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Dependency of Crops on Pollinators and Pollination Deficits: An Approach to Measurement Considering the Influence of Various Reproductive Traits

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Abstract: Pollinators provide crucial ecosystem services, i.e., pollination, which determines crops' reproductive fitness and yield. As pollinators decline, flowering crops might face pollination-deficit stress depending on their dependence on pollinators and pollinator availability. Here, we assessed the dependency of some crops (belonging to diverse plant families) on biotic pollinators based on their maximum reproductive potential in a supplementary pollination treatment and minimum reproductive success in a pollinator exclusion treatment. Additionally, we determined the pollen transfer limitation of the crops in open field conditions. We also determined the influence of the different reproductive traits with the index of dependency of crops on pollinators (*IDP*) and the coefficient of pollination deficit (*D*). Based on the values of *IDP*, members of Cucurbitaceae are obligatorily dependent on pollinators for their fruit set. Members of Brassicaceae and Rutaceae are highly reliant on pollinators. A few crops, like *Lablab purpureus* and *Nigella sativa*, are less dependent on pollinators. In open field conditions, most crops have a low pollination deficit, some without pollen transfer limitations, and only a few crops (*Citrus × limon* and *Citrus maxima*) show a higher pollination deficit. The *IDP* is negatively influenced by the pollen–ovule ratio, which also negatively affects the pollination deficit of the crops. This study will be useful in understanding and mitigating the effects of pollinator losses, as well as in choosing crops (those under pollination deficit stress and largely dependent on pollinators for fruit set) for supplemental pollination services to increase agricultural production.

Keywords: crop; dependency on pollinators; fruit set; pollen–ovule ratio; pollination deficit

1. Introduction

Pollination is a vital ecosystem service required for the reproductive success (fruit and seed sets) of flowering plants, including crops. Over 80% of the world's primary food crops rely to some extent on animal-mediated pollination in agricultural systems, accounting for 5–8% of worldwide output volumes [1,2]. According to Rodger et al. [3], in the absence of animal-mediated pollination, one-third of flowering plant species would produce no seeds, while the other half, including many crops, may have a reduced seed set of 80% or more.

The availability of pollinators, which influences flower-visiting frequency, can be a decisive selection factor in plant mating systems [4,5]. The fitness obtained from autonomous selfing is thought to compensate for the costs of inbreeding depression in conditions when producing selfed seed is preferable to having no seed at all, that is, the 'reproductive assurance' concept [6–8]. A decline in pollinator richness and abundance, which leads to higher pollination constraints [9], may facilitate transitions from outcrossing to autonomous selfing, despite inbreeding costs [6,10,11].

The world's pollinators are vanishing for a host of reasons, including loss of habitat, use of pesticides and climate change [12–15]. As a consequence, there may be a risk of pollination deficits compromising crop yield and quality [16]. However, demand for crops that rely on biotic pollinators is increasing on a global scale [17]. We are becoming more aware of pollinators' essential functions in the global food supply, particularly for nutritionally important crops [18]. However, pollination deficits are species- and region-specific [19–21]. For example, Holland et al. [22] found pollination deficiencies in sunflowers and oilseed rape but not in pears or pumpkins. Considering regional variation, blueberry pollination deficits were discovered in Michigan, Oregon and British Columbia, but not in Florida [21]. Therefore, it is important to investigate pollinators and pollination for particular crops or varieties in their agroecological context. It is imperative for future sustainable food production to distinguish between supply and demand for this crucial ecosystem service.

Sustainable crop production depends on approaches that help predict a crop's pollinator dependence and potential risks of yield losses arising from pollination shortfalls. Unfortunately, there are few studies [23–26] on the dependency of crops on pollinators and the pollination deficit of crops. It is also unknown whether the different floral traits have significant relationships with a crop's dependency and pollination deficits. Therefore, the present study was designed to assess the dependency of crops on pollinators and the pollination deficits of crops; in addition, we estimated some reproductive traits of the crops. We set out to answer the following research questions: (1) How dependent are crops on biotic pollinators? (2) How do crops face pollination deficit stress? (3) Do reproductive traits influence the dependency of crops on pollinators and pollination deficits?

2. Materials and Methods

2.1. Experimental Site

The study was conducted in open fields of Bankura, Birbhum and Hooghly districts, West Bengal, India (Figure 1). The selected regions are in rural habitats with prevailing agricultural activities. The major crops grown here are cereals (e.g., *Oryza sativa* L., *Triticum aestivum* L.), oilseeds (*Brassica juncea* (L.) Czern. and *Sesamum indicum* L.), pulses (*Cicer arietinum* L. and *Pisum sativum* L.) and other vegetables (*Brassica oleracea* L., *Capsicum annuum* L., *Raphanus sativus* (L.) Domin, *Solanum lycopersicum* L., *Solanum melongena* L., and *Solanum tuberosum* L.), which occupy a significant portion of the total cropped area. Several fruit plants (*Mangifera indica* L., *Syzygium* spp., and *Citrus* spp.) are also frequently found within the study areas.
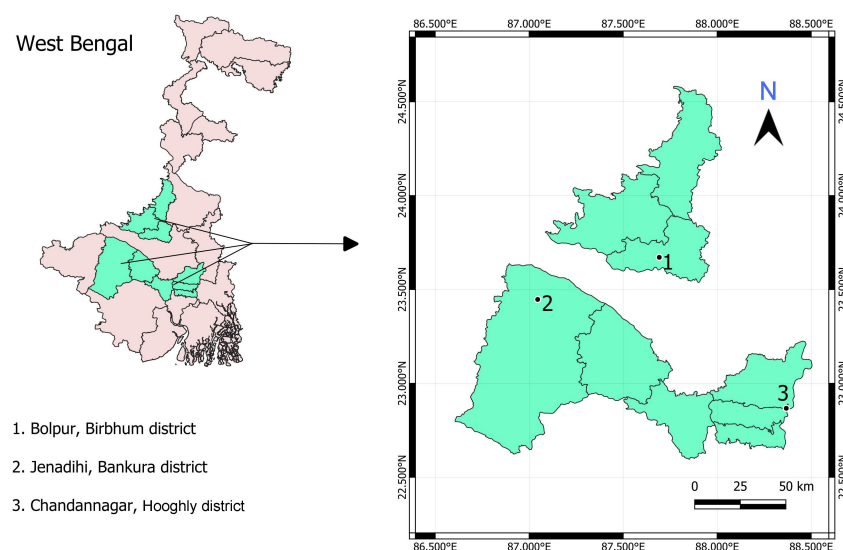

Figure 1. Map showing the study areas (black circles).

2.2. Selected Crops

We selected 17 crop species from seven plant families (Supplementary Table S1) for the present study. Our selection was based on the availability within the study regions and the suitability of data collection. We did not include cereals (as members of Poaceae are anemophilous) in our study. The selected crops (including beans, oilseed, spices and other fruits and vegetables) have diverse floral traits, including floral architectures.

2.3. Dependency of Crops on Pollinators and Pollination Deficit

To determine the dependency of flowering crops on pollinators and pollination deficit, we estimated fruit sets in three pollination treatments: (1) Open pollination: used as a control to test the pollination success in nature provided by native pollinators; (2) Pollinator exclusion: reproductive success in the absence of a biotic pollinator; (3) Supplementary pollination (i.e., in addition to routine pollination services provided by native pollinators, we also manually transferred pollen without bagging): to estimate the maximum potential of reproductive success of the crop. We chose 100 mature flower buds for each pollination treatment upon ten sampling days (10 flower buds per sampling day). Individual flower buds were recognized by marking their pedicel or scribbling a black ink dot on the calyx, and inflorescences were labeled. For the pollinator exclusion treatment, all selected buds were bagged with a nylon net until the senescence of the flower or the set of fruit. We counted the number of fruits for each treatment and then estimated the fruit set percentage. Then, we calculated the index of dependency of flowering plants (here crops) on pollinators (*IDP*) as follows:

$$IDP = 1 - \frac{Re}{Rs},$$

where *Re* is the reproductive success (fruit set or seed set, here we considered the percentage of fruit set) in the pollinator exclusion treatment, and *Rs* means reproductive success in supplementary pollination. The value of *IDP* ranges from 0 to 1, and a higher value indicates a higher dependency of the plant on pollinators. Based on the *IDP* values, we classified the crops into five categories (Table 1).

Table 1. Categories of plants based on the index of dependency of the flowering plants on pollinators (*IDP*) and the coefficient of pollination deficit (*D*).

Value of *IDP*	Category
>0.9–1	Obligatory dependence on biotic pollinators
0.6–0.9	Highly dependent on biotic pollinators
>0.3–<0.6	Moderately dependent on biotic pollinators
0.1–0.3	Low dependence on biotic pollinators
<0.1	Negligible dependence on biotic pollinators

Value of *D*	Category
>0.5	High pollination deficit
0.3–0.5	Medium pollination deficit
$0.3 > D \geq 0.1$	Low pollination deficit
<0.1	Negligible pollination deficit

To determine whether crop species experience pollen transfer constraints in open field circumstances, we estimated the 'coefficient of pollination deficit (*D*)' using the method of Layek et al. [26] as follows:

$$D = 1 - \frac{Ro}{Rs},$$

where *Ro* denotes reproductive success (here, percentages of fruit set) in open pollination. The *D* has a value between 0 and 1. Based on the *D* values, we classified the crops into four categories (Table 1).

2.4. Data Collection about Reproductive Traits

We recorded several reproductive traits like the longevity of the flowers, pollen production per flower, ovule number, pollen–ovule ratio, pollen viability, stigma receptivity (time duration) and number of visits received by an individual flower throughout its lifespan. To record flower longevity, mature flower buds were marked with black ink dots on their calyx ($n = 10$ for each crop). We noted the date and time of flower opening and senescence (in the case of legumes, we considered the time of closing of petals as determining its longevity). In the case of cucurbits, we recorded flower longevity of female flowers. We extracted the anthers from a mature flower bud ($n = 10$ flower buds for each crop) and placed them in a vial to count the pollen grains per flower. Then, we added 1–5 mL of 0.4 M sucrose solution and crushed the anthers with a glass rod. The solution was then filtrated (using a nylon net) to remove the debris. After that, we added 50 µL to 2 mL of *Lycopodium* solution (standardized with 756 spores per 10 µL) and shook it to homogenize. We took 10 µL of the solution on a glass slide and counted the spores and pollen grains of *Lycopodium* and the targeted crops ($\geq$100 pollen grains or spores) in the microscopic field. Then, we calculated the total pollen production of the flower (*Pt*) as follows:

$$Pt = \frac{Pc \times Slt}{Sl},$$

where *Pt* is the total amount of pollen produced by the flower, *Pc* is the pollen count of the crop species, *Sl* is the spore count of *Lycopodium* and *Slt* is the number of spores of *Lycopodium* which were added to the solution.

The number of ovules per flower was counted by rupturing the ovary wall and observed using a hand lens. Then, the pollen–ovule ratio was calculated. Pollen viability was tested using a staining method at the flower's opening time. For staining purposes, we used an aqueous solution of 1% TTC, i.e., 2,3,5-triphenyl tetrazolium chloride [27]. On a glass slide, a drop of this solution was placed. Then, we added pollen grains to this solution and roofed it with a coverslip. We examined the pollen grains under a light microscope after 2 h of incubation. Pollen grains stained orange or bright red were considered viable. The H_2O_2 test was used to assess stigma receptivity [28]. For short-lived flowers, we tested receptivity at 2 h intervals; for long-lasting flowers, we conducted the test at 6 or 12 h intervals.

To record the number of visits received by a flower, we marked freshly opened flowers ($n = 10$ for each crop). On a 10 min observation (covering different times throughout the flower's lifespan), we counted the number of visits received by the flower. Then, we estimated the total number of visits received by the flowers as follows:

$$Vt = Va \times 6 \times Fl,$$

where *Vt* is the estimated total number of visits received by a flower throughout its lifespan, *Va* is the average number of visits received by the flower in a 10 min observation period and *Fl* is the flower's longevity (in hours).

2.5. Statistical Analyses

To obtain the mean and standard deviation, descriptive data analyses were performed. We used the 'Shapiro–Wilk' tests to determine whether the data were normally distributed. Fruit set data of different crops (heterogeneous data) were analyzed using a non-parametric Kruskal–Wallis test. Some data on floral traits (flower longevity, pollen viability and stigma receptivity) were analyzed using the parametric test 'One-way ANOVA', and $p \leq 0.05$ was considered statistically significant. If the obtained *p*-value was significant, we used Duncan's multiple range test for post-hoc comparisons. To estimate the relationship between various reproductive traits with the index of dependency of crop plants on pollinators (*IDP*) and the coefficient of pollination deficit (*D*) of crops, we followed the correlation (Karl Pearson's) method. For factor analysis, we checked sampling adequacy (using the

Kaiser–Mayer–Olkin test) and sphericity using Bartlett's test. On satisfying, we carried out structural equation modeling (SEM) to resolve the impacts of different reproductive traits (treated as manifest variables) on the *IDP* and *D*. SEM was conducted using IBM AMOS ver. 21. Other statistical analyses were performed using SPSS (ver. 25.0) statistical packages.

3. Results

3.1. Dependency of Crops on Pollinators and Pollination Deficit

Fruit set percentages significantly differed among the three pollination treatments, i.e., open pollination, pollinator exclusion and supplementary pollination, except *Lablab purpureus* (Kruskal–Wallis $H = 5.279$, $df = 2$, $p = 0.071$). The fruit set percentage remained higher in the supplementary pollination treatments than in the other two treatments (Supplementary Table S2). Pollinator exclusion significantly reduced the fruit set. In monoecious cucurbits, fruit sets did not take place in the absence of biotic pollinators.

Based on the index of dependency of flowering plants on pollinator (*IDP*) values, cucurbitaceous crops showed obligatory dependence on biotic pollinators for their fruit set (*IDP* = 1). Fruit plants of Rutaceae (*Citrus* spp.) and members of Brassicaceae also showed higher dependency on pollinators (Table 2). Some crops like *Cajanus cajan*, *Cicer arietinum*, *Foeniculum vulgare*, *Pisum sativum*, *Solanum lycopersicum* and *Solanum melongena* showed moderate dependency on pollinators. Only a few crops (e.g., *Lablab purpureus* and *Nigella sativa*) showed little dependence on biotic pollinators for their fruit set.

Table 2. Index of dependency of flowering plants (here crops) on pollinators (*IDP*) and coefficient of pollination deficit (*D*) of crops.

Crop Species	*IDP*	*D*
Foeniculum vulgare Mill.	0.47	0.19
Brassica juncea (L.) Czern.	0.67	0.09
Raphanus sativus (L.) Domin	0.70	0.11
Cucumis sativus L.	1	0.17
Cucurbita maxima Duchesne	1	0.11
Luffa aegyptiaca Mill.	1	0.11
Momordica charantia L.	1	0.12
Cajanas cajan L. Millsp.	0.55	0.21
Cicer arietinum L.	0.45	0.12
Lablab purpureus (L.) Sweet	0.11	0.06
Pisum sativum L.	0.53	0.06
Nigella sativa L.	0.18	0.06
Citrus × aurantiifolia (Christm.) Swingle	0.74	0.15
Citrus × limon L. Osbeck	0.81	0.59
Citrus maxima (Burm.) Merr.	0.57	0.28
Solanum lycopersicum L.	0.47	0.13
Solanum melongena L.	0.48	0.14

Among the selected crops, *Citrus × limon* and *Citrus maxima* showed higher pollen transfer limitations (the values of the coefficient of pollination deficit *D* were 0.59 and 0.28 for *Citrus × limon* and *Citrus maxima*, respectively) in open conditions (Table 2). The majority of the selected crops (e.g., *Cajanus cajan*, *Cicer arietinum*, *Citrus × aurantiifolia*, *Cucumis sativus*, *Cucurbita maxima*, *Foeniculum vulgare*, *Luffa aegyptiaca*, *Momordica charantia*, *Raphanus sativus*, *Solanum lycopersicum* and *Solanum melongena*) showed only a slight pollination deficit. A few crops (e.g., *Brassica juncea*, *Lablab purpureus*, *Pisum sativum* and *Nigella sativa*) did not show significant pollen transfer limitations.

3.2. Reproductive Traits of the Crops

The different reproductive traits significantly varied among the studied crops (flower longevity: $F_{16, 153} = 121.43$, $p < 0.001$; pollen production: $F_{16, 153} = 315.54$, $p < 0.001$; ovule

number: $F_{16, 313} = 1814.12$, $p < 0.001$; pollen viability: $F_{16, 153} = 13.43$, $p < 0.001$; stigma receptivity: $F_{16, 153} = 149.20$, $p < 0.001$; visits received: $F_{16, 153} = 72.49$, $p < 0.001$). Flower longevity remains higher in Rutaceae fruit plants, moderate for Brassicaceae members and lower for cucurbitaceous crops (Table 3). Pollen yield and the number of ovules were higher for the members of Cucurbitaceae and Solanaceae. A smaller number of ovules were found in members of Apiaceae and Fabaceae. The pollen–ovule ratios were higher in *Cajanus cajan* (6135.76:1), *Solanum lycopersicum* (6087.05:1), *Foeniculum vulgare* (4891.96:1), *Pisum sativum* (4786.58:1) and *Raphanus sativus* (4324.43:1). The ratios were low for the members of Cucurbitaceae and Rutaceae. The percentage of viable pollen was higher in *Brassica juncea* (89.28 ± 4.03), *Raphanus sativus* (86.69 ± 4.46), *Lablab purpureus* (86.55 ± 6.31), *Citrus × limon* (85.18 ± 5.19) and *Foeniculum vulgare* (85.63 ± 2.80), whereas lower percentages of viability were obtained in *Momordica charantia* (69.93 ± 3.95), *Cicer arietinum* (71.21 ± 3.38) and *Nigella sativa* (71.31 ± 6.17). Longer periods of stigmatic receptivity were obtained for the members of Rutaceae (*Citrus × aurantiifolia*: 86.40 ± 12.39; *Citrus × limon*: 82.80 ± 8.85; *Citrus maxima*: 66 ± 6.32) and Apiaceae (*Foeniculum vulgare*: 64.80 ± 8.39). Shorter times of stigmatic receptivity were obtained in *Cucurbita maxima* (6.60 ± 0.97), *Cucumis sativus* (7.20 ± 1.03), *Luffa aegyptiaca* (9.20 ± 1.03) and *Nigella sativa* (9.60 ± 1.58). Stigmatic receptivity (time span) was positively correlated with flower longevity (r = 0.662, $p < 0.01$, n = 17). The number of visits received by a flower throughout its lifespan were also positively correlated with the flower's longevity (r = 0.809, $p < 0.001$, n = 17). On average, a higher number of visits per flower were seen in *Foeniculum vulgare* (223.26 ± 41.05 visits/flower), *Brassica juncea* (183.32 ± 41.50 visits/flower), *Raphanus sativus* (172.97 ± 36.28 visits/flower), *Nigella sativa* (172.80 ± 48.44 visits/flower), *Citrus × aurantiifolia* (153.96 ± 25.53 visits/flower) and *Solanum melongena* (141.47 ± 48.44 visits/flower). Lower visitation was seen in *Solanum lycopersicum* (17.39 ± 9.29 visits/flower), *Momordica charantia* (22.38 ± 6.75 visits/flower), *Cajanus cajan* (24.20 ± 8.82 visits/flower), *Cicer arietinum* (38.56 ± 11.36 visits/flower), *Lablab purpureus* (43.34 ± 11.88 visits/flower) and *Pisum sativum* (47.19 ± 11.32 visits/flower).

Table 3. Some reproductive traits of the crops.

Crops	Flower Longevity (h)	Pollen/Flower	Ovule/Flower	Pollen–Ovule Ratio	Pollen Viability	Stigma Receptivity Time (h)	Visits/Flower
Foeniculum vulgare	68.40 [d] ± 11.38	9783.92 [e] ± 1307.57	2.00 [g] ± 0	4891.96	85.63 [ab] ± 2.80	64.80 [b] ± 8.39	223.26 [a] ± 41.05
Brassica juncea	49.20 [f] ± 14.37	36,612.88 [de] ± 5952.24	20.20 [g] ± 0.95	1812.52	89.28 [a] ± 4.03	43.20 [c] ± 10.12	183.32 [b] ± 41.50
Raphanus sativus	52.80 [ef] ± 11.59	32,433.25 [de] ± 3554.95	7.50 [g] ± 0.89	4324.43	86.69 [ab] ± 4.46	44.40 [c] ± 8.10	172.97 [bc] ± 36.28
Cucumis sativus	7.80 [h] ± 1.48	7511.91 [e] ± 1026.68	79.85 [def] ± 11.59	94.08	75.53 [efg] ± 6.88	7.20 [g] ± 1.03	34.73 [gh] ± 17.10
Cucurbita maxima	7.20 [h] ± 1.40	112,829.10 [c] ± 17,942.11	248.70 [b] ± 35.83	453.68	88.49 [a] ± 5.98	6.60 [g] ± 0.97	38.88 [gh] ± 16.22
Luffa aegyptiaca	9.60 [h] ± 1.58	28,530.46 [de] ± 6572.95	176 [c] ± 28.43	162.10	77.72 [def] ± 5.39	9.20 [fg] ± 1.03	44.87 [g] ± 18.35
Momordica charantia	11.20 [h] ± 1.93	17,485.52 [e] ± 2817.90	22.05 [g] ± 3.02	792.99	69.93 [h] ± 3.95	10.80 [fg] ± 1.40	22.38 [gh] ± 6.75
Cajanas cajan	7.80 [h] ± 1.48	30,371.99 [de] ± 4500.18	4.95 [g] ± 0.83	6135.76	82.54 [bcd] ± 5.41	21.60 [e] ± 7.59	24.20 [gh] ± 8.82
Cicer arietinum	15.60 [h] ± 5.80	5835.61 [e] ± 842.80	2.35 [g] ± 0.49	2483.24	71.21 [gh] ± 3.38	14.40 [f] ± 5.06	38.56 [gh] ± 11.36
Lablab purpureus	33.60 [g] ± 8.58	28,419.86 [de] ± 5278.89	8.30 [g] ± 1.03	3424.08	86.55 [ab] ± 6.31	27.60 [e] ± 5.80	43.34 [gh] ± 11.88
Pisum sativum	34.80 [g] ± 8.39	34,224.06 [de] ± 5424.89	7.15 [g] ± 0.81	4786.58	84.74 [abc] ± 6.19	34.80 [d] ± 6.81	47.19 [g] ± 11.32
Nigella sativa	115.20 [a] ± 18.07	55,420.15 [d] ± 6804.97	96.25 [de] ± 13.57	575.79	71.31 [gh] ± 6.17	9.60 [fg] ± 1.58	172.80 [bc] ± 48.44
Citrus × aurantiifolia	97.20 [b] ± 14.37	7536.38 [e] ± 673.64	27.40 [g] ± 5.70	275.05	74.06 [fgh] ± 6.01	86.40 [a] ± 12.39	153.96 [cd] ± 25.53
Citrus × limon	79.20 [c] ± 11.59	9090.86 [e] ± 918.03	70.25 [ef] ± 9.50	129.41	85.18 [ab] ± 5.19	82.80 [a] ± 8.85	124.22 [e] ± 23.07
Citrus maxima	61.20 [de] ± 8.85	10,805.85 [e] ± 2996.74	104.25 [d] ± 13.18	103.65	84.37 [abc] ± 5.36	66 [b] ± 6.32	84.37 [f] ± 5.36
Solanum lycopersicum	13.80 [h] ± 1.75	345,440.30 [b] ± 81,326.76	56.75 [f] ± 9.04	6087.05	81.93 [bcd] ± 7.23	11 [fg] ± 1.41	17.39 [h] ± 9.29
Solanum melongena	58.80 [e] ± 14.37	681,055.60 [a] ± 95,092.40	2180.60 [a] ± 219.56	312.32	79.45 [cde] ± 6.92	45.60 [c] ± 12.39	141.47 [de] ± 38.01

Values given as mean ± standard deviation. Means in the column followed by same superscript letters do not differ significantly by DMRT at 5%.

3.3. Influence of Reproductive Traits on a Crop's Dependency on Pollinators and Pollination Deficit

The value of the correlation coefficient (r) showed that the crop's dependency on pollinators (*IDP*) was negatively correlated with flower longevity, pollen yield, ovule number, pollen–ovule ratio, pollen viability, stigma receptivity and the number of visits received by an individual flower (Table 4). The number of ovules, periods of stigma receptivity and pollen viability had no significant correlation with *IDP* (ovule number:

r = −0.082, *p* = 0.755; pollen viability: r = −0.070, *p* = 0.789; stigma receptivity: r = −0.071, *p* = 0.785; *n* = 17 for each trait). Some other traits (e.g., flower longevity, pollen yield and number of visits received by a flower) have a low negative correlation with the *IDP*. The pollen–ovule ratio has a medium negative correlation with the dependency of crops on pollinators (r = −0.43, *n* =17).

Table 4. Correlation of different reproductive traits with the index of dependency of flowering crop plants on pollinators (*IDP*) and coefficient of pollination deficit (*D*).

Reproductive Traits	Value of Correlation Coefficient (r)	
	IDP	*D*
Flower longevity	−0.398	0.227
Pollen/flower	−0.185	−0.119
Ovule/flower	−0.082	−0.035
Pollen–ovule ratio	−0.430	−0.253
Pollen viability	−0.070	0.139
Stigma receptivity time	−0.071	0.562
Numberof visits received by a flower	−0.234	0.101

The coefficient of pollination deficit (*D*) has a moderate correlation with stigma receptivity (r = 0.562, *p* < 0.05, *n* = 17) and a lower correlation with flower longevity (r = 0.227, *n* = 17) and the pollen–ovule ratio (r = −0.253, *n* = 17). The remaining studied traits (e.g., pollen and ovule yield, pollen viability and number of visits received by an individual flower) did not significantly correlate with the crop's pollination deficiency (Table 4).

When we draw a model through the SEM, connecting the different reproductive traits with the index of dependency of flowering crops on pollinators (*IDP*), the pollination deficit (*D*) and the covariance between traits, the generated model (Figure 2) is moderately fitted (CMIN/DF: 2.786, CFI: 0.481, GFI: 0.705, TLI: 0.017, RMSEA: 0.334).
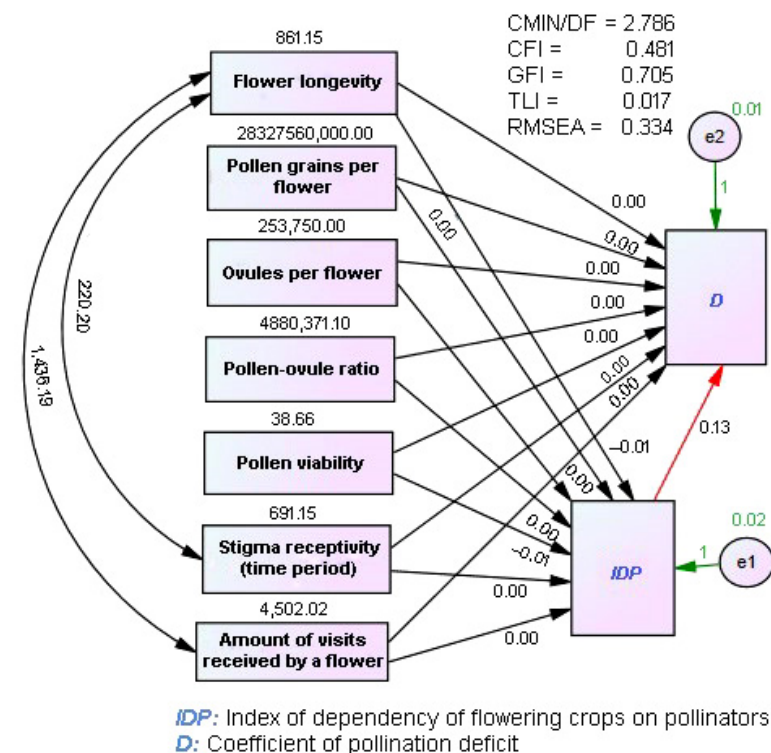

IDP: Index of dependency of flowering crops on pollinators
D: Coefficient of pollination deficit

Figure 2. The relationship of reproductive traits with the index of dependency of flowering crops on pollinators (*IDP*) and pollination deficit (*D*) in a model (path diagram).

4. Discussion

Over the last few decades, the world's reliance on pollinator-dependent crops has risen [29]. However, both wild and managed pollinators have declined in many areas [12,30], raising concerns that pollinator limitation could jeopardize yield stability and food security [2,17]. In this sense, in our present study we estimated an index of dependency of flowering crops on pollinators (*IDP*) and the actual pollination deficit (*D*). Based on the obtained values of the *IDP*, most crops (non-cereal crops) depend on biotic pollinators for their fruit set. Several researchers [1,21,29] have also reported that agricultural production depends to varying extents on pollinators for fruit and seed production. The Cucurbitaceae crops obligatorily depend on pollinators for fruit set due to their unisexual flowers. Other crops having bisexual flowers are also partially dependent on biotic pollinators. In addition to the total mass of production, the nutritional contribution (in terms of proteins, vitamins and minerals content) of many animal-pollinated crops may be an important factor in human diets [31–33]. To support this point, Gallai et al. [34] reported that the value of a ton of pollinator-dependent crops was about five times larger than that of non-dependent crops. Ongoing pollinator reductions might exacerbate current challenges in supplying a nutritionally healthy diet for the world's human population [32].

Considering the value of the coefficient of pollination deficit (*D*) of the crops, both obligatorily and partially dependent crops showed pollen transfer limitations in open field conditions. However, there is no strong relation between a crop's dependency on pollinators and the crop's pollination deficit, as obligatorily dependent Cucurbitaceae crops showed small pollination deficits. The pollination deficit varies according to crop species and geographical regions, mainly depending on the abundance of effective pollinators and the number of visits received by the flowers. In our experiments, some crops (e.g., *Brassica juncea*, *Nigella sativa* and *Pisum sativum*) did not show pollen transfer limitations, although they depend on pollinators for fruit set.

The various reproductive traits (e.g., flower longevity, pollen production, ovule number, pollen–ovule ratio, pollen viability, stigma receptivity time span and number of visits received by an individual flower) varied among the crop species. Ranunculaceae spices and Rutaceae fruit crops have long-lived flowers. The longer lifespan of flowers was also previously reported for different species of Ranunculaceae [35] and Rutaceae [25]. Long-lived flowers have a greater opportunity to interact with pollinators. Furthermore, the longer lifespan of an individual flower enhances the floral display size and reproductive fitness of a plant species. For that reason, it may expected that they do not face any pollination deficit, as our results in the case of Ranunculaceae indicate. Pollen production and ovules per flower were higher in members of Solanaceae and *Cucurbita maxima*. The pollen–ovule ratio was higher in Fabaceae members, *Raphanus sativus* and *Solanum lycopersicum*. According to Cruden [36], the pollen–ovule ratio is a good indicator of a plant's reproductive system. However, it has been proposed that the association between the pollen–ovule ratio and the breeding system is only approximate [37,38] and reflects the relative allocation of resources to male–female functions [39,40]. Pollen viability varied among the crops, even within a plant family. For example, more viable pollen was obtained in *Cucurbita maxima* and less viable in *Momordica charantia*. Pollen viability is primarily determined by cytogenetics rather than pollen morphologies, including pollen size. However, during pollen presentation (i.e., pollination), environmental conditions strongly influence pollen viability [41]. Pollen viability includes multiple aspects of pollen performance, including germinability and fertilization ability [42]. Stigma receptivity also varied, with a more significant period in long-lived flowers of Rutaceae fruit crops. The activity of enzymes, such as peroxidase, esterase and dehydrogenase, influences stigma receptivity [43,44]. These enzymes are important in pollen grain germination, pollen tube penetration in the stigma, and, most likely, incompatibility responses [45,46]. Thus, stigma receptivity directly affects the reproductive success of plants. The number of visits received by a flower in its lifespan highly varied among crops. In general, long-lived flowers (e.g., *Citrus* spp., *Foeniculum vulgare*, *Solanum melongena*, etc.) received more visits. However, this also depends on the

abundance of floral visitors and their foraging behavior, which is again influenced by other floral traits (like floral rewards, color and floral volatile organic compounds) and also by abiotic factors [47–49].

We conclude that different reproductive traits can be determinants of the crop's dependency on pollinators and have a link with pollen transfer limitations. The flower longevity and the pollen–ovule ratio have a moderate negative correlation with the *IDP*. The flower's longevity directly influences the number of visits a flower receives during its lifespan. Stigma receptivity (time span) has an unexpected positive correlation with the pollination deficit (*D*). The possible explanation for the pattern we observed is that long-lived flowers (especially Rutaceae fruit crops) have a longer time span of stigmatic receptivity, and some are highly dependent on biotic pollinators; non-optimum pollinator activity can result in significant pollination deficits. Thus, pollination success is determined by multiple factors, like the density of pollinators in the area, their foraging behavior (e.g., foraging rate, legitimate and illegitimate visits, floral constancy, etc.), the plant species' self- or cross-compatibility and the number of receptive flowers per plant.

5. Conclusions

We measured an index of dependency of flowering crop plants on pollinators (*IDP*) and the coefficient of pollination deficit (*D*) for some crops. We also addressed the influence of several reproductive traits on *IDP* and *D*. Members of Cucurbitaceae (having unisexual flowers) are obligatorily dependent (*IDP* = 1) on biotic pollinators for their reproductive success. Members of Brassicaceae and Rutaceae are highly reliant on biotic pollinators (*IDP* values range from 0.67 to 0.85). A few crops, like *Lablab purpureus* and *Nigella sativa* are less dependent on pollinators. In open field conditions, most crops have low pollination deficits, some (e.g., *Brassica juncea*, *Lablab purpureus*, *Pisum sativum* and *Nigella sativa*) were without pollen transfer limitations, and only a few crops (*Citrus × limon* and *Citrus maxima*) showed higher pollination deficits. The *IDP* is negatively influenced by the pollen–ovule ratio, which also negatively affects the pollination deficit of the crops. The current study will be helpful in understanding and mitigating the consequences of pollinator declines. It also helps to select crops (those highly dependent on biotic pollinators for their fruit set and demand for additional pollination services) for providing supplementary pollination services and promoting pollinator conservation measures to enhance crop yields.

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