exhibited a narrower range of temperature and humidity than those shown by the orchard and glassine bags which could impact the success of crossing and seed set rate. Similarly, the duraweb® crossing tent had greater control on temperature and humidity than the isolation chambers resulting in better average seed number. These findings are consistent with the previous studies with duraweb® bags in different crops[25,26]. The duraweb® bags made from non-woven fabrics seem to allow air-permeability and moisture absorption for micro-environmental adjustments conducive for better seed set and development.

Another important role of pollination bags is to save the hybrid seed from bird damage. Most importantly, the bird-resistant spun polyethylene bags reduce the need for labourers to repeatedly walk the field to cover plants with additional bags as bird damage occurs during seed development because conventional paper bags are prone to bird damage and climatic vagaries such as rains and high winds[24]. The new synthetic materials made of polyester fabrics such as duraweb® have greater strength for bird or wind-resistance as verified in sorghum[27,28]. High damage from migratory birds on sorghum crop has been reported in Minas Gerais state of Brazil where protecting the breeding germplasm and hybrid seeds from bird damage by pollination bags revealed that paper bags offered little protection compared to the polyester bags[28]. The growing panicles of *Miscanthus* may also pierce through the week bags made of paper or similar materials to cause contaminations. However, no piercing was noticed from the expanding panicles within duraweb® bags because of their strong fibre than other type of bags. While no data are available on bird damage in the present experiment but the greater strength of duraweb® fabrics suggested that these polyester bags would be a better option against bird damage in field conditions.

Though indicative yet a preliminary comparison of relative benefit of different types of pollination bags could be made from the approximate cost estimate of £50 for one *Miscanthus* cross as determined by plant breeders at the Aberystwyth University using data from IBERS (Table 7). The actual cost of the crossing bag is insignificant when factors such as glasshouse space, person-hours, storage, consumables, selecting the plant for crossing etc., are considered. Admittedly, the glasshouse costs are high and would be less in an industrial setting but the overall trend would remain the same and the most economical bags are those of duraweb® type which are cheaper by £43 over glassine and £16 over orchard type for a cross. By using duraweb® bags there is relative gain of 28% over glassine and 13% over orchard type (Table 7). The benefit accrued from economical bag type would be significantly higher if 500 or 1000 crosses are made.

CONCLUSION

This investigation has clearly established the improvement of materials for pollination bags and the new duraweb® bags made from non-woven polyester fabrics were superior in seed set success rates compared with glassine or orchard wholesale bags. The duraweb® bags can withstand climatic conditions better than other type of bags, are easy to re-use with an autoclave cycle in between than the single use orchard wholesale and glassine bags that were unable to withstand an autoclave cycle, could withstand the overgrowth of plants within them and were not damaged by slugs. The duraweb® tent also excelled for properties similar to pollination bags with much higher seed set rate from crossing compared to isolation chambers. In conclusion, the duraweb® material whether used in manufacturing pollination bags or tents provides an environment that is conductive for higher seed set in *Miscanthus*. These preliminary results need confirmation with more robust experiments as similar data are not yet not available particulary for the isolation chambers.

SIGNIFICANT STATEMENT

- The influence of fabric quality of three pollination bags on seed set in *Miscanthus* crosses was assessed
- The non-woven polyester fabric of duraweb® bags was superior in seed set success by 15% over glassine and 7% over orchard wholesale bags. The duraweb® bags were re-usable and resistant to slug damage
- The duraweb® crossing tent resulted in much higher seed set rate compared to isolation chambers. The average seed number in tent was 82% higher than in isolation chamber in glasshouse
- The polyester duraweb® material whether used as pollination bags or tent provides an environment that is conductive for higher seed set in *Miscanthus*

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REFERENCES

1. Lewandowski, I. and U. Schmidt, 2006. Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agric. Ecosyst. Environ., 112: 335-346.
2. Clifton-Brown, J.C., Y.C. Chiang and T.R. Hodkinson, 2008. Miscanthus: Genetic Resources and Breeding Potential to Enhance Bioenergy Production. In: Genetic Improvement of Bioenergy Crops, Vermerris, W. (Ed.). Springer, New York, USA., ISBN-13: 9780387708058, pp: 273-294.
3. Greef, J.M. and M. Deuter, 1993. Syntaxonomy of *Miscanthus*×*giganteus*. Angewandte Botanik, 67: 87-90.
4. Hodkinson, T.R. and S. Renvoize, 2001. Nomenclature of *Miscanthus* x *giganteus* (Poaceae). Kew Bull., 56: 759-760.
5. Clifton-Brown, J.C., J. Breuer and M.B. Jones, 2007. Carbon mitigation by the energy crop, *Miscanthus*. Global Change Biol., 13: 2296-2307.
6. Hastings, A., J. Clifton-Brown, M. Wattenbach, P.C. Mitchell, P. Stampfl and P. Smith, 2009. Future energy potential of *Miscanthus* in Europe. GCB Bioenergy, 1: 180-196.
7. Clifton-Brown, J.C., I. Lewandowski, B. Andersson, G. Basch and D.G. Christian*et al*., 2001. Performance of 15 *Miscanthus* genotypes at five sites in Europe. Agron. J., 93: 1013-1019.
8. Clifton-Brown, J., P. Robson, G. Allison, S. Lister and R. Sanderson *et al*., 2008. *Miscanthus*: Breeding our way to a better future. Proceedings of the International Conference Biomass and Energy Crops III, December 10-12, 2008, Sand Hutton, UK., pp: 199-206.
9. Bentley, A.R., E.F. Jensen, I.J. Mackay, H. Honicka and M. Fladung *et al*., 2013. Flowering Time. In: Genomics and Breeding for Climate-Resilient Crops, Volume 2: Target Traits, Kole, C. (Ed.). Chapter 1, Springer-Verlag, Heidelberg, Germany, ISBN-13: 9783642370472, pp: 1-66.
10. Vogel, K.P., G. Sarath and R.B. Mitchell, 2014. Micromesh fabric pollination bags for switchgrass. Crop Sci., 54: 1621-1623.
11. England, F.J.W., 1972. Isolation chambers for controlled pollination in grasses. Euphytica, 21: 523-524.
12. Adhikari, L., M.P. Anderson, A. Klatt and Y. Wu, 2015. Testing the efficacy of a polyester bagging method for selfing switchgrass. BioEnergy Res., 8: 380-387.
13. Griffiths, D.J. and R.A.D. Pegler, 1963. Pollen proof fabrics for isolation purposes. Rept. Welsh Plant Breeding Station, pp: 74.
14. Foster, C.A., 1968. Ryegrass hybridisation: The effect of artificial isolation materials on seed yield and floral environment. Euphytica, 17: 102-109.
15. McAdam, N.J., J. Senior and M.D. Hayward, 1987. Testing the efficiency of pollination bag materials. Plant Breed., 98: 178-180.
16. Snedecor, G.W. and W.G. Cochran, 1974. Statistical Methods. 6th Edn., Iowa State University Press, USA.
17. Rounsaville, T.J., D.H. Touchell and T.G. Ranney, 2011. Fertility and reproductive pathways in diploid and triploid *Miscanthus sinensis*. HortScience, 46: 1353-1357.
18. Pickering, R.A., 1982. The effect of pollination bag type on seed quality and size in *Hordeum* inter-and intraspecific hybridization. Euphytica, 31: 439-449.
19. Ball, S.T., G.S. Campbell and C.F. Konzak, 1992. Pollination bags affect wheat spike temperature. Crop Sci., 32: 1155-1159.
20. Wyatt, R., S.B. Broyles and G.S. Derda, 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). Am. J. Bot., 79: 636-642.
21. McGranahan, G.H., D.G. Voyiatzis, P.B. Catlin and V.S.I. Polito, 1994. High pollen loads can cause pistillate flower abscission in walnut. J. Am. Soc. Horti. Sci., 119: 505-509.
22. Del Rio,C. and J.M. Caballero, 1999. A new bag for olive pollination studies. Acta Horticulturae, 474: 233-236.

23. Neal, P.R. and G.J. Anderson, 2004. Does the old bag make a good wind bag?: Comparison of four fabrics commonly used as exclusion bags in studies of pollination and reproductive biology. Ann. Bot., 93: 603-607.
24. Gitz, D.C., J.T. Baker, Z. Xin, J.J. Burke and R.J.Lascano, 2015. The microenvironment within and pollen transmission through polyethylene sorghum pollination bags. Am. J. Plant Sci., 6: 265-274.
25. PBS International, 2013. The first PBS international research update. http://www.pbsinternational.com/wp-content/uploads/2013/09/PBS-Research-Flyer_AW.pdf
26. PBS International, 2016. Why our bags are best for cereals and grasses? http://www.pbsinternational.com/our-products-new/
27. Gitz, D.C., J.T. Baker, Z. Xin, R.J. Lascano, J.J. Burke and S.E. Duke, 2013. Bird-resistant pollination bags for sorghum breeding and germplasm maintenance. Am. J. Plant Sci., 4: 571-574.
28. PBS International, 2014. The second PBS international research update. http://www.pbsinternational.com/knowledge/research-update2-2/