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# Wind Pollination in Indian Mustard [*Brassica juncea* (L.) Czern & Coss]

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**ABSTRACT** The experiments to assess the extent of wind pollination in *Brassica juncea* cv.Pusa Bold were conducted at IARI, New Delhi for three years (1996-97—1998-99). Dispersal of pollen grains by wind was noticed up to 35 meters from the pollen source. Air-borne pollen grains may pass through insect proof net and the effective pollination may occur. The extent of wind pollination was recorded up to 11 to 17.5 per cent. In want of pollen the flowers of male sterile plants may develop multistigmas to intercept effectively wind-borne pollen.

**Keywords:** Mustard, pollination, seed setting

Wind is the only agent of pollination in crops like pearlmillet, maize and spinach. Wind pollination is likely to be successful if certain idealized conditions are met which include, large numbers of pollen grains with light weight and small in size, moderate wind velocity, relatively low humidity, close spacing of plants, stigmatic surfaces structured and positioned to maximize pollen collection efficiency etc. In Brassica group the amphiploid species like *B.juncea, B.napus* and *B.carinata* are largely self pollinated. There exists a considerable level of genetic and environmental variation for the extent of out crossing [1]. The pollen grains of brassicas are relatively large, sticky and suitable for insect pollination. Role of wind alone in pollination of brassica crop is not clear; however, Pearson [2] and Olsson [3] concluded that wind is the main agent of pollination of oilseed rape. Eisikowitch [4] conducted an experiment to assess the effects of wind pollination alone on seed setting in oil seed rape (*B. napus*).He found that 22.2% flower giving rise to pod and 3.3 seeds per pod were due to wind pollination. Under natural condition, 20 to 40% out crossing can occur in *B. napus* and *B. campestris*, due to mediation by wind [5]. In *B. juncea*, out crossing ranging from 7.6 to 18.1 per cent has been demonstrated [6]. *B. juncea* is largely self-pollinated, however, insects have a major role in out crossing. The present studies were planned to assess extent of wind pollination in *B.juncea.* cv. Pusa Bold.

## MATERIALS AND METHODS

Cytoplasmic male sterile line with *B. oxyrrhina* cytoplasm and *B.juncea* genome maintained by B-line var. Pusa Bold and variety Pusa Bold as pollinator were used as experimental material to assess the extent of wind pollination. Three experiments were conducted.

**Experiment-1**: *Dispersal of air-borne pollen grains at different distances from the source:*

The wooden poles of 2m height were fixed in the leeward direction of experimental field up to 40 meters at an intervals of 5m i.e., 5m, 10m, 15m, 20 m, 25m, 30m, 35m and 40m. One meter long stick was tied horizontally to each pole at the approximately same height to that of mustard plants. Three glass slides were hung to each stick with the help of cello tape. Glycerol was smeared on one side of glass slide which was facing towards experimental field. These slides were kept hanging in the field for whole day i. e. 0800 to 1800 hrs. in the year 1996-97. During the next two years experimental slides were kept hanging in field only for two hours at different intervals. After

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every two hours slides were taken to laboratory to be examined for the presence of number of pollen grains under the microscope. The number of pollen grains under 25x25mm square shaped cover glass was counted. This counting procedure was followed for seven days during peak flowering days. These experiments were repeated over three years.

**Experiment-2:** *Counting of air-borne pollen in different blending ratios :*

To record the concentration of air-borne pollen grains in different blending ratio i.e. 90:10, 80:20, 70:30, 60:40, 50:50 and 0:100(male fertile and male sterile plants) the same procedure was followed as described under experiment 1 except that the glass slides were hung inside the net 10 cm. above plants. This study was conducted for two years.

**Experiment-3:** *Wind pollination estimated by observing yield and yield attributes*

This experiment was conducted in two ways:

**a.** In this experiment 5m long eight rows of oxy CMS line were sown in a plot with three replications. The row spacing was 45 cm. On both the sides of this plot of eight rows of male fertile Pusa Bold were sown at a distance of 1.5m to allow movement of pollen grains to CMS line. The plots of CMS rows only were covered with insect proof nylon net before initiation of flowering to prevent entry of insect pollinators, mainly honeybees. Further, to prevent the pollination by minor insect, the sprays of insecticides were applied twice a week during entire flowering period. As the entry of honeybees and other insect pollinators was prevented by nylon net and minor insects inside the net were killed with the help of insecticidal sprays, it was expected that any seed set in CMS rows, if it is there, would happen only by wind pollination. In order to estimate the extent of wind pollination the observation on siliquae per terminal branch, seeds per siliqua and seed yield were recorded at the time of maturity. The experiment was carried out for two years.

**b.** Experimental rows of CMS line and pollinator were sown in 5F:1M planting ratio in the same plot in three replications. The spacing between CMS lines was 45cm while 90cm gap was kept between pollen parent line and adjacent CMS line to prevent intermixing of branches. Whole experiment was covered with insect proof nylon net before initiation of flowering and to kill minor insects inside net, insecticidal sprays was applied at frequent intervals. Observation on siliquae per terminal branch, seeds per siliqua and seed yield were recorded at the time of maturity The experiment was conducted for two years.

## RESULTS

**Experiment 1:** *Dispersal of air-borne pollen grains at different distances from the source:*

During the year 1996-97 glass slides were kept hanging from 0800 to 1800 hrs after which pollen grains were counted under microscope. The maximum number of pollen grains (27.5) were trapped on glass slides at 5m distance from the experimental field. The number of pollen grains trapped thereafter reduced gradually and the least average number of pollen grains (0.66) were found at 35m distance. At 40m distance no pollen grains was trapped (Table 1.) During the year 1997-98 and 1998-99 trapped pollen grains were counted after every two hours starting from 0800 to 1800 hrs. Every time a fresh slide was kept for trapping the pollen grains. In the morning, the number of pollen grains trapped on glass slide was less (5.24) at 5m and pollens were found only up to 15m from the pollen sources. The maximum number of pollen-grains (14.3) were trapped on slides which were kept hanging from 1000 to 1200 hrs at 5m distance from pollen source. There was gradual decrease with increase in distance and only 1.22 pollens were found at 35m in 1997-98 while there was no pollen at 35m distance in 1998-99. The pollen grains were found absent at 40m distance during all the three years experiments and pollen grains dispersal were also absent during 1600 to 1800 hrs in the year 1998-99 (Table 2).

**Experiments: 2** *Counting of air-borne pollen in different blending ratios:*

The density of pollen grains in air increased with the increase in proportion of pollinator plants. The maximum number of pollen-grains were found in the hundred per cent pollen parent. The lowest density of pollen grains was recorded in 90:10 blending ratio. In case of time of pollen dispersal highest average number of pollen grains was recorded between 1000 to 1200 hrs, followed by 1200-1400 hrs (Table 3).

**Experiment 3:** *Wind pollination estimated by observing yield and yield attributes*

**a.** In this experiment, the data recorded for two years showed that pollens were able to reach up to two rows on both sides from pollinator's i.e. windward and leeward directions. However, per

**Table 1. Dispersal of pollen grains by wind**

| Distance from pollen source (m) | No. of pollen grains/ 25x25mm coverslip/two hours | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1996-1997 | 1997-1998 | | | | | 1998-1999 | | | | | |
| | Total/ day | 0800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | Total/ day | 0800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | Total/ day |
| 5 | 27.50 | 5.24 | 14.30 | 8.40 | 5.74 | 1.2 | 34.88 | 2.4 | 13.0 | 4.5 | 3.00 | - | 22.90 |
| 10 | 17.23 | 4.00 | 11.90 | 7.70 | 5.00 | - | 28.60 | 1.0 | 5.0 | 3.90 | 2.53 | - | 12.43 |
| 15 | 13.20 | 1.50 | 10.00 | 6.30 | 2.23 | - | 20.53 | - | 4.8 | 3.83 | 1.77 | - | 10.40 |
| 20 | 9.66 | - | 9.50 | 5.58 | 1.03 | - | 17.21 | - | 4.5 | 2.73 | 1.00 | - | 8.23 |
| 25 | 7.39 | - | 7.30 | 3.50 | - | - | 10.14 | - | 2.10 | 2.15 | - | - | 4.25 |
| 30 | 3.25 | - | 5.05 | 2.84 | - | - | 8.55 | - | 1.70 | 0.40 | - | - | 2.10 |
| 35 | 0.66 | - | 1.22 | - | - | - | 1.22 | - | - | - | - | - | - |
| 40 | - | - | - | - | - | - | - | - | - | - | - | - | - |

**Table 2. Pollen density (number of pollen grains/coverslip/two hours) in the air inside nets under different blending ratios in the year 1997-98**

| Blending ratio $F_1$: pollinator | Time intervals | | | | |
|---|---|---|---|---|---|
| | 0800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | Mean |
| 90:10 | 0.28 | 2.27 | 1.88 | 1.15 | 1.36 |
| 80:20 | 1.07 | 3.57 | 2.99 | 2.40 | 2.51 |
| 70:30 | 2.43 | 5.80 | 4.83 | 3.23 | 4.08 |
| 60:40 | 3.94 | 6.22 | 6.00 | 5.00 | 5.29 |
| 50:50 | 4.33 | 6.77 | 6.25 | 5.30 | 5.67 |
| 00:100 | 7.00 | 13.17 | 8.00 | 5.67 | 8.46 |
| Mean | 3.18 | 6.28 | 4.99 | 3.79 | |

$F_1$-MH-7 (Pusa Barani CMSxPSR-7) Pollinator-PSR-7(non-restorer pollen parent)

| | | | |
|---|---|---|---|
| CD at 5% level | Blending ratios (B) | : | 1.18 |
| | Time (T) | : | 0.96 |
| | BxT | : | 2.36 |

cent seed bearing siliqua, seeds per siliqua and seed yield per row were considerably more in both the years on rows which-were on leeward direction of pollinators. In general, the data recorded on second row was nearly 50 per cent less than the first row (Table 4).

**b.** The pollen source was sown inside the net with 5:1 (CMS: pollinator) ratio. The distance of 90 cm was maintained between pollinator and first adjacent CMS row. The first row from pollinator on leeward direction had maximum seed bearing siliquae on terminal branch, average number of seeds per siliqua and seed yield per row in both the years of study. The lowest seed bearing siliquae, average number of seed per siliqua and seed yield were recorded in fourth row from pollinator on leeward direction. In fact, pollinator was also planted at 90 cm distance from fifth row, but due to unfavorable wind direction all three attributes were found to be very low as compared to first row (Table 5).

Table 3. Pollen density (number of pollen grains/coverslip/two hours) in the air inside nets under different blending ratios in the year 1998-99

| Blending ratio $F_1$: pollinator | Time intervals | | | | |
|---|---|---|---|---|---|
| | 0800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | Mean |
| 90:10 | 0.00 | 1.13 | 2.10 | 0.00 | 0.81 |
| 80:20 | 0.66 | 2.53 | 3.00 | 1.73 | 1.98 |
| 70:30 | 1.50 | 5.77 | 4.73 | 3.27 | 3.82 |
| 60:40 | 3.77 | 6.05 | 5.97 | 4.43 | 5.06 |
| 50:50 | 4.27 | 7.79 | 6.33 | 5.33 | 5.93 |
| 00:100 | 7.17 | 13.27 | 8.17 | 5.83 | 8.61 |
| Mean | 2.89 | 6.09 | 5.05 | 3.43 | |

$F_1$-MH-7 (Pusa Barani CMSxPSR-7) Pollinator-PSR-7(non-restorer pollen parent)

| | | | |
|---|---|---|---|
| CD at 5% level | Blending ratios (B) | : | 1.08 |
| | Time (T) | : | 0.88 |
| | BxT | : | 2.15 |

Table 4. Pollen dispersal in relation to wind direction by observing seed set

| Row from pollinator | Wind direction | Per cent seed bearing siliquae on terminal branch | | Seeds/siliqua | | Seed yield (g)/ 5m row | |
|---|---|---|---|---|---|---|---|
| | | 1997-98 | 1998-99 | 1997-98 | 1998-99 | 1997-98 | 1998-99 |
| I | ↓ | 10.33 | 12.50 | 3.92 | 4.20 | 32.50 | 35.00 |
| II | | 4.99 | 5.66 | 2.13 | 2.19 | 18.00 | 24.50 |
| III | | - | - | - | - | - | - |
| IV | | - | - | - | - | - | - |
| IV | | - | - | - | - | - | - |
| III | | - | - | - | - | - | - |
| II | | 3.12 | 3.16 | 1.25 | 1.40 | 15.66 | 19.00 |
| I | | 8.20 | 9.00 | 2.50 | 3.33 | 28.33 | 29.70 |

Table 5. Pollen dispersal study by observing yield attributes.

| Row from pollinator | Distance from pollen source (cm) | Per cent seed bearing siliquae on terminal branch | | Seeds/siliqua | | Seed yield (g)/ row (5m) | |
|---|---|---|---|---|---|---|---|
| | | 1997-98 | 1998-99 | 1997-98 | 1998-99 | 1997-98 | 1998-99 |
| Pollen source (one row) | | | | | | | |
| I | 90 | 19.79 | 20.22 | 4.97 | 5.32 | 56.23 | 61.60 |
| II | 135 | 16.22 | 17.90 | 3.58 | 3.97 | 46.66 | 56.63 |
| III | 180 | 12.86 | 13.50 | 2.07 | 2.05 | 43.30 | 43.00 |
| IV | 225 | 8.69 | 9.40 | 0.50 | 0.52 | 31.36 | 34.36 |
| V | 270 | 10.89 | 11.60 | 1.30 | 1.40 | 43.33 | 44.16 |
| Pollen source (one row) | | | | | | | |
| C.D. at (P=0.05) | | 4.53 | 7.04 | 2.11 | 1.70 | 6.51 | 2.30 |

Table 6. Comparative study of open pollination and wind pollination

| Row from pollinator | Distance from pollen source (cm) | Per cent seed bearing siliquae on terminal branch | | Seeds/siliqua | | Seed yield (g)/ row (5m) | |
|---|---|---|---|---|---|---|---|
| | | O.P | W.P | O.P | W.P | O.P | W.P |
| Pollen source (one row) | | | | | | | |
| I | 90 | 96.00 | 19.79 | 15.20 | 4.97 | 406 | 56.23 |
| II | 135 | 94.33 | 16.22 | 14.33 | 3.58 | 395 | 46.66 |
| III | 180 | 93.00 | 12.86 | 13.00 | 2.07 | 390 | 43.30 |
| IV | 225 | 97.00 | 8.69 | 13.50 | 0.50 | 108 | 31.36 |
| V | 270 | 98.50 | 10.89 | 15.00 | 1.30 | 410 | 43.33 |
| Mean | | 95.77 | 13.69 | 19.21 | 2.48 | 401.8 | 44.38 |

O.P.-Open pollination ; W.P.-Wind pollination

## DISCUSSION

Sufficient number of pollen were dispersed up to 30-35m distance from pollen source in the three years of study. As regards time of observations during the day, maximum concentration of pollen (41 to 57 per cent) in the air was at 1000 to 1200 hrs. Timmons *et al.* [7] found pollen grains of rape at a distance of 36m and the density of pollen was 10% of that at the field margin. McCartney and Lacey [8] with 20x20m plot, reported 77% decrease in pollen concentration at 6m down wind and 90% decrease at 10m in oilseed rape. Further, Monasse and Kareiva [9] reported 0.22 per cent out crossing rate in rapeseed at 50m and 0.011 per cent at 100m whereas Scheffler *et al.* [10] reported 0.0033 per cent out crossing at 47m. However, the present study indicated that 40m distance is sufficient to avoid wind pollination in Delhi conditions, Timmons *et al.* [7] observed maximum pollen concentration between 1100 and 1300 hrs. However, in the present study maximum concentration of pollen was recorded between 1000 and 1200hrs, which suggests that there is a relation between time of flower opening and concentration of air-borne pollen. The different density of wind-borne pollen in air may be due to difference in plot size, bee activity, prevailing wind velocity, and environmental conditions such as air humidity and temperature. Pollen grain density in air increased with the increase in proportion of male plants in the field. The average number of pollen grains observed in different blending ratio was less than that found at 5m from the pollen source in open area. This happened because the wind velocity and micro environment in net were different from the open area. This may be the reason for less number of pollen grains under net. However, honeybees were present inside the net, whose activities might have disturbed the pollen to make it air-borne. Williams [11] and Eisikowitch [4] demonstrated that in rape, pollen grains which initially disturbed by insects may become truly air-borne and get dispersed. Langridge and Goodman [12] and Williams [11] reported 48 to 646 pollen grains per cubic meter of air above oilseed rape crop. The amount of pollen grains fluctuated greatly, both with time of the day and with the stage of flowering of the crop. The results revealed that seed bearing sliquae were formed on first and second rows from pollen source even though pollen source was not present inside net. However, pollen parent was present at 1.5m distance from first row of CMS plot but outside the net. Thus, it can be concluded that pollen grains were able to reach on stigmas on CMS plants through insect proof net up to two rows (1.95 m) on both sides and are able to set seeds. In this experiment the CMS plants in the third and fourth rows (2.40m and 2.85m) from pollen source, where pollen grains were not able to reach, showed continuous flowerings and multistigmas in their flowers (Fig. 1). These flowers were pollinated by hand and seeds were collected to find out whether this character is inherited due to mutation or it is an adaptive measure created against the unfavorable environment. In the progeny this character disappeared and plants had normal flowers. Thus, it may be concluded that in nature plants develop such temporary characters for survival. In response to non-availability of pollen for pollination, CMS plants developed multistigma to increase unit area to capture pollen grains, if any, in air. Where pollen source was provided inside the insect proof net with 5:1 planting ratio, seed set was found in all five rows which clearly indicated the effect of wind pollination. All the yield components were comparatively more in first and second rows than that obtained from the experiment where pollen source was kept outside the net. That may be due to the hindrance created by the net in flow of pollen but could not prevent it completely. A comparison between open pollination and wind pollination has been made and presented in Table 6. Average

Multistigmas in a flower

number of seeds bearing siliquae on terminal branch, seeds per siliqua and seed yield were 14.29%, 17.45% and 10.99% respectively in wind pollination to that of open pollination in the CMS line of mustard. Jenkinson and Glynne Jones [13] found that rape plants kept in relatively still air of green house produced only a third to half as much seed as in normal plant kept outside. They concluded that wind or insects are needed to obtain high seed set in self fertile plants. Mesquida and Renard [14] reported that in cages, without insect pollinators, male sterile plants adjacent to male fertile row had 26 per cent seed set of those equivalent plants in open pollination. The present study indicated that about 11 to 17.5 % pollination may take place due to wind. In want of pollen sterile plants developed different adaptive characters like increased length of terminal branch, more number of flowers, termination of flowering delayed, stigma remained receptive for longer period and interestingly, the multistigma habit to capture pollen, if available, in air.

## REFERENCES


1. BECKER, H.C., C. DAMGAARD & B. KARLSON (1992). Environmental variation for out crossing rate in rapeseed. In: *Proc. 6th Inter. Rapeseed Conf. Poznan.* **5**: 1454-1459.
2. PEARSON, B. (1956). Obsevationner rorande blommornas biology hos raps. *Statens vaxttskyddsanstalt Meddelande,* **66**: 1-67.
3. OLSSON, G. (1955). Investigation of degree of cross pollination in white mustard and rape *Sver, Utsaedesfoeren Tidskr,* **62**: 311-322.
4. EISIKOWITCH, O. (1981). Some aspects of pollination of oilseed rape (*Brassica napus* L.). *Agric. Sci.(Camb).,* **96**: 321-326.
5. LABANA, K. S. & S.S. BANGA (1984). Floral biology in Indian mustard [*Brassica juncea* (L.) Coss.]. *Genetica Agraria,* **38**: 131-138.
6. WILLIAMS, I.H. (1978). The pollination requirements of swede rape (*Brassica napus* L.) and of tunip rape (*Brassica campestris* L). *J.Agric. Sci. (Camb).,* **91**: 343-348.
7. TIMMONSAM., E.T.O.BRIEN, Y.M. CHARTERS, S.J. DUBBLES, & J. WILKINSON (1995), Assessing the risk of wind pollination from fields of genetically modified *Brassica napus* (oleifera). *Euphytica,* **85**: 417-423.
8. Mc CARTNEY, H.A. & M.E.LACEY (1991). Wind dispersal of pollen from crops of oilseed rape (*Brassica napus* L.). *J. Aerosol Sci.,* **22**(4): 467-477.
9. MONASSE, R. & P. KAREIVA (1991). Quantifying the spread of recombinant-genes and organisms. In : *Assessing Ecological Risk of Biotecnology, (ed)* I. Ginzburgh, pp. 215-231.
10. SCHEFFER, J.A., R. PARKINSON. & P.J.DALE (1993). Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Trans. Res.,* **2**: 356-364.
11. WILLIAMS, I. H. (1984). The concentrations of air-borne rape pollen over a crop of oilseed rape (*Brassica napus* L.). *J. Agric. Sci.,* **103**: 353-357.
12. LANGRIDGE, D. E. & R.D. GOODMAN (1982). Honeybee pollination of oilseed rape, cultivar Midas. *Aust. J. Exp. Agric. Anim. Husb.,* **22**: 124-126.
13. JENKINSON, J.P. & G.P. GLYNNE-JONES (1953). Observations on the pollination of oil rape and broccoli. *Bee Wld.,* **34**: 173-177.
14. MESQUIDA, J. & M.RENARD (1978). Entomophilous pollination of male-sterile strains of winter rapeseed (*B. napus* L. Metzager var. olifera) and preliminary study of alternating device. In: Proc. 4th Int. Symp. Poll. Maryland Agricultural Experimental Station, Miscellaneous publication. **1**: 49-57.