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Increasing seed set and pollen proofing in *Brassica juncea* and *Brassica napus* through novel non-woven synthetic pollination control bags

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ABSTRACT

Brassica juncea and *B. napus* species are predominantly self-pollinated. Parchment paper or muslin cloth bags are used for controlled selfed or cross-seed production. We evaluated three non-woven synthetic pollination control bags (PCBs) for two years as replacement for the commonly used PCBs. The new synthetic PCBs were fully pollen proof since the molecular marker analysis of the random seeds set on two cytoplasmic male sterile (CMS) lines isolated with PCBs were all maternally originated. DWB03 bags returned 47% significantly more seeds per bud than the parchment paper bags. Larger DWB03 bags showed 38% higher mean seed yield (g/plant) than the muslin cloth bags. Re-used DWB03 bags were statistically on par with new bags for the seed yield per plant. On average, DWB03 bags produced 57% more hybrid seed set on CMS lines than the muslin cloth bags. The micro-climate in DWB03 bags appeared to be more conducive for good seed set as compared to the outside and muslin cloth bags. Non-woven PCBs offer better options for replacing paper or muslin cloth bags in the breeding of Brassicas.

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Biological output; Indian mustard; non-woven synthetic fabrics; oilseed rape; pollination control bags (PCBs)

Introduction

Indian mustard (*Brassica juncea* L.) and oilseed rape (*B. napus* L.) are cultivated worldwide as key edible and industrial oilseed crops. Both are predominantly self-pollinated crops with varying amounts of outcrossing in the entomophilous flowers, which attract honeybee foraging (Labana and Banga 1984; Becker, Damgaard, and Karlsson 1992). Breeders maintain genetic integrity of thousands of breeding stocks by isolating floral shoots or complete plants with pollination control bags (PCBs) made of parchment paper or muslin cloth. The number of *Brassica* germplasm accessions alone exceeds 74,000 globally (Singh and Sharma 2007). PCBs are also used to manage bird damage in the breeding stocks (Ormerod and Watkinson 2000;

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Gitz et al. 2013, 2015; Schaffert, Virk, and Senior 2016). However, seed set in standard bags is generally low due to poor aeration, elevated temperatures, high humidity, reduced light transmission, and high incidence of pathogens and pests (Foster and Wright 1970; Gitz et al. 2015; Schaffert, Virk, and Senior 2016, 2018, 2019; Yun et al. 2017). Furthermore, the parchment paper bags are prone to tearing due to high intensity winds, rains, and the pressure of growing inflorescences (Bridgwater et al. 1998; Gitz et al. 2013). Muslin cloth bags made of loosely woven fabric carry the added risk of stray pollen grains infiltrating through its holes. Of late, PCBs made from novel non-woven fabrics are becoming popular as these avoid alien pollen ingress through randomly laid multiple fabric layers, which create a "tortuous path" and not allow the pollen to infiltrate (Hayes and Virk 2016; Clifton-Brown et al. 2018). Synthetic PCBs with greater air permeability and strength, and re-usability (PBS International 2022a, b) have been found to be more effective than standard PCBs in sorghum (Gitz et al. 2015; Schaffert, Virk, and Senior 2016, 2018, 2019; Gaddameedi et al. 2017), sugar beet, wheat, and *Arabidopsis* (Clifton-Brown et al. 2018), *Miscanthus* (Hayes and Virk 2016), grasses (Vogel, Sarath, and Mitchell 2014; Adhikari et al. 2015), and oil palm (Bonneau et al. 2017). However, no corresponding studies on synthetic PCBs are available in *Brassicas*. Therefore, the major objectives of the present investigation, conducted on *ogura*-cytoplasm-based male sterile lines and fully fertile genotypes across two years, were to assess the efficiency of novel non-woven synthetic fabric PCBs for pollen proofing and biological output in comparison to the standard practices in Indian mustard and oil-seed rape.

Materials and methods

Pollination control experiments were conducted during winters of 2019–2020 and 2020–2021 at the Oilseeds Research Farms of Punjab Agricultural University (PAU), Ludhiana (30.9010° N, 75.8573° E), India (Figure S1, S2). In the first year, three PCBs were tested, followed by more detailed confirmatory studies on the best PCB in the second year. The flowering time of the crop from mid-February to mid-March in both years coincided with the COVID-19 pandemic. Despite work interruptions resulting in forced unbalanced experimental structure, data were amenable to robust statistical analyses.

Types of pollination bags used

Pollen-size variations in Brassica have been reported by various authors, e.g., Arora and Modi (2011), and Saha and Begum (2020) reported *B. juncea* pollen = 13 µm polar (P) axis and 11 µm equatorial (E) axis);

and *B. napus* pollen = 16 P × 16 μm E. Perveen, Qaiser, and Khan (2004) estimated pollen sizes of *B. napus* = 20 P × 20 E μm; and *B. juncea* = 33 P × 30 E μm. We selected three non-woven fabrics with pore sizes little larger than the Brassica pollens. Physical properties of these materials (named DWB01, DWB03 and DWB23) are available in Table 1. PCBs were made in two sizes: (a). Two-dimensional (2-D) small bags (40 × 15 cm) to cover an inflorescence with counted number of floral buds (25 to 50 buds) and (b). Three-dimensional (3-D) bags (130 × 24.5 × 24.5 cm) to cover a whole plant for selfing or two plants (one plant of cytoplasmic male sterile (CMS) line plus one plant of fertility-restorer line) together for hybrid seed production. The standard controls were parchment paper bags for the smaller PCBs and muslin cloth bags for the larger PCBs. An open-pollinated control was also included to estimate reduction in selfed-seed output following covering of the whole plants. The best performing PCB (DWB03) from 2019–20 trials was studied more extensively during 2020–21 by increasing the sample size to 35 plants. Used DBW03 bags from the first year were saved and tested for re-usability (Hayes and Virk 2016). Data on per cent relative humidity (RH %) and temperature in Centigrade (^{0}C) were recorded, by placing electronic data loggers inside the PCBs from 15 February 2020 to 15 March 2020 (Figure S3A-F) and from 10 February 2021 to 15 March 2021, at intervals of 30 min (Figure S4A-F). This generated 1435 and 1615 data points for 2020 and 2021, respectively. Estimates of outside temperature and RH for the corresponding outside environment were obtained from the Metrological Station, PAU Ludhiana. The estimates of daily average, maximum and minimum temperature and RH were plotted separately for the two years (Figures S3, S4). The variance estimates of the daily temperature and RH recordings for new and re-used DWB03 bags were plotted for 2021 only (Figure S5). A two-tailed paired *t*-test was used to test the significance of difference of means of average, maximum and minimum daily temperatures (^{0}C) and RH (%) within DWB03 new and Muslin cloth bags from the outside estimates over the two years (31 days in 2020 and 34 days in 2021).

Table 1. Properties of nonwoven fabrics used for pollination bags.

Parameter	Estimation unit	DWB01	DWB03 = SG1	DWB23
Polymer		Polyester	Polypropylene	Polyester
Manufacturing technique		Heat bonded	Point Bonded	Spun bond
Thickness	mm	0.2	0.36	0.4
Mass per unit area/weight	g m^{-2}	101	60	110
Air Permeability	l/m^2/s	110	192	1470
Light transmittance	% (350–800 nm)	35%	34%	39%
Max pore size	microns	31.7	34	219
Fiber cross section		Simple	Simple	Complex

However, this comparison for the re-used DWB03 bags was only possible for 2021.

Description of experiments

Experiment 1: Three synthetic PCBs (DBW01, DBW03, DBW23) along with muslin cloth bags were tested for pollen-proofing ability. Whole plants from CMS lines, DTM81 (*B. juncea*) and AG24 (*B. napus*) were bagged. These experiments were conducted across two years in two different hybrid seed-production isolation plots, where CMS lines were surrounded by their respective fertility restorer lines. During 2019–20, nine and ten bags of each type (DBW01, DBW03, DBW23 and muslin cloth) were used for covering CMS plants of *B. juncea* and *B. napus*, respectively. During 2020–21, 35 bags each for DBW03 and muslin cloth were used to cover individual plants of the same two male sterile lines of *B. juncea* and *B. napus*. Any seeds set on enclosed male sterile plants were collected from bags and germinated to extract genomic DNA to test their hybridity using previously reported *Rfo*-specific Kompetitive Allele Specific PCR (KASP) marker (Gudi et al. 2020).
Experiment 2: This experiment tested the selfed-seed production in fully fertile plants of *B. napus* cv. GSC22 and *B. juncea* cv. AJR102B. Small 2-D bags were used to isolate a counted number of floral buds on inflorescences (25 to 50 floral buds), and the large 3-D bags were used to isolate single plants to study the impact of reduced light transmission due to relative opacity of the PCB fabric on total seed yield per plant (Figures S1, S2). Parchment paper or muslin cloth bags were used as the control treatments for small bags and large bags, respectively. Open-pollinated seed set per plant was also included as a treatment during 2019–20 to investigate plant productivity under normal conditions. Total number of seeds from each bag was counted and divided by the number of buds isolated to arrive at the number of seeds per bud for statistical analysis. Number of pods per bag was also recorded. This allowed the computing of number of seeds per pod for analysis during 2020–21. Two biological traits, plant height (PH in cm) and seed yield (SY) per plant in grams (g) were recorded at maturity for plants under large PCBs. Used bags from 2019–20 were included as an extra treatment with small and large bags on *B. napus* cv. GSC22. A combined statistical analysis was performed using Minitab-17 statistical package (ANOVA in Tables S1, S2).
Experiment 3: We enclosed one male sterile plant and one fertility restorer plant in large synthetic PCBs to study hybrid seed set on the male sterile plants. Muslin cloth bags were used as control. CMS plants produced hybrid seeds due to pollination by the fertility restorer. In contrast, seeds set on fertility restorer plants were due to self-pollination. Data were recorded at maturity on plant height (cm) and seed yield (g per plant) from the male

sterile as well as fertility restorer plants. During 2019–20, an analysis of variance was performed (ANOVA in Table S3) for number of hybrid seeds collected from CMS plants and selfed-seeds from the fertility restorer plants as factors. However, during 2020–21, analysis of variance (ANOVA in Table S4) was performed only on hybrid seed set on CMS plants since no data could be recorded for selfed-seed on restorer-line plants.

Mean values for different bag treatments from experiments 1 and 2 were tested for significant differences using Fisher's pairwise least significant difference (LSD) comparison at 95% probability.

Results

Restricting to overall best performing DWB03 bag, its daily average temperature in both years was lower than outside but either higher than or closer to muslin cloth (Figure S3). The re-used bags showed a slightly lower average temperature than the new bags. The maximum temperature was higher in DWB03 in both years but equal or higher than muslin cloth, with re-used bags showing lower temperature than the new bags (Figure S3). The minimum temperature inside DWB03 was lower than the outside but comparable to re-used bags and higher than muslin cloth in both years (Figure S3). Average and maximum RH in DWB03 was higher than outside but close to muslin cloth, with re-used bags showing highest estimates. The minimum RH inside DWB03 was close to muslin cloth but re-used bags showed higher estimates compared to the outside, which showed large fluctuations (Figure S4). The variances of daily temperature and RH for DWB03 new and muslin cloth bags were very similar, but re-used bags showed the lowest estimates (Figure S5).

New and re-used DWB03 bags showed significantly lower average temperature by 1 to 2°C than the outside temperature, in line with muslin cloth bags (Table 2). However, the mean maximum temperature of DWB03 bags was about 5 degrees higher, which was slightly higher than muslin cloth but about 2 degrees higher than DWB03 re-used bags. All PCBs showed about 2 degrees lower minimum temperature than the outside. RH estimates in PCBs were generally higher than the outside (Table 2). The mean for average RH was 14%, 16% and 22% higher for muslin cloth, DWB03 new and DWB03 re-used bags compared with the outside. The mean maximum RH was 12%, 15%, 15% higher than the outside for DWB03 new, muslin cloth and DWB03 re-used bags. The mean minimum RH for DWB03 new and muslin cloth bags was on par with the outside but the DWB03 reused bags showed significantly (13%) more RH than the outside (Table 2).

Table 2. Comparative mean of daily average, maximum and minimum temperature (^0C) and mean of daily average, maximum and minimum relative humidity (RH %) within DWB03 bags (new and re-used) and outside over two years (2020 and 2021).

Parameter	Year	Bag	Average	Outside Diff†	Max	Outside Diff†	Min	Outside Diff†
Temperature	2020,21	DWB03 New	17.63	−0.83	29.59	4.72	9.66	−2.24
	2020,21	Muslin	17.33	−1.12	29.11	4.24	9.21	−2.69
	2020,21	Outside	18.45		24.87		11.90	
	2021	DWB03 Reused	18.10	−1.55	29.44	2.87	10.47	−1.99
	2021	Outside	19.65		26.58		12.47	
RH (%)	2020,21	DWB03 New	86.05	16.00	101.47	12.31	54.97	2.60NS
	2020,21	Muslin	84.23	14.18	104.54	15.37	51.67	−0.70NS
	2020,21	Outside	70.05		89.17		52.37	
	2021	DWB03 Reused	91.48	22.24	104.21	14.68	65.01	13.33
	2021	Outside	69.24		89.53		51.68	

†Diff = difference from outside. All differences were significant at $P < 0.01$ except NS = non-significant using a two-tail paired t-test. Number of days = 31 in 2020 and 34 in 2021.

Pollen-proofing ability of pollination control bags

No seed set was recorded on the isolated male sterile plants of *B. napus* CMS line AG24 (both during 2020 and 2021) and *B. juncea* CMS line DTM81 (*B. juncea*) during 2021. However, sporadic seed set was recorded on a few plants of CMS DTM81, isolated with different bag sizes during 2020. The lowest number of 14 seeds was observed following isolation with DWB23 and DWB01 bags versus 39 from DWB03 and 36 from the muslin cloth bags. The latter two bags also showed pre-harvest sprouting of seeds. Only 28 of the 103 seeds germinated and 27 of these were found to be of maternal origin as per KASPar marker assays (Figure 1). However, the plant germinated from one seed obtained under the muslin cloth bag was heterozygous (*Rfo rfo*) for fertility restoration gene (*Rfo*) (Figure 1).

Comparing relative biological outputs in plants enclosed with different PCBs

A combined analysis of variance (across two years) showed that the main effects of years, genotypes and bag types were significant for the numbers of seeds per bud and seeds per pod for the plant inflorescences covered by small bags (Table S1). However, the seed output for new vs. re-used PCBs did not differ significantly (Table S1). Interactions years × genotypes and genotypes × bags were significant for both seed traits, but they had little consequence because of their low (2 to 7%) contributions toward total sum of squares (Table S1) compared with large contributions of the main effects.

A comparison of pairwise combination of least squares-fitted mean values revealed that DWB03 was significantly superior to all bag types

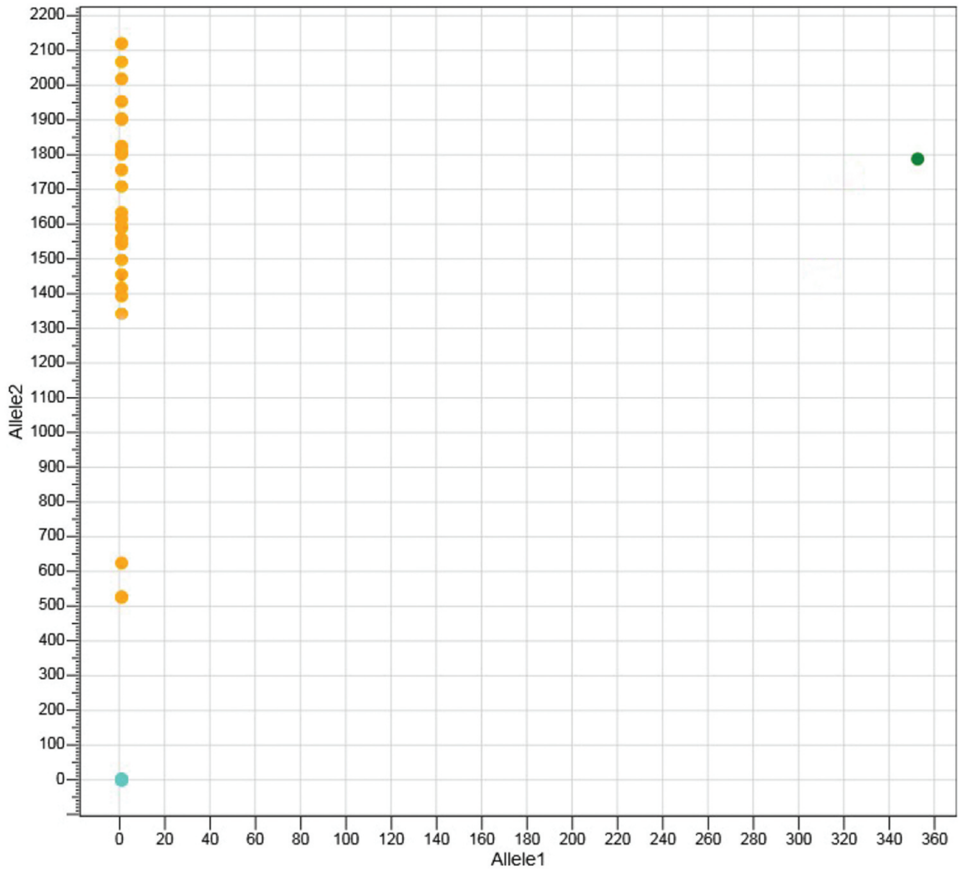

Figure 1. Snapshot showing genotyping with *Rfo* KASPar assays in the germinated 28 plants. The plant (progeny of plant enclosed in muslin cloth bag) in the upper right corner as green dot is heterozygous (*Rforfo*) for the fertility restorer gene (*Rfo*) for *Ogura* CMS. Plants clustered as yellow dots on the left corner are maternal in origin (*rforfo*). Sky blue dot indicates non-template control.

evaluated in the overall analysis for seed number per bud and seed number per pod (Table 3). Compared with the parchment paper control, it yielded 58% more seeds per bud in both years separately. Overall, its superiority across two years was 47%, less than the average of two years because of the unequal number of bag samples for DWB03 compared with parchment paper bags, especially in 2021 (Figure 2, Table 3). In contrast, the commonly used parchment paper bags performed better than DWB01 and DWB23 synthetic bags, which showed 40% and 52% lower mean seeds per bud (Table 3).

A combined analysis of variance for plant height and seed yield per plant across two years from the fully covered plants with large bags revealed significant differences between the years and bag types (Table S2). Genotypes differed significantly for plant height but not for seed yield.

Table 3. Fitted mean values with standard errors (± SE) over 2019–2020 and 2020–2021 for seed output traits with different bag treatments.

Factor	Level	N	Mean (seeds/bud)	No. of Seeds/pod	Mean (Seeds/pod)[a]
Bag age	New	240	7.09 ± 0.23A	140	10.15 ± 0.20A
	Reused	35	6.12 ± 0.64A	35	9.25 ± 0.52A
Year	2020	100	10.28 ± 0.43A		
	2021	175	2.93 ± 0.43B		
Genotype	GSC22	165	8.72 ± 0.37A	105	11.62 ± 0.28A
	AJR102B	110	4.49 ± 0.48B	70	7.77 ± 0.39B
Bags	DWB01	25	4.43 ± 0.67C		
	DWB03	130	10.97 ± 0.36A	105	10.26 ± 0.28A
	DWB23	25	3.57 ± 0.67C		
	Paper	95	7.44 ± 0.46B	70	9.14 ± 0.39B

[a]Recorded in 2021 only. Pairwise grouping using Fisher LSD method and 95% confidence. Means that do not share a letter are significantly different.

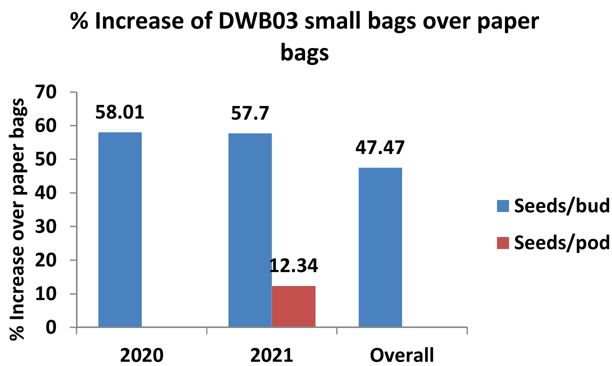

Figure 2. Per cent increase in seed number per bud and seed number per pod for the small DWB03 bags over standard parchment paper bags averaged over two varieties: GSC22 *B. napus* and ARB102 *B. juncea*.

Interestingly, there were no significant differences between the new and reused DWB03 bags for plant height and seed yield (Table S2). The interactions genotype × bag type and genotype × year were significant for both plant height and seed yield. However, interactions contributed little (0.2 to 7.5%) to the total SS for both the traits (Table S2).

The fitted mean values across two years showed that the mean plant height was significantly higher for open-pollinated plants than the most bagged plants, except for those covered by DWB03 bags (Table 4). The mean seed yield per plant (g) and plant height (cm) were highest in the plants enclosed with DWB03 bag. These values were comparable with those in open-pollinated control (Table 4). DWB03 was as good as open-pollinated condition, with 6% overall increase in plant height over muslin cloth bags (Figure 3a). DWB03 returned 8% more seed yield than the corresponding open-pollinated plants and 38% more seed yield than the muslin cloth bags (Table 4, Figure 3b). The standard practice of using muslin cloth bags led to

Table 4. Fitted mean values over 2019–2020 and 2020–2021 for different biological traits with different treatments.

Factor	Level	N	Mean PH (cm) ±SE	Mean SY (g/pl) ±SE
Bag age	New	244	178.46 ± 0.77A	13.30 ± 0.39A
	Reused	30	177.04 ± 2.20A	13.40 ± 1.11A
Year	2020	125	180.93 ± 1.38A	10.66 ± 0.70B
	2021	149	174.58 ± 1.51B	16.04 ± 0.76A
Genotype	GSC22	169	172.09 ± 1.25B	13.13 ± 0.63A
	AJR102B	105	183.41 ± 1.62A	13.57 ± 0.81A
Bags	DWB01	25	176.55 ± 2.21B	9.61 ± 1.11C
	DWB03	115	182.60 ± 1.24A	17.18 ± 0.62A
	DWB23	25	174.95 ± 2.21B	11.61 ± 1.11BC
	OP	25	182.60 ± 2.21A	15.89 ± 1.11A
	MC	84	172.38 ± 1.56B	12.46 ± 0.79B

Pairwise Grouping Using Fisher LSD Method and 95% Confidence. Means that do not share a letter are significantly different. OP = open pollinated; MC = Muslin cloth.

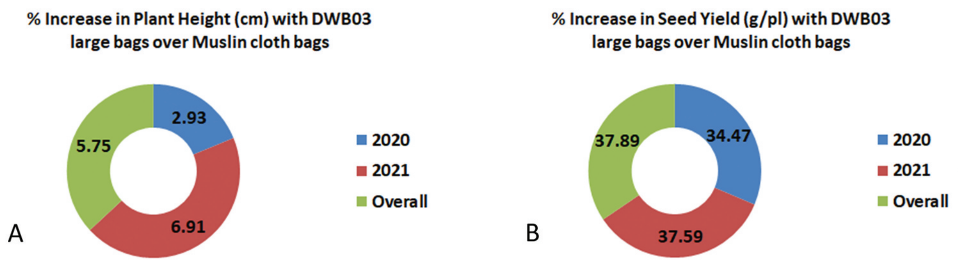

Figure 3. Per cent increase in (A) plant height (cm) (left) and (B) seed yield (g/plant) (right) for the large DWB03 bags over the standard muslin cloth bags averaged over two varieties: GSC22 *B. napus* and ARB102 *B. juncea.*

plants with the lowest plant height and 22% percent reduction in the seed yield compared with the open-pollinated situation. Seed yield reduction compared to the open-pollinated condition was even greater when DWB01 and DWB23 bags were used (Table 4).

Comparative hybrid seed set on male sterile plants, enclosed with corresponding fertility restorers

Analysis of variance showed significant differences between bag types for the overall number of seeds set on both male sterile and fertile lines in 2019–20 (Table S3). The number of seeds set on male fertile Vs male sterile plants was also significant (Table S3). The mean number of selfed-seeds on restorer plants was significantly higher (by 165%) than the hybrid seed mean on male sterile plants (Table 5). DWB03 retuned significantly higher (48%) seed set over the next best, muslin cloth bags (Figure 4, Table 5). The other bags (DWB01 and DWB23) were far inferior to the control.

Table 5. Mean seed set on male and female inflorescences enclosed in the same large bags for producing hybrid seeds during 2019–2020.

Genotype/bag	N	Mean seed number ± SE
Plant type		
Fertile	17	566.9 ± 66.0A
Male Sterile	20	213.6 ± 60.7B
Bag type		
DWB03	9	648.5 ± 90.7A
Muslin cloth	10	438.0 ± 90.7AB
DWB23	9	277.3 ± 85.9B
DWB01	9	197.3 ± 90.7B

† Mean values that do not share a letter are significantly different on Fisher's pairwise comparison at 95%.

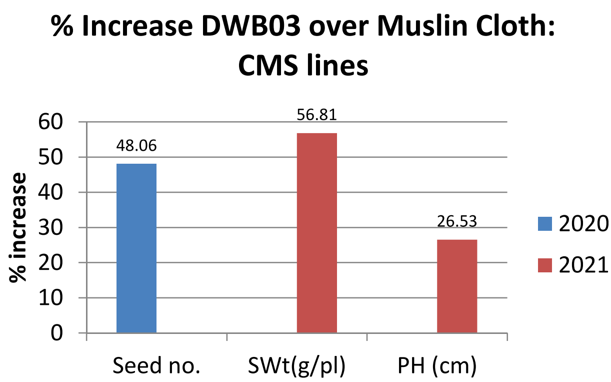

Figure 4. Percent increase for seed number, seed weight (SWt = seed yield) and plant height (PH) for DWB03 over muslin cloth large bags when a male sterile plant was enclosed in the same large bag with a restorer plant.

The analysis of variance for 2020–21 also revealed significant differences between the two male sterile lines for the quantity of hybrid seeds produced (Table S4). DWB03 and muslin cloth bags differed significantly for plant height and seed yield produced (Table S4). CMS lines × bag type interaction was significant for both the traits, though having small contribution to the total sum of squares, i.e., 6% for plant height and 4% for seed yield (Table S4). DWB03 bags produced 57% higher seed yield, with plants taller by 27% in 2020–21 over the the muslin cloth bags (Figure 4, Table 6).

Discussion

Parchment paper or muslin cloth bags are commonly used for controlled seed set in oilseeds Brassicas, despite their inherent limitations. Recently, PCBs made of non-woven synthetic fibers have been found to be very effective in many crops (Gitz et al. 2015; Hayes and Virk 2016; Bonneau

Table 6. Fitted mean values for 2020–2021 for plant height and seed yield (g/plant) on CMS lines bagged together with restorer lines.

Factor	Level	N	Mean PH (cm) ±SE	Mean SY (g/pl) ±SE
CMS line	AG24 *B. napus*	70	154.53 ± 1.42A	6.176 ± 0.138A
	DTM10 *B. juncea*	70	143.46 ± 1.42B	3.218 ± 0.138B
Bag type	DWB03	70	166.44 ± 1.42A	5.736 ± 0.138A
	Muslin cloth	70	131.54 ± 1.42B	3.658 ± 0.138B

Pairwise Grouping Using Fisher LSD Method and 95% Confidence. Means that do not share a letter are significantly different.

et al. 2017; Clifton-Brown et al. 2018; Schaffert, Virk, and Senior 2019). The non-woven materials offer more properties by flexing polymer, fiber size, length, shape and manufacturing technique and are known to have high strength to withstand plant over-growth, damage from bad weather (heavy rains and high winds), field pests and bird attack; good air permeability to balance moisture and temperature inside the bag. Sufficient light transmissibility and appropriate porosity to prevent external pollen ingress are other important factors (PBS Intl 2022a, b; Figure S6). Many types of non-woven synthetic fabrics have been found to meet these requirements, which have been shown to increase plant breeding efficiency by producing higher and better-quality seed outputs than the controls (Townson, Virk, and Senior 2020; Trammell et al. 2020). We identified three non-woven fabrics (DWB01, DWB03, and DWB23) based on their physical properties that make them more suitable as PCBs for Brassica crops. Because of different properties of fabrics, all these bags did not perform equally well in the two years of study suggesting a need to identify crop specific fabrics for PCBs (Clifton-Brown et al. 2018). However, at least one bag type (DWB03) performed outstandingly better than the control practices, and in some cases better than the open pollinated conditions, for different biological parameters. Apparently, DWB03 provided a micro-environment that was more conducive to higher seed set even when compared with the outside conditions. This might be attributed to breathability of DWB03 fabrics that led to inside temperature and humidity to levels more suitable for seed production (Table 1). Since a PCB covers reproductive structures of the plant, elevated inside temperature (Ball, Campbell, and Konzak 1992; Gitz et al. 2015) is bound to impact the seed set because of reduced pollen viability (Chowdhury and Wardlaw 1978; Harsant et al. 2013) and or seed set (Prasad, Boote, and Allen 2006). Along with rise in temperature, high humidity can also directly reduce the seed set (Foster and Wright 1970) or by creating micro-climate conditions that are more favorable for diseases and pests to develop (Yun et al. 2017). Further, Brassica specificity of DWB03 fabric is strengthened from the similar microclimate and seed output performance of the re-used and new bags.

Bagging completely male sterile (MS) plants should normally result in no seed set. However, sporadic seed set may occur on bagged MS plants by pollen ingress or due to the reversion of male sterility resulting in maternally produced selfed-seeds. Our studies showed that DWB03 was completely pollen proof and selfed-seed set, if any, on CMS plants was of parthenogenetic maternal origin as KASPar marker analysis for fertility gene (*Rfo*) showed the *rfo rfo* genotype. Only one seed (out of 28 germinated) produced on CMS plant covered with muslin cloth bag presented a heterozygous (*Rfo rfo*) genotype that was expected from outcrossing. DWB03 was previously proved to be superior for seed output in sorghum (Schaffert, Virk, and Senior 2016, 2018; Gaddameedi et al. 2017) and grasses (Hayes and Virk 2016: Clifton-Brown et al. 2018). DWB03 are made of the fiber, known commercially as duraweb® SG1. This material could significantly reduce bird damage and mold infection in sorghum by being strong but highly breathable. It is proven to halt unwanted pollen, reduce contamination and increase seed yield in sorghum (PBS International 2022c). Completely pollen proof characteristic of DWB03 was primarily due to the physical complexity caused by weave architecture of the non-woven fabric used, as pore sizes of PCBs evaluated in our studies were larger than the pollen size of Brassica species investigated. Thermally bonded nonwoven fabric filter samples are characterized by multiple filtration layers of interconnected pores and tortuous pore paths through the fabric thickness (Wang and Gong 2006). This torturous but purposefully effective filtration of pollen through larger pore size may not assure totally impermeable conditions, yet it provides a trade-off in pollination performance while allowing exchange of air and moisture. All the fabrics of PCBs clearly provide an acceptable filtering level of co-optimization of pollen exclusion in the present experiment. DWB03 bags also showed no adverse effects with respect to reduced seed production in fully covered Brassica plants. The better biological performance of DWB03 may be attributed to its higher light transmissibility, especially in 400 to 1000 nm wavelength range, compared with kraft paper (Hannah Senior, PBS International, Pers. Comm.; Figure S6).

Another useful outcome of our study is the possibility of re-using the DWB03 PCBs more than once (Hayes and Virk 2016; Schaffert, Virk, and Senior 2018) which would reduce the actual cost and make them more economical than might first appear from the initial higher investment. However, optimization of the number of times of re-use of non-woven PCBs needs to be established. Although PCBs enclose reproductive plant parts and are critical in artificial hybridization, plant breeders tend to allocate minimal resources to this in relation to the total cost per cross. This tendency exists even though cheap PCBs made of paper and cellulose are prone to damage by birds (Gitz et al. 2013), insects (Demirel and Cranshaw 2006), wind (Bridgwater et al. 1998); require deliberate daily bag shaking for pollen dispersion, and are affected by water, and diseases (Windham and Williams 2007) or slugs. The parchment paper PCBs are also prone to stress tearing as the plant's growth causes the seams of the parchment bags to give way. Muslin cloth bags collapse on the plant and get wet due to dew in the night, creating a favorable environment for diseases and pests. It is estimated that a medium-sized rapeseed mustard breeding program uses ~30,000 standard PCBs per season, of which around 40–50% may get damaged due to bad weather. Although economic analysis was not envisaged in this research, some conclusions can be derived (Table 7) following Schaffert, Virk, and Senior (2016) and Gaddameedi et al. (2017). Parchment papers are prone to various losses compared with non-woven PCBs, which gave 47% higher seed yield (Table 7). Extrapolations with these assumptions provide a ratio 2.43 paper bags: 1 non-woven PCB per season. Since the latter are re-usable, the cumulative ratio for the second season = 4.86:1 and for the third season = 7.29:1. Clearly, across three seasons, 100 non-woven PCBs can replace 729 paper bags. In addition, using fewer bags also requires less labor, which may reduce direct and indirect (supervisory) cost. The muslin cloth bags are re-usable but collapse on plants, and attract moisture, increase diseases, and reduce seed purity. These assumptions provide a ratio of 1.86:1 per season, i.e., 100 large non-woven bags can replace 186 muslin cloth bags. Plant breeding programs mandate the

Table 7. Factors for comparing pollination control bags (PCBs) for economic relativity.

Bag type	Seed yield	Effect of natural factors	Bird damage	Seed Quality (diseases, vigor)	Loss of genetic purity	Reuse	Relative cost†
Parchment paper-small bag	1	Wind, rain may tear or blow away, = 40%	10%	10%	5%	Nil	$
Nonwoven-small bag	2.43 of paper	Nil	Nil		Nil	Yes	$$
Muslin cloth – large bag	1	Collapse on plant, tear from plant overgrowth, moisture, diseases = 10%	Nil	20%	5%	Yes	$$
Nonwoven-large bag	1.86 of muslin cloth	Nil	Nil	Nil	Nil	Yes	$$$

† The dollar ($) sign indicates relative costing of each method. The method with one $ has minimum cost, $ has highest price.

production of large quantities of selfed or cross seeds with high genetic integrity and germinability. Failures in these may result in losing a season for testing of new hybrids/ entries in multi-location trials.

Conclusions

DWB03 pollination bag made of non-woven synthetic fiber was found superior for selfed or hybrid seed production under controlled conditions as micro-climate conditions within DWB03 bags were found to be more conducive for higher biological output in comparison to standard practices and outside conditions. Further, this bag can be re-used at least once, as no significant differences were detected between used and new PCBs in the present studies. Despite the need for careful economic analysis of new and standard pollination control practices, the benefits of synthetic PCBs are apparent and Brassica breeders may well adopt them at least on a test scale while scientific and economic impact is assessed.

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Authors' contributions

Mehak Gupta and Gurpreet Kaur conducted field and laboratory experiments. Surinder Singh Banga provided the supervisory support, planning of experiments and editing the manuscript. D.S. Virk contributed to planning, statistical analysis and preparing the draft manuscript. All authors contributed to the final preparation of manuscript.

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References

Adhikari, L., M. P. Anderson, A. Klatt, and Y. Q. Wu. 2015. "Testing the Efficacy of a Polyester Bagging Method for Selfing Switchgrass." *Bioenergy Research* 8 (1): 380–387. doi:10.1007/s12155-014-9528-3.

Arora, A., and A. Modi. 2011. "Pollen Morphology of Some Desertic Crucifers." *Indian Journal of Fundamental and Applied Life Sciences* 1: 11–15.

Ball, S. T., G. S. Campbell, and C. F. Konzak. 1992. "Pollination Bags Affect Wheat Spike Temperature." *Crop Science* 32 (5): 1155–1159. doi:10.2135/cropsci1992.0011183X003200050019x.

Becker, H. C., C. Damgaard, and B. Karlsson. 1992. "Environmental Variation for Outcrossing Rate in Rapeseed." *(Brassica Napus) Theor. Appl. Genet* 84 (3–4): 303–306. doi:10.1007/BF00229487.

Bonneau, L., D. Eli, P. Vovola, and D. S. Virk. 2017. "Comparing Pollination Bag Types for micro-environmental Parameters Influencing Seed Production in Oil Palm." *Journal of Oil Palm Research* 29 (2): 168–179. doi:10.21894/jopr.2017.2902.02.

Bridgwater, F. E., D. L. Bramlett, T. D. Byram, and W. J. Lowe. 1998. "Controlled Mass Pollination in Loblolly Pine to Increase Genetic Gains." *Forestry Chronicles* 74 (2): 185–189. doi:10.5558/tfc74185-2.

Chowdhury, S. I., and I. F. Wardlaw. 1978. "Effect of Temperature on Kernel Development in Cereals." *Australian Journal of Agricultural Research* 29 (2): 205–223. doi:10.1071/AR9780205.

Clifton-Brown, J. C., H. Senior, S. J. Purdy, R. Horsnell, B. Lankamp, A. K. MuEennekhoff, D. Virk, et al. 2018. "Investigating the Potential of Novel Nonwoven Fabrics for Efficient Pollination Control in Plant Breeding." *Plos ONE* 13 (9): 1–21, e0204728. doi:10.1371/journal.pone.0204728.

Demirel, N., and W. Cranshaw. 2006. "Permethrin Treatment of Pollination Bags as a Protecting from False Chinch Bug, *Nysius Raphanus* (Howard) (Hemiptera: Lygaeidae), Injury to Canola in Colorado." *Crop Protection* 25 (9): 1062–1064. doi:10.1016/j.cropro.2006.01.009.

Foster, C. A., and C. E. Wright. 1970. "Variation in the Expression of self-fertility inLolium Perenne L." *Euphytica* 19 (1): 61–70. doi:10.1007/BF01904667.

Gaddameedi, A., A. A. Kumar, R. M. Phuke, D. S. Virk, and H. Senior. 2017. "Evaluating the Efficacy of Synthetic Fibre Pollination Control Bags in Sorghum during the Rainy Season." *International Journal of Plant Breeding and Genetics* 11: 35–54.

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." *American Journal of Plant Science* 6 (2): 265–274. doi:10.4236/ajps.2015.62030.

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." *American Journal of Plant Science* 4 (3): 571–574. doi:10.4236/ajps.2013.43074.

Gudi, S., C. Atri, A. Goyal, N. Kaur, J. Akhtar, M. Mittal, K. Kaur, G. Kaur, and S. S. Banga. 2020. "Physical Mapping of Introgressed Chromosome Fragment Carrying the Fertility Restorer *(Rfo)* Gene for Ogura CMS in *Brassica Juncea* L. Czern & Coss." *Theoretical and Applied Genetics* 133 (10): 2949–2959. doi:10.1007/s00122-020-03648-3.

Harsant, J., L. Pavlovic, G. Chiu, S. Sultmanis, and T. L. Sage. 2013. "High Temperature Stress and Its Effect on Pollen Development and Morphological Components of Harvest Index in the C3 Model Grass *Brachypodium Distachyon*." *Journal of Experimental Botany* 64 (10): 2971–2983. doi:10.1093/jxb/ert142.

Hayes, C., and D. S. Virk. 2016. "Assessing the Relative Efficacy of Polyester Pollination Bags and Crossing Tents, and Isolation Chambers for Seed Harvest in Miscanthus Crosses." *International Journal of Plant Breeding and Genetics* 10 (2): 79–90. doi:10.3923/ijpbg.2016.79.90.

Labana, K. S., and S. S. Banga. 1984. “Floral Biology in Indian Mustard.” *(Brassica juncea (L.) Coss) Genetica Agraria* 30: 131–138.

Ormerod, S. J., and A. R. Watkinson. 2000. “Editor’s Introduction: Birds and Agriculture.” *Journal of Applied Ecology* 37 (5): 699–705. doi:10.1046/j.1365-2664.2000.00576.x.

PBS International. (2022a). “Intelligent design-why Our Bags are Best.” Accessed 7 February 2022. https://www.pbsinternational.com/our-products/intelligent-design/

PBS International. (2022b). “Grasses research-why Our Bags are Best.” Accessed 7 February 2022. https://www.pbsinternational.com/our-products/grasses-products/grasses-research/

PBS International. (2022c). “Our Products: Scientifically Proven Performance.” Accessed 7 February 2022. https://www.pbsinternational.com/our-products/

Perveen, A., M. Qaiser, and R. Khan. 2004. “Pollen flora of Pakistan -XLII. Brassicaceae.” *Pakistan Journal of Botany* 36 (4): 683–700.

Prasad, P. V. V., K. J. Boote, and L. H. Allen. 2006. “Adverse High Temperature Effects on Pollen Viability, seed-set, Seed Yield and Harvest Index of grain-sorghum [*Sorghum Bicolor* (L.) Moench] are More Severe at Elevated Carbon Dioxide Due to Higher Tissue Temperatures.” *Agriculture and Forestry Meteorology* 139 (3–4): 237–251. doi:10.1016/j.agrformet.2006.07.003.

Saha, S., and K. N. A. Begum. 2020. “Comparative Analysis on Pollen Morphology of the Genus *Brassica* L. (Brassicaceae) in Bangladesh.” *Intentional Journal of Botany Studies* 5 (5): 191–194.

Schaffert, R. E., D. S. Virk, and H. Senior. 2016. “Comparing Pollination Control Bag Types for Sorghum Seed Harvest.” *Journal of Plant Breeding and Crop Science* 8 (8): 126–-137.2006-9758 doi:10.5897/JPBCS2016.0580.

Schaffert, R. E., D. S. Virk, and H. Senior. 2018. “Are Nonwoven Synthetic Pollination Bags a Better Choice for Sorghum Breeding?” *Journal of Plant Breeding and Crop Science* 10 (3): 58–68. doi:10.5897/JPBCS2017.0704.

Schaffert, R. E., D. S. Virk, and H. Senior. 2019. “Are Nonwoven Synthetic Pollination Bags a Better Choice for Sorghum Breeding? In.” *Current Research in Agriculture and Horticulture* 1 (9): 82–95. doi:10.9734/bpi/crah/v1.

Singh, R., and S. K. Sharma. 2007. “Evaluation, Maintenance and Conservation of Germplasm.“ *Advances in Botanical Research* 45: 465–481. doi:10.1016/S0065-2296(07)45017-0.

Townson, P., D. S. Virk, and H. Senior. 2020. “Evaluating the Pollen Proofing of Nonwoven Synthetic Fabric Pollination Control Tents for Sugar Beet.” *Journal of Plant Breeding and Crop Science* 12 (3): 228–236. doi:10.5897/JPBCS2020.0904.

Trammell, M., D. G. Pittman, D. S. Virk, and H. Senior. 2020. “Assessing the Effectiveness of Nonwoven Fabric Pollination Tents for Improved Grass Breeding.” *Journal of Plant Breeding and Crop Science* 12 (3): 200–218. doi:10.5897/JPBCS2020.0893.

Vogel, K. P., G. Sarath, and R. B. Mitchell. 2014. “Micromesh Fabric Pollination Bags for Switchgrass.” *Crop Science* 54 (4): 1621–1623. doi:10.2135/cropsci2013.09.0647.

Wang, W. H., and R. H. Gong. 2006. “Thermally Bonded Nonwoven Filters Composed of Bi-Component Polypropylene/Polyester Fiber. II. Relationships between Fabric Area Density, Air Permeability, and Pore Size Distribution.” *Journal of Applied Polymer Science* 102 (3): 2264–2275. doi:10.1002/app.24169.

Windham, G. L., and W. P. Williams. 2007. “Note: Effect of Ear Bagging Systems on *Aspergillus Flavus* Kernel Infection and Aflatoxin Contamination of Corn Hybrids Grown in the Field.” *Phytoparasitica* 35 (3): 277–281. doi:10.1007/BF02981161.

Yun, H. G., D. J. Kim, W. S. Gwak, T. Y. Shin, and S. D. Woo. 2017. “Entomopathogenic Fungi as Dual Control Agents against Both the Pest Myzus Persicae and Phytopathogen Botrytis Cinerea.” *Mycobiology* 45 (3): 192–198. doi:10.5941/MYCO.2017.45.3.192.