

1 Optimum time for hand pollination in yam (*Dioscorea* spp.)

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14 Abstract

15 Hand pollination success rate is low in yam (*Dioscorea* spp.), due partly to suboptimal weather
16 conditions. Thus, determining the most suitable time for pollination could improve the pollination
17 success in yam breeding programs. We performed continuous hand pollination within flowering
18 windows of *D. rotundata* and *D. alata* for two consecutive years to determine the most appropriate
19 month, week, and hours of the day allowing maximum pollination success. In *D. alata* crossing
20 block, we observed significant differences among crossing hours for pollination success ($p =$
21 0.003); morning hours (8–12 a.m.) being more conducive than afternoons (12–5 p.m.). No
22 significant differences existed between crossing hours in *D. rotundata*, though the mid-day seemed
23 optimal. For both species, the time interval 11–12 a.m. was more appropriate for crossing while
24 4–5 p.m. was the poorest. However, *in vitro* pollen germination tests showed that mid-day pollen
25 collection (12 noon – 2 p.m.) had better results than both extremes, though there were strong
26 genotypic effects on outcomes. Pollination success rates differed significantly among months for
27 *D. alata* ($p < 0.001$) but not for *D. rotundata* ($p > 0.05$). Differences in pollination success existed
28 across weeks within flowering windows of both *D. alata* ($p < 0.001$) and *D. rotundata* ($p = 0.004$).
29 The seed production efficiency (SPE) had a similar trend as the pollination success rate. No clear
30 pattern existed between the pollination time and the seed setting rate (SSR) or seed viability (SV),
31 though their dynamics varied with weeks and months. This study provided an insight on the
32 dynamics of pollination outcomes under the influence of pollination times and allows detecting
33 months, weeks, and hours of the day when hybridization activities should be focused for better
34 results.

35
36 **Keywords:** Pollination success, dioecy, seed setting rate, seed viability, pollen viability, weather
37 conditions.

40 Introduction

41 Yam (*Dioscorea* spp.) is a multispecies staple crop with significant contributions to food security
42 and poverty alleviation in tropics and subtropics, especially in West Africa where it is extensively
43 produced (Asiedu and Sartie, 2010). Its cultivation faces several yield restricting and quality
44 reducing factors related to poor crop husbandry, biotic and abiotic stresses, and postharvest losses

45 that widen the gap between the farmer yields ($\sim 10 \text{ t ha}^{-1}$) and the crop potential ($40\text{--}50 \text{ t ha}^{-1}$) and
46 reduce the market penetration (Frossard et al., 2017). Plant breeding research is an integral
47 component of addressing these challenges by development and delivery of resilient, productive,
48 and high-quality varieties. However, improved cultivar development through breeding in yam is
49 challenged by sexual reproduction abnormalities resulting from sparse, irregular, and
50 asynchronous flowering, cross compatibility barriers as the vegetative propagation is favored at
51 the expense of botanical seeds during the domestication and subsequent cultivation process
52 (Mondo et al., 2020). Yam plant exhibits extremely low levels of fruit-to-flower and seed-to-ovule
53 ratios, partly because of the sensitivity of its reproductive phases to suboptimal weather conditions
54 (Mondo et al., 2022). Important climatic factors such as temperature, rainfall, relative humidity,
55 and light intensity fluctuate from one location to the other and even from time to time in the same
56 location. These fluctuations dictate the limit within which controlled pollination can be
57 successfully conducted in any given location for a given species (Salami, 2016; Maity et al., 2019;
58 Raina and Kaul, 2019; Katano et al., 2020; Mondo et al., 2022).

59 Several attempts were undertaken to improve hand pollination success rate in yam breeding
60 programs by determining the appropriate time for crossing (Akoroda et al., 1981; Akoroda, 1983;
61 Abraham and Nair, 1990; Bakayoko et al., 2019; Mondo et al., 2020). However, the optimum time
62 for yam pollination is location-specific and depends on local environmental conditions (Mondo et
63 al., 2020). High relative humidity, well-distributed rainfall, sunshine, and moderate atmospheric
64 temperatures are the leading climatic factors for successful pollination in *D. alata* and *D. rotundata*
65 yams (Abraham and Nair, 1990; Mondo et al., 2022). Recommended time for hand pollination in
66 Nigeria (12 noon–3 p.m.) was set ~ 40 years ago (Akoroda, 1983), thus, there is a chance that trends
67 recorded four decades ago may have changed. Besides, due to predominant sunny conditions at
68 the previously recommended crossing hours, crossing activities are seldom undertaken at mid-day
69 (Mondo et al., 2020). Pollinators most conveniently operate in morning hours (8 a.m.–12 noon).
70 Yet, no study assessed the pollination success rates at those hours compared to the mid-day hours
71 recommended by the literature.

72 Most yam species, including *D. alata* and *D. rotundata*, are dioecious with male and female
73 flowers on separate individuals (Agre et al., 2020; Mondo et al., 2020; 2021a; 2022). The gene
74 flow between and among these species to meet breeding objectives depends, therefore, on cross-
75 pollination success. The cross-pollination involves three phases: the release of pollen from the
76 anther, transfer of pollen from the anther to the stigma, and successful placement of pollen on
77 receptive stigma surface, followed by germination (Di-Giovanni and Kevan, 1991; Bhattacharya
78 and Mandal, 2004; He et al., 2017). The transfer of yam pollen from the anther to the stigma is
79 either by the assistance of local insects (natural) or human hand (artificial) since the sticky nature
80 of yam pollen renders the wind pollination impossible (Mondo et al., 2020). However, the insects'
81 inefficiency is a major factor of low natural pollination success in yam (Akoroda, 1985; Segnou et
82 al., 1992). This insects' inefficiency is associated with low visitation rate, limited movements, and
83 selectivity (Martin et al., 1963; Mondo et al., 2020). Hand pollination is used as an alternative
84 solution; it is 2–3 times more efficient than natural pollination by insects (Akoroda, 1983; Segnou
85 et al., 1992). Whether natural or artificial, the pollination success is associated with other factors
86 such as pollen viability, stigma receptivity, cross compatibility, and the prevailing weather
87 conditions (Lebot et al., 2019; Mondo et al., 2020; 2022).

88 This study aims at improving pollination success in yam breeding programs by assessing the
89 optimum time of pollination, when the pollen is fully viable, the stigma receptive, and the weather

90 is conducive in *D. alata* and *D. rotundata* crossing blocks. It uses crossing block, *in vitro* pollen
91 germination, and weather data for assessment.

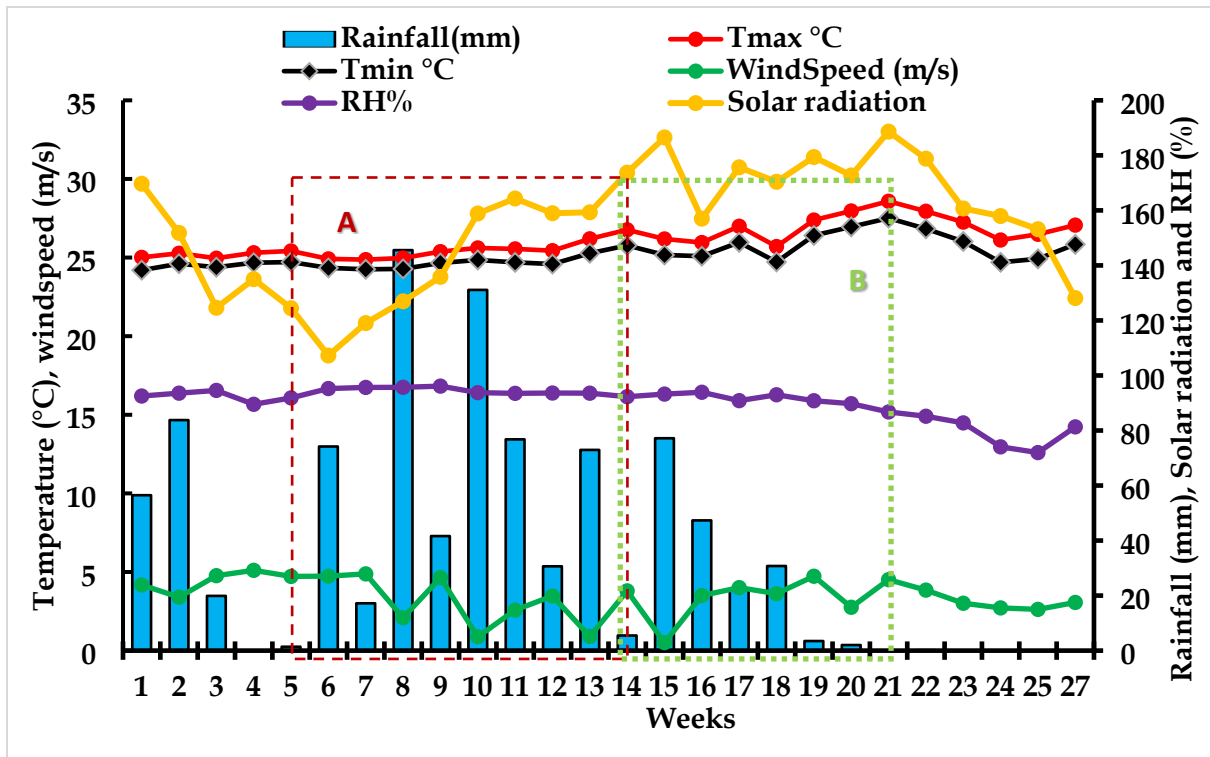
92 **Materials and methods**

93 **Study site, plant material, and field establishment**

94 Two-year experiment was conducted at the International Institute of Tropical Agriculture (IITA)
95 Ibadan (7°29' N and 3°54' E), Nigeria, from April 2020 to February 2022. Six female parents
96 (three *D. rotundata* and three *D. alata*) were selected based on the length of their flowering window
97 and flowering intensity. On the other hand, six male parents (three per species) were used as pollen
98 sources. All these materials were breeding lines maintained by the IITA Yam Breeding Unit (S1
99 Table). The cross-compatibility among selected genotypes and their ploidy statuses were based on
100 historical data information (Mondo et al., 2022).

101 The planting was done in April for both species and seasons. Male and female crossing blocks
102 were grown at appropriate spacing (1 m × 1 m). Recommended field management was conducted,
103 including individual plant staking, fertilizer application, supplemental irrigation, regular weeding,
104 etc. Pollination on *D. rotundata* crossing blocks were carried out from August to mid-October
105 while it started in late September and ended in early December for *D. alata*. Weather data in the
106 field was recorded using a data logger for the entire research period (Fig 1).

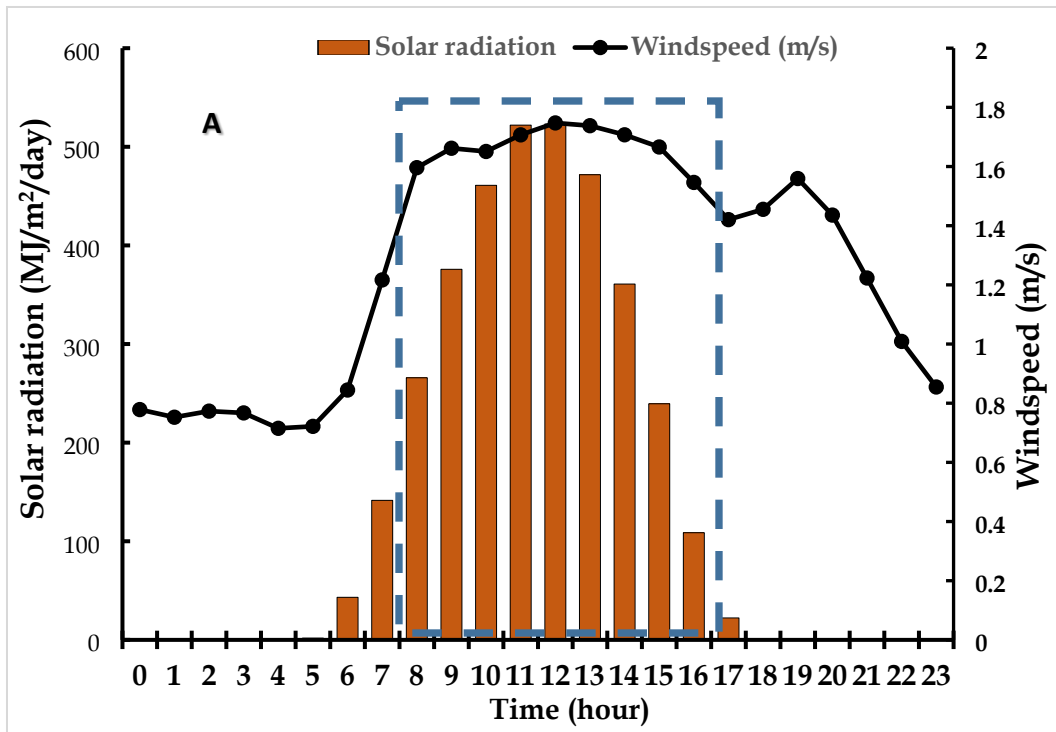
107 Among weather conditions, the solar radiation fluctuated much across flowering weeks (107.3 MJ
108 m⁻² day⁻¹ in week 6 to 188.6 MJ m⁻² day⁻¹ in week 21). The wind speed varied with weeks (week
109 15 had lowest wind speed: 0.5 km ha⁻¹ while week 4 had highest speed: 5.1 km ha⁻¹). Maximum
110 and minimum temperatures followed similar trends across weeks: week 21 had the highest
111 minimum (27.5°C) and maximum temperatures (28.6°C). The relative humidity ranged from 72.0
112 to 96.1% (Fig 1).



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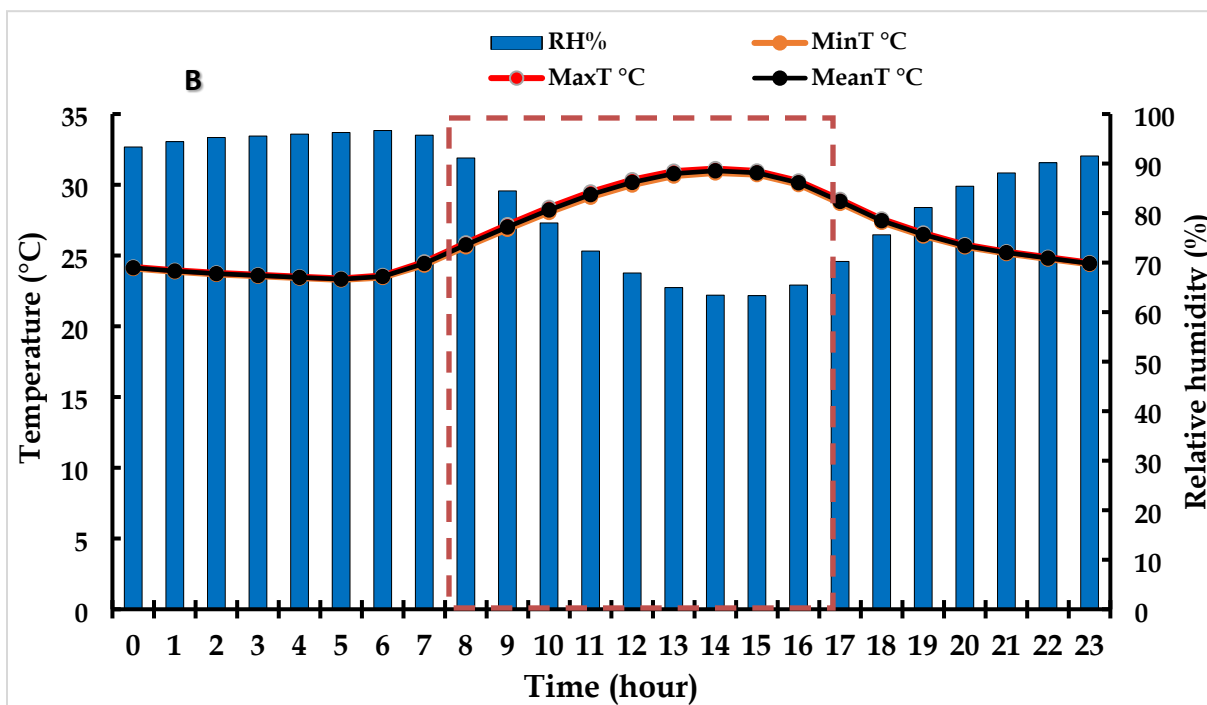
114 **Fig 1. Weekly weather data information of 2020 and 2021 crossing windows, IITA Ibadan station.**
 115 Week 1 corresponds to 1st week of July while Week 27 corresponds to the last week of December. (A) *D.*
 116 *rotundata* crossing window started early August (Week 5) and ended mid-October (Week 14). (B) *D. alata*
 117 crossing window started in mid-October (Week 14) and ended early December (Week 21).

118 Weather conditions varied with hours of the day: solar radiation, maximum and minimum
 119 temperatures were highest at the midday (11 a.m.–3 p.m.). The relative humidity had an opposite
 120 trend than temperatures, midday (1–4 p.m.) having lowest values (63.3–65.5%) than morning and
 121 night hours (Fig 2).



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125 **Fig 2. Variations in weather conditions throughout the day during 2020-2021 flowering windows at**
126 **IITA, Ibadan, Nigeria.** On the time axis, 0 refers to midnight while 23 refers to 11 p.m. of the same day.
127 Highlighted hours (8 a.m. to 5 p.m.) correspond to crossing hours. (A) trends of daily variations in solar
128 radiation and windspeed, (B) trends of daily variations in temperatures and relative humidity.

129 **Pollen viability assessment**

130 *In vitro* pollen germination testing was performed regularly to ensure pollination results were not
131 influenced by the pollen viability status. The previously optimized pollen germination testing
132 protocol by Mondo et al. (2021a) was used. This consisted of culturing anthers with pollen on Petri
133 dishes containing a nutritive medium made of 10% sucrose, 100 ppm H₃BO₃, 300 ppm
134 Ca(NO₃)₂·4H₂O, 200 ppm MgSO₄·7H₂O, and 100 ppm KNO₃. This medium was supplemented
135 with 0.5% agar and adjusted at pH 6.5. The culture was incubated at dark for 3 h under 25°C. The
136 pollen germination output was visualized under a fluorescence microscope (Olympus BX51,
137 Tokyo, Japan) at 10× magnification. The stigma receptivity was determined using visual
138 observation of the female flowers prior the crossing.

139 **Hand pollinations**

140 At flowering, female flowers were bagged with thrip-proof cloth-bags five days before pollination.
141 Hand pollinations between selected male and female plants were carried out from 8:00 a.m. to 5:00
142 p.m. for the entire flowering window. A cumulative number of 9,775 *D. rotundata* and 6,565 *D.*
143 *alata* female flowers were hand-pollinated with fresh pollen across crossing hours for the two
144 seasons. At each pollination day, an equal number of female flowers were pollinated hourly. The
145 pollinated flowers were then kept bagged for two weeks to ensure the purity of offspring from
146 crosses.

147 Following data were collected to assess the optimal time for hand pollination: (1) date of
148 pollination, (2) time of pollination, (3) fruit set (evaluated two weeks after pollination), and (4) the
149 seed set at plant physiological maturity. After fruit processing, the seed viability was also assessed.

150 Data collected on the fruit and seed sets were further used to calculate the pollination success rate,
151 the seed setting rate (SSR), the seed production efficiency (SPE), and the seed viability (SV) as in
152 Mondo et al. (2021b, 2022). The pollination success rate was calculated as follows:

$$153 \quad \text{Pollination success (\%)} = \frac{\text{Number of fruits set}}{\text{Number of flowers pollinated}} \times 100 \quad (1)$$

154 The seed setting rate (SSR) was the ratio between the number of seeds from a cross and the number
155 of fruits multiplied by six (which is the expected number of seeds in a yam fruit):

$$156 \quad \text{Seed setting rate (\%)} = \left(\frac{\text{Number of seeds set}}{\text{Number of fruits set} \times 6} \right) \times 100 \quad (2)$$

157 The seed production efficiency (SPE) for a cross was calculated as the number of viable seeds
158 divided by six times the number of pollinated flowers multiplied by 100:

$$159 \quad \text{SPE (\%)} = \frac{\text{Number of viable seeds}}{\text{Number of flowers pollinated} \times 6} \times 100 \quad (3)$$

160 The seed germination rate was estimated by dividing the seedling stand count in nurseries by the
161 number of seeds sown multiplied by 100:

$$162 \quad \text{Seed viability (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100 \quad (4)$$

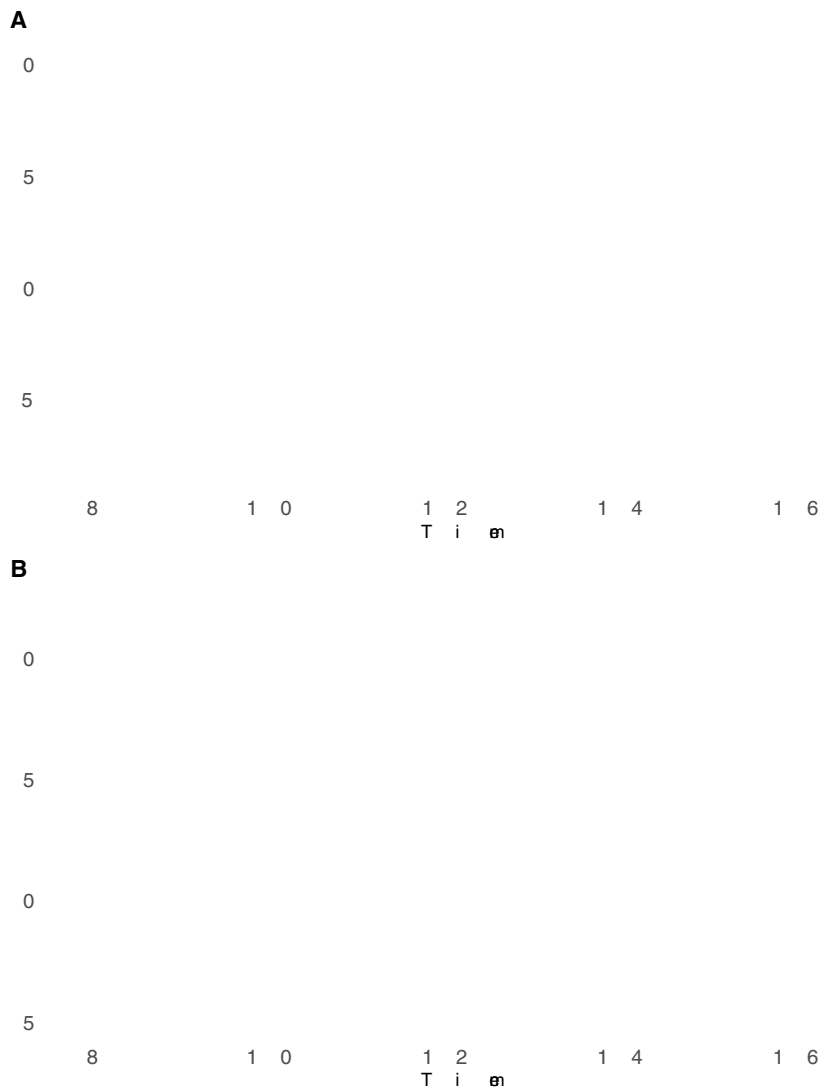
163 **Statistical data analysis**

164 The analysis of variance (ANOVA) was performed to detect differences among pollination
165 successes, SPE, SSR, and SV using the pollination month, week, and hour of the day as factors.
166 When necessary, means were separated by the least significant difference (LSD) test at 0.05 p-
167 value threshold. Analyses and plotting of graphs were performed using ggplot2 package in R.

168 **Results**

169 **Pollination success across crossing time**

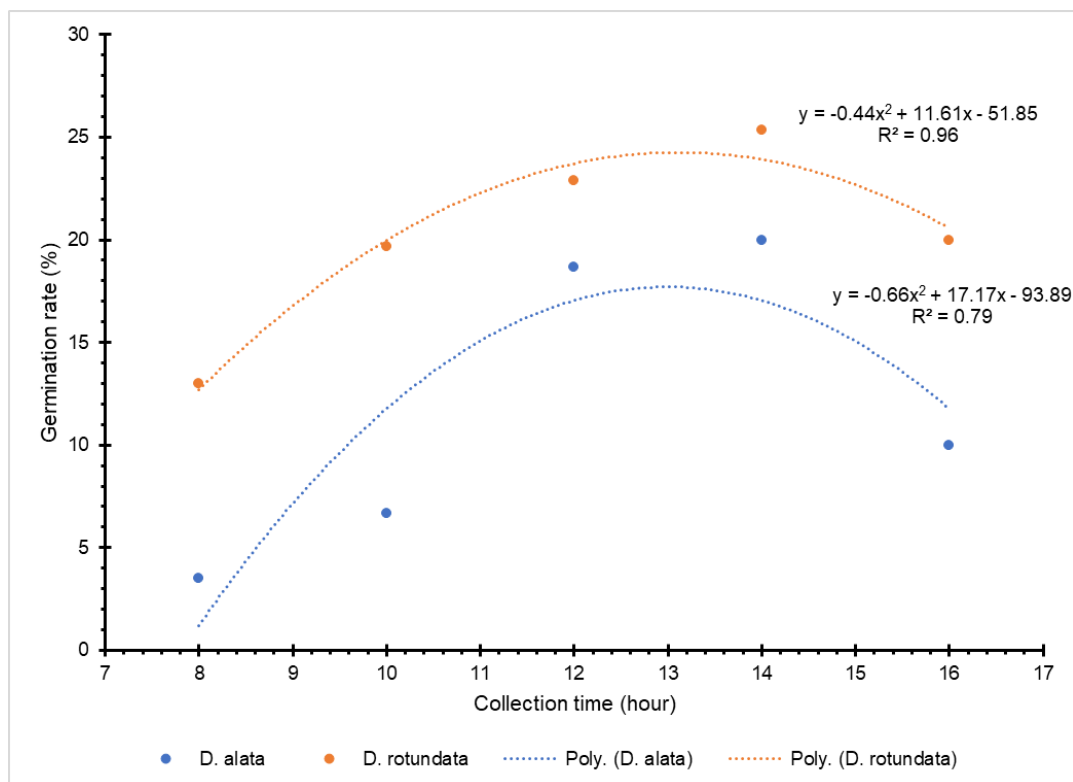
170 There were significant differences among crossing hours for pollination success in *D. alata* ($p =$
171 0.003), morning hours (8–11 a.m.) being better than afternoons (12–4 p.m.) (Fig 3A, S2 Table).
172 No significant difference existed between crossing hours in *D. rotundata* crossing blocks ($p =$
173 0.618 , Fig 3B) though the mid-day seemed optimal for hand pollination. Based on the crossing
174 block data, 11 a.m. could be considered as optimal for both species (18.6% for *D. alata* and 40.3%
175 for *D. rotundata*). Lowest rates were recorded at 4 p.m. for both species (3.3% for *D. alata* and
176 30.5% for *D. rotundata*). Though both crossing time and genotype had a significant effect on the
177 pollination outcome ($p < 0.001$), their interaction was not significant (S2 Table). *In vitro* pollen
178 germination data showed, however, that mid-day pollen collection had better results than both
179 extremes though the response was genotype-specific ($p = 0.0014$; Fig 4, S1 Fig). Pollen germinated
180 most between 12 noon and 2 p.m. (18.7–20% for *D. alata* and 22.9–25.3% for *D. rotundata*).



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Fig 3. Pollination success across crossing hours: (A) *D. alata*, (B) *D. rotundata*



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Fig 4. Dynamics of pollen germination rates across day hours

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Year, month and weekly effects on pollination success and seed set

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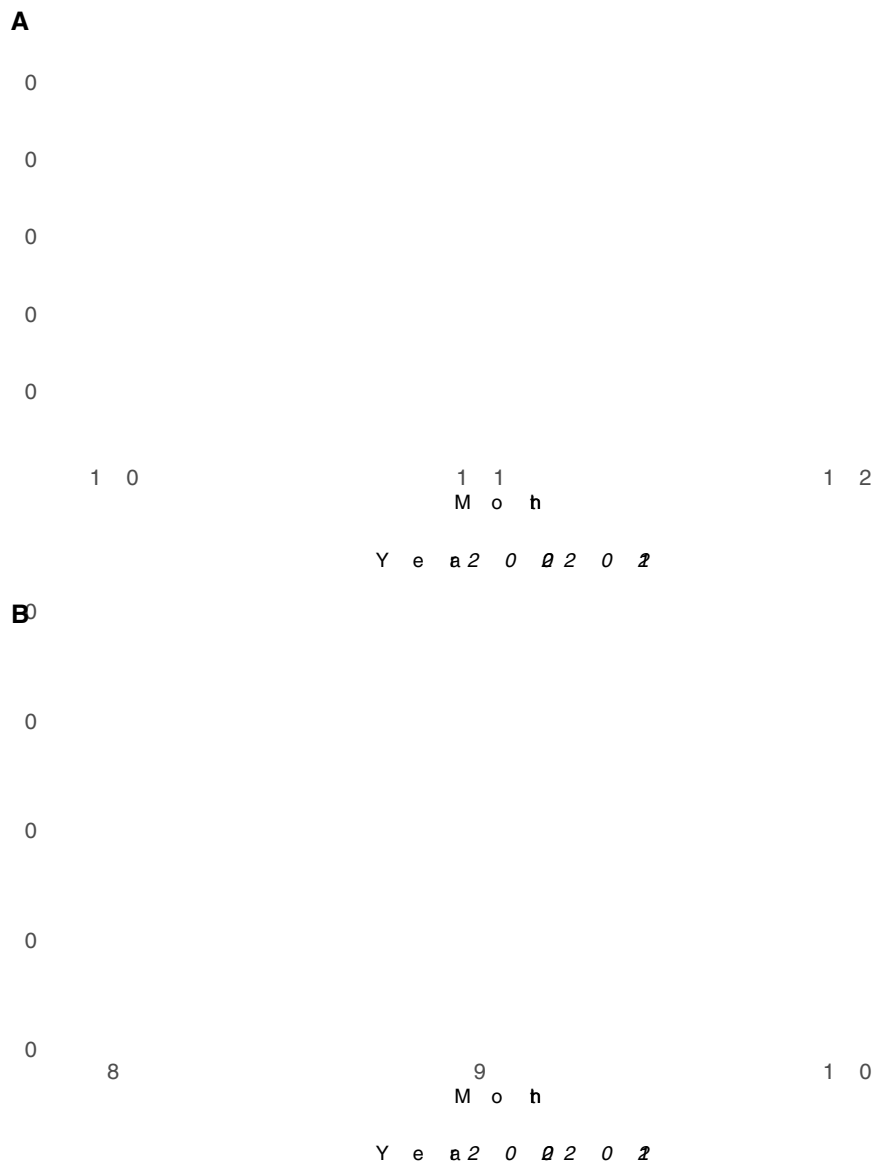
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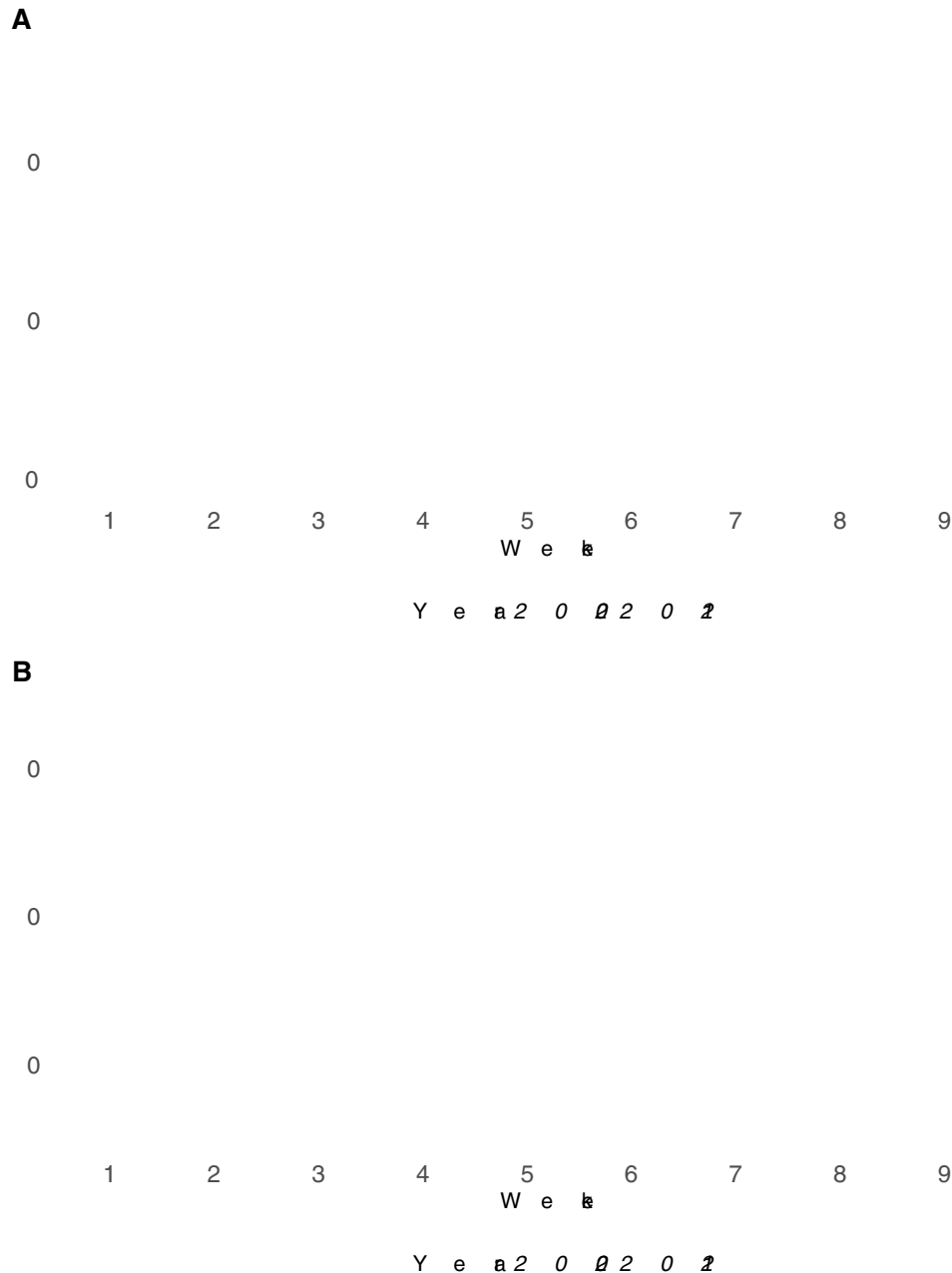
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Pollination success rate was higher in 2021 (29.1%) than 2020 (20.5%) regardless of the species (Fig 5). There were significant differences in pollination success rates among months for *D. alata* ($p < 2.2e-16$) but not for *D. rotundata* ($p = 0.053$). During *D. alata* crossing window, October (32.9%) was consistently the optimum month for pollination across both years while November (6.9%) and December (5.6%) had poor pollination success rates. For *D. rotundata* on the other hand, September had relatively best results across years while rates were lowest in October. The pollination success rate varied across weeks within the flowering window ($p < 2.2e-16$ for *D. alata* and $p = 0.004$ for *D. rotundata*) (Fig 6). The peak for *D. alata* (35.5–51.0%) was reached during the third week and then started decreasing. *D. rotundata* peak (36.6–68.9%) was observed at week 5 and then started decreasing to reach its minimum pollination rate at week 8 (15.3–19.5%).



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197 **Fig 5. Pollination success rates across crossing months for 2020 and 2021. (A) *D. alata* and (B) *D.***
 198 ***rotundata*.** The number 8 refers to August and 12 to December.



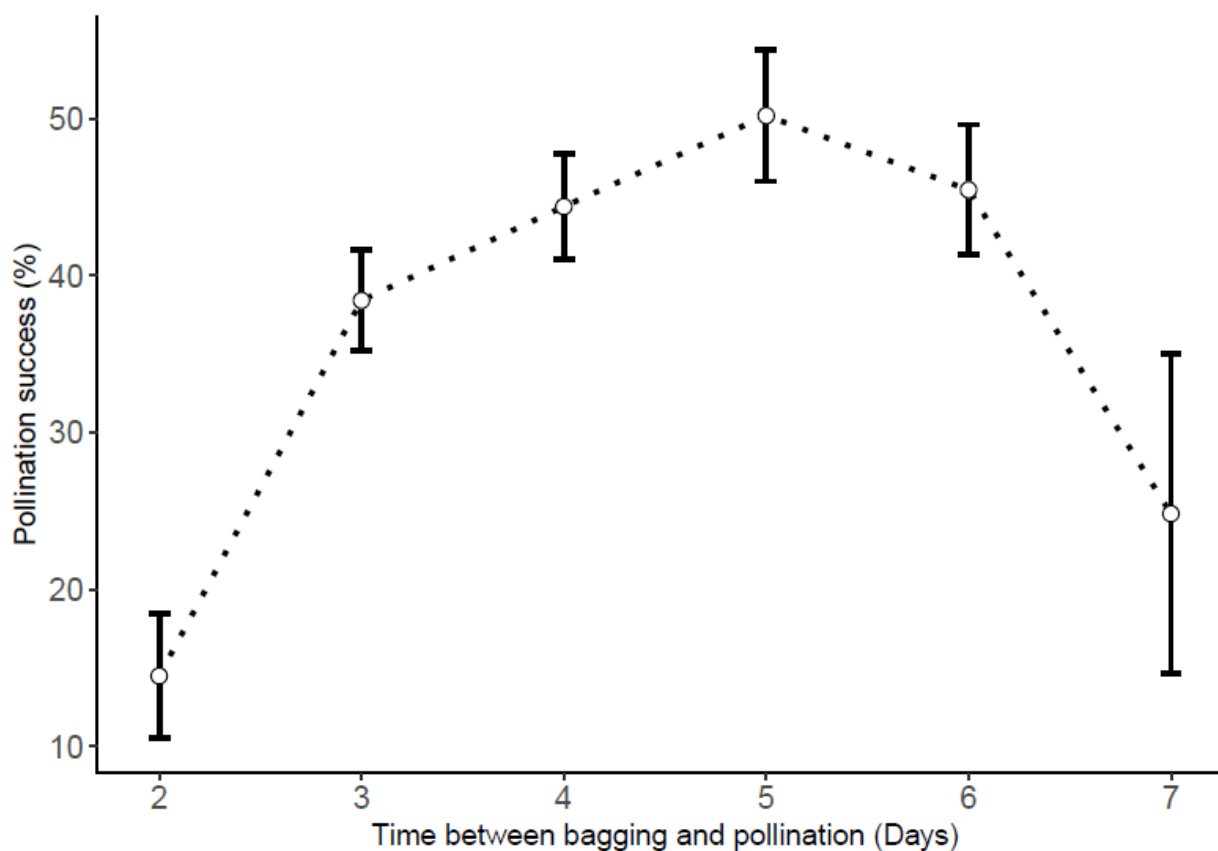
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200 **Fig 6. Pollination success rates across crossing weeks for 2020 and 2021. (A) *D. alata* and (B) *D.***
201 ***rotundata*.** Week 1 for *D. alata* corresponded to the second week of October while week 9 was the
202 second week of December. Week 1 for *D. rotundata* referred to second week of August while
203 week 9 corresponded to the second week of October.

204 **Bagging-to-crossing time interval and pollination success**

205 There was an association between the pollination success rate and the bagging-to-crossing time
206 interval ($R^2=0.97$, $p<0.001$). Results suggested that 4–6 days is the optimal interval between flower
207 bagging and crossing time (Fig 7, S2 Fig). The pollination success within that interval ranged from

208 44.4–50.2% while the lowest success rate was recorded for crosses made within two days after
209 bagging (14.5%).



210
211 **Fig 7. Influence of the time interval between flower bagging and crossing on pollination success rates.**

212 **Dynamics of SPE, SSR, and SV across crossing times**

213 Only the SPE was influenced by the hour of pollination, no particular pattern existed for SSR and
214 SV regardless of the species (Table 1). For SPE, the trend was comparable to the one of the
215 pollination success rate: 11–12 a.m. had highest SPE values (6.01% for *D. alata* and 22.97% for
216 *D. rotundata*). Lowest values were recorded at 4–5 p.m. for both *D. alata* (0.11%) and *D.*
217 *rotundata* (9.51%). At the monthly basis, the SPE varied with months, October being optimal for
218 *D. alata* (10.3%) and September for *D. rotundata* (18.1%). October (12.2%) and December (0.2%)
219 were worst for *D. rotundata* and *D. alata*, respectively (S3 Fig). For *D. alata*, October had once
220 again the highest SSR (28.1%) and December the lowest (2.3%). August had highest SSR (60.5%)
221 for *D. rotundata* and October the lowest (30.1%) (S4 Fig). Seed viability was indifferent to
222 monthly variabilities and ranged from 70.6–75.6% for *D. alata* and 75.3–81.6% for *D. rotundata*
223 (S5 Fig).

224 At weekly basis, there were significant differences among weekly SPE (Fig 8), SSR (Fig 9), and
225 SV (Fig 10). Second (10.8) and third (11.8%) weeks had highest SPE for *D. alata* while highest

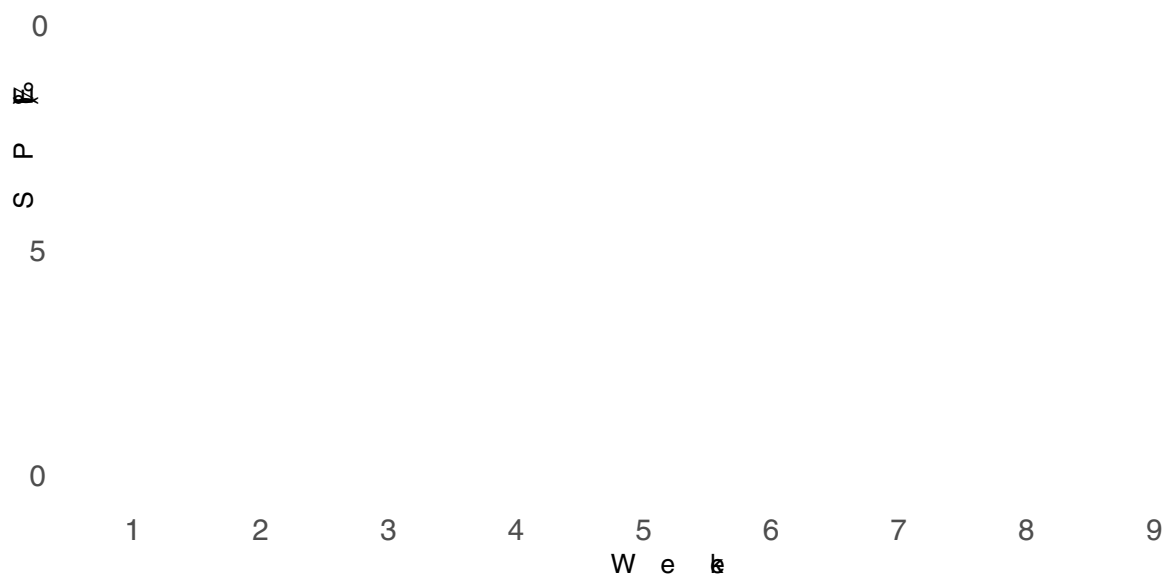
226 SPE were on fourth (21.9%) and fifth (22.8%) weeks for *D. rotundata*. Lowest SPE were in the
 227 6th to 8th weeks (0%) for *D. alata* and second (7.2%) and 8th (9.9%) weeks for *D. rotundata*. SSR
 228 values were not significantly different for weeks 1 to 5 (17.1–29.0%) after which it decreased
 229 significantly in *D. alata* crossing blocks (0.0–8.3%). For *D. rotundata*, SSR had a similar trend as
 230 for SPE, the second week having lowest SSR (23.3%) and week 1 (65.3), week 4 (60.5%) and
 231 week 5 (63.1%) had the highest SSR. *D. alata* seed viability did not vary much across weeks (Fig
 232 10) while for *D. rotundata*, the second week (56.8%) had significantly lower seed viability than
 233 all other weekly means (74.8–82.3%).

234 **Table 1. Pollination time and seed production efficiency (SPE), seed setting rate (SSR) and seed**
 235 **viability (SV)**

Species	Crossing time	SPE (%)	SSR (%)	SV (%)
<i>D. alata</i>	8–9 h	5.16	20.78	68.94
	9–10h	3.43	21.65	78.24
	10–11h	5.63	27.49	79.95
	11–12h	6.01	23.47	70.84
	12–13h	2.11	18.43	64.16
	13–14h	4.91	27.21	82.58
	14–15h	2.94	16.46	80.88
	15–16h	1.98	22.17	66.07
	16–17h	0.11	0.00	41.67
	Mean (%)	3.96	22.53	74.26
<i>D. rotundata</i>	8–9h	15.27	56.62	78.67
	9–10h	16.66	48.54	75.36
	10–11h	17.57	55.59	76.95
	11–12h	22.97	56.70	77.00
	12–13h	20.83	59.96	78.21
	13–14h	16.73	61.05	82.23
	14–15h	12.75	41.37	77.29
	15–16h	18.70	45.99	76.08
	16–17h	9.51	47.99	78.57
	Mean (%)	17.65	52.83	77.02
	Overall mean (%)	9.99	42.51	76.22

236

A



B

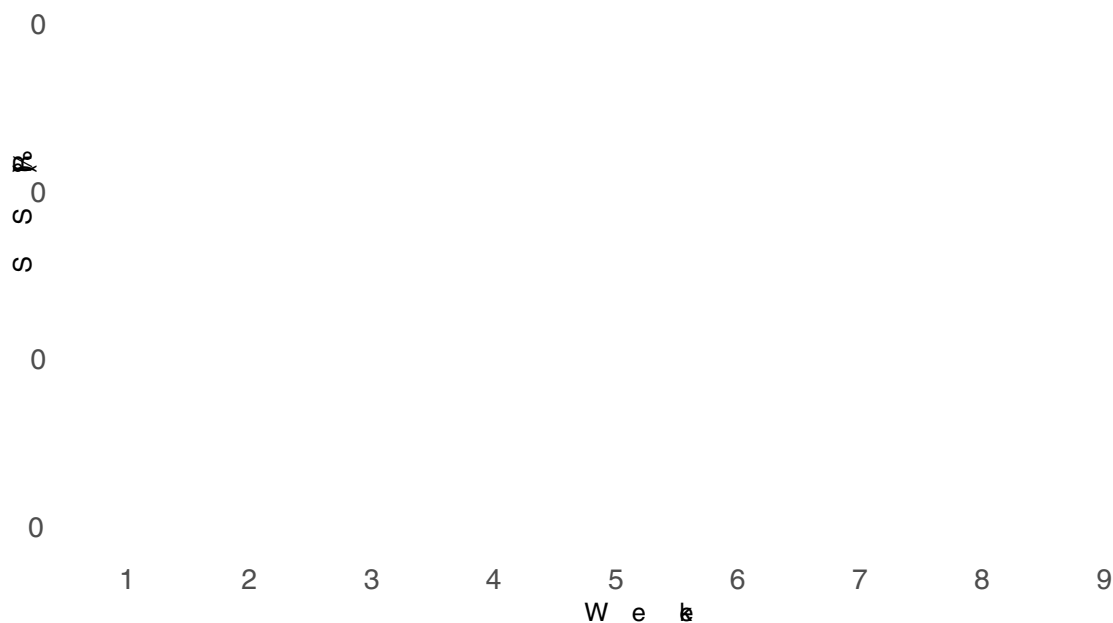


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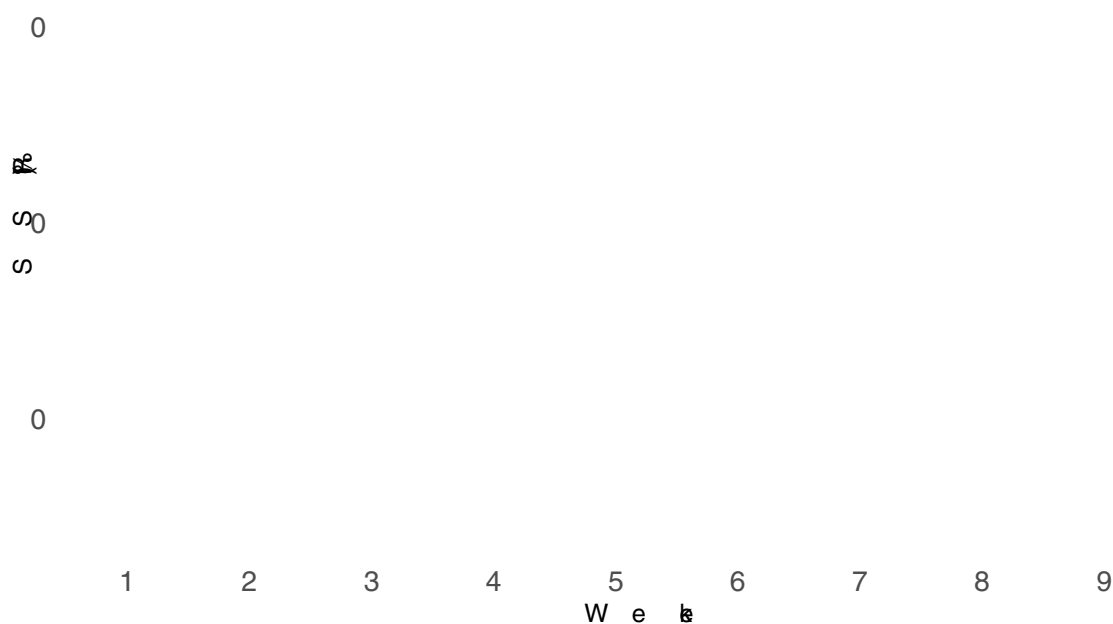
238 **Fig 8. Weekly seed production efficiency across the crossing window: (A) *D. alata* and (B) *D.***
239 ***rotundata*.** Week 1 for *D. alata* corresponded to the second week of October while week 9 was the

240 second week of December. Week 1 for *D. rotundata* referred to second week of August while
241 week 9 corresponded to the second week of October.

A



B



242

243 **Fig 9. Weekly seed setting rate (SSR) across the crossing windows: (A) *D. alata* and (B) *D. rotundata*.**
244 Week 1 for *D. alata* corresponded to the second week of October while week 9 was the second
245 week of December. Week 1 for *D. rotundata* referred to second week of August while week 9
246 corresponded to the second week of October.

A

0
%
S
0

0

1

2

3
W e e k

4

5

B

0

%
S
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1

2

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4

5
W e e k

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8

9

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248 **Fig 10. Weekly seed viability rate across the crossing windows: (A) *D. alata* and (B) *D. rotundata*.**
249 Week 1 for *D. alata* corresponded to the second week of October. We had no seed for viability
250 test for weeks 6–9. Week 1 for *D. rotundata* referred to second week of August while week 9
251 corresponded to the second week of October.

252 **Discussion**

253 **Pollination success depended on crossing time**

254 Yam breeding is challenged by sexual reproduction abnormalities (sparse, irregular, and
255 asynchronous flowering, cross compatibility barriers and low fertility) because of the
256 domestication process that favored vegetative propagation at the expense of botanical seeds
257 (Mondo et al., 2020). The International Institute of Tropical Agriculture (IITA) has devised a series
258 of studies to control those reproduction abnormalities. This study is a continuation of such efforts
259 and aimed to update recommendations on optimum time for hand pollination in yam breeding. We
260 realized that the pollination success varied with the year, month, week, and hour of the day when
261 the hand pollination is performed. The year, month, week, and hour of the day with well-distributed
262 rainfall, high relative humidity and moderate temperatures were conducive to hand pollination for
263 both *D. alata* and *D. rotundata* yam species. The year 2021 was more wet than 2020 and thus
264 recorded higher pollination success rate than 2020. That finding agreed with Mondo et al. (2022)
265 who showed variability in pollination success across years while using 2010–2020 crossing block
266 information. *D. alata* was more sensitive to the time of pollination than *D. rotundata*. For instance,
267 Mondo et al. (2021a) showed that *D. rotundata* pollen had a wide range of germination
268 temperatures (15–35°C) compared to *D. alata* that gave better results at 25°C. The weather
269 parameters' fluctuation with time could have explained the difference in pollination outcomes. A
270 previous study on yam showed that weekly variability in rainfall, temperature, relative humidity,
271 sunshine, and the number of rainy days within the yam flowering window (July to November)
272 significantly influenced the pollination outcomes in either the *D. rotundata* or *D. alata* crossing
273 blocks (Mondo et al., 2022). Abraham and Nair (1990) also reported that successful pollination in
274 yam is significantly associated with high relative humidity and moderate atmospheric
275 temperatures. As supported by this study, making crosses in some weeks of August or in the second
276 half of October for *D. rotundata* and after 15th November for *D. alata* could result in low
277 pollination success due to the suboptimal weather conditions. As recommended by Mondo et al.
278 (2022) there is the need for supplemental irrigation in yam crossing blocks to reduce the water
279 deficit's adverse effects on yam reproductive phases during these months. Since *D. alata* presents
280 no dormancy at harvest, options of establishing the crossing block as early as March (with
281 supplemental irrigation) could help avoiding the coincidence of its flowering window with harsh
282 environmental conditions, as it was the case the last two years.

283 In contrast to most reports on yam pollination, *D. rotundata* had higher crossability rate and higher
284 seed production efficiency than *D. alata* for both 2020 and 2021. However, *D. alata* had
285 consistently higher values (31%) than *D. rotundata* (23%) when bulking 2010–2020 crossing
286 block information at IITA (Mondo et al., 2022). For our study period (2020–2021), months
287 corresponding with the *D. alata* flowering window (October–December) were drier compared to

288 those of *D. rotundata* (August–October) which benefited from relatively high and well-distributed
289 rains and moderate temperatures. It is noteworthy that *D. alata* is sensitive to rainfall distribution,
290 sunshine, relative humidity, and temperatures (Abraham and Nair, 1990; Mondo et al., 2022)
291 which were suboptimal during *D. alata* flowering window.

292
293 For both seasons, crosses made in morning hours (8–12 a.m.) had better results than those from
294 afternoons for *D. alata*. Though not significant, mid-day seemed optimal for *D. rotundata*. Based
295 on the crossing block data, 11 p.m. could be considered as optimal for both species while lowest
296 rates were recorded at 4 p.m. for both species. However, *in vitro* pollen germination tests supported
297 the mid-day (12 noon–2 p.m.) as the optimal time for pollen collection for both species while both
298 morning and evening extremes should be avoided. This result aligned with findings by Akoroda
299 (1983, 1985) that showed that *D. rotundata*'s better pollination success was achieved when crosses
300 are made between 12 noon and 2 p.m. at IITA Ibadan, Nigeria. These findings partly dismissed
301 our hypothesis that the optimal time for hand pollination, recommended four decades ago, might
302 have been affected by climate changes. Since weather conditions are conducive for human labor
303 in morning hours than the mid-day, we could recommend concentrating crossing activities at 11–
304 12 a.m. interval for both species since morning was better than afternoons for *D. alata* and there
305 were no significant differences between morning and mid-day hours for *D. rotundata*. Results
306 showed an influence of the flower bagging on pollination outcomes, 4–6 days being the optimal
307 interval between flower bagging and crossing time. Further investigations are necessary to
308 elucidate reasons behind the influence of bagging-to-crossing time interval in yam crossing blocks.

309 **The seed setting rate and the seed viability were less affected by the hour of crossing but**
310 **varied with the month and week of crossing**

311 Only the pollination success rate and the SPE varied with the hour of pollination, no particular
312 pattern existed for the SSR and SV regardless of the species. For the SPE, the trend was
313 comparable to the one of the pollination success rate: 11–12 a.m. had highest SPE values for both
314 species while lowest values were recorded at 4–5 p.m. At the monthly basis, the SPE varied with
315 months, October being optimal for *D. alata* and September for *D. rotundata*. October and
316 December were worst for *D. rotundata* and *D. alata*, respectively. For *D. alata*, October had once
317 again the highest SSR and December the lowest. August had highest SSR for *D. rotundata* and
318 October the lowest. Seed viability was indifferent to monthly variabilities for both species.

319 There were significant differences among weekly SPE, SSR, and SV, weeks with conducive
320 climatic conditions provided the best outcomes. Bandeira e Sousa et al. (2021) showed that
321 environmental factors (temperature, rainfall, and photoperiod) contribute to a post-zygotic barrier
322 in crops like cassava. They showed that high temperatures induced flower abortion and reduced
323 the number of female flowers per inflorescence and seed setting rate. There was also a decreased
324 pollen tube growth rate at higher average temperatures than lower temperatures, supporting the
325 hypothesis that environmental conditions affect the efficiency of sexual reproduction, and that
326 appropriate planning of planting dates and locations can maximize seed production (Ramos Abril

327 et al., 2019; Bandeira e Sousa et al., 2021). Environmental factors such as rainfall and temperature
328 had also affected flowering, pollen production, and fruit development in cocoa (Omolaja et al.,
329 2009).

330 **Conclusion**

331 This study, based on two-year crossing data, showed that the time of pollination had an influence
332 on the pollination success rates. The year, month, week, and hour of the day with well-distributed
333 rainfall, high relative humidity and moderate temperatures were conducive to hand pollination for
334 both yam species. Crossing block data, weather information, and *in vitro* pollen germination
335 seemed to encourage morning to mid-day hybridization for better pollination results. Special
336 measures should be devised for *D. alata* as it was the most sensitive to weather conditions, and the
337 months corresponding to its flowering window had globally suboptimal climatic conditions for
338 both years.

339 **Supporting information**

340 **S1 Fig. Genotypic variability in pollen germination in *D. alata* and *D. rotundata***
341 **S2 Fig. Relationship between the bagging–crossing time interval and pollination success**
342 **S3 Fig. Monthly variability in SPE for *D. alata* and *D. rotundata***
343 **S4 Fig. Monthly variability in SSR for *D. alata* and *D. rotundata***
344 **S5 Fig. Monthly variability in seed viability for *D. alata* and *D. rotundata***
345 **S1 Table. Description of yam genotypes used in hand pollination experiment**
346 **S2 Table. ANOVA table for pollination success across crossing hours for *D. alata***

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355 files.

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362
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375 References

- 376 1. Abraham K, Nair PG. Floral biology and artificial pollination in *Dioscorea alata* L. *Euphytica*.
377 1990; 48: p. 45–51.
- 378 2. Agre P, Nwachukwu C, Olasanmi B, Obidiegwu J, Nwachukwu E, Adebola P, De Koeyer D,
379 Asrat A. Sample preservation and plant sex prediction in white guinea yam (*Dioscorea*
380 *rotundata* Poir.). *Journal of Applied Biotechnology Reports*. 2020; 7(3): p. 145–151.
- 381 3. Akoroda MO. Floral biology in relation to hand pollination of white yam. *Euphytica*. 1983;
382 32: p. 831–838.
- 383 4. Akoroda MO. Pollen management for controlled hybridization of white yam. *Scientia*
384 *Horticulturae*. 1985; 25: p. 201–209.
- 385 5. Asiedu R, Sartie A. Crops that feed the world 1. Yams. *Food Security*. 2010; 2: p. 305–315.
386 <https://doi.org/10.1007/s12571-010-0085-0>
- 387 6. Babayoko Y, Kouakou AM, Dibi KEB, Ehounou AE, Essis BS, Nzue B, Kouassi AB, Nguetta
388 AS, Adebola PO. Effet de la période de pollinisation manuelle sur la fructification de l'igname
389 (*Dioscorea rotundata* Poir; Dioscoreaceae) en Côte d'Ivoire. *European Scientific Journal*.
390 2019; 15(27): p. 448–464.
- 391 7. Bandeira e Sousa M, Andrade LR, Souza EH, Alves AA, de Oliveira EJ. Reproductive barriers
392 in cassava: Factors and implications for genetic improvement. *PloS one*. 2021;
393 16(11):e0260576. <https://doi.org/10.1371/journal.pone.0260576>
- 394 8. Bhattacharya A, Mandal S. Pollination, pollen germination and stigma receptivity in *Moringa*
395 *oleifera* Lamk. *Grana*. 2004; 43(1): p. 48–56.
- 396 9. Di-Giovanni F, Kevan PG. Factors affecting pollen dynamics and its importance to pollen
397 contamination: a review. *Canadian Journal of Forest Research*. 1991; 21(8): p.1155–1170.
- 398 10. Frossard E, Aighewi BA, Aké S, Barjolle D, Baumann P, Bernet T, Dao D, Diby LN, Floquet
399 A, Hgaza VK, Ilboudo LJ, Kiba DI, Mongbo RL, Nacro HB, Nicolay GL, Oka E, Ouattara
400 YF, Pouya N, Senanayake RL, Six J, Traoré OI. The challenge of improving soil fertility in
401 yam cropping systems of West Africa. *Frontiers in Plant Science*.2017; 8: p.1953.
402 doi:10.3389/fpls.2017.01953
- 403 11. He G, Hu F, Ming J, Liu C, Yuan S. Pollen viability and stigma receptivity in *Lilium* during
404 anthesis. *Euphytica*. 2017; 213(10): p.1–10.
- 405 12. Katano K, Oi T, Suzuki N. Failure of pollen attachment to the stigma triggers elongation of
406 stigmatic papillae in *Arabidopsis thaliana*. *Frontiers in Plant Science*. 2020; 11: p.989.
- 407 13. Maity A, Chakarbarty SK, Pramanik P, Gupta R, Parmar SS, Sharma DK. Response of stigma
408 receptivity in CMS and male fertile line of Indian mustard (*B. juncea*) under variable thermal
409 conditions. *International Journal of Biometeorology*. 2019; 63(2): p.143–152.
- 410 14. Martin FW, Cabanillas E, Ortiz S. Natural pollination, hand pollination and crossability of
411 some Mexican species of *Dioscorea*. *Tropical Agriculture*. 1963; 40: p. 135–141.

- 412 15. Mondo JM, Agre PA, Asiedu R, Akoroda MO, Asfaw A. Genome-wide association studies
413 for sex determination and cross-compatibility in water yam (*Dioscorea alata* L.). *Plants*.
414 2021a; 10(7): p.1412.
- 415 16. Mondo JM, Agre PA, Asiedu R, Akoroda MO, Asfaw A. Optimized Protocol for In Vitro
416 Pollen Germination in Yam (*Dioscorea* spp.). *Plants*. 2021b; 10(4): p.795.
- 417 17. Mondo JM, Agre PA, Edemodu A, Adebola P, Asiedu R, Akoroda MO, Asfaw A. Floral
418 Biology and Pollination Efficiency in Yam (*Dioscorea* spp.). *Agriculture*. 2020; 10(11):
419 p.560.
- 420 18. Mondo JM, Agre PA, Edemodu A, Asiedu R, Akoroda MO, Asfaw A. Cross compatibility in
421 intraspecific and interspecific hybridization in yam (*Dioscorea* spp.). *Scientific Reports*. 2022;
422 12(1): p. 1–13.
- 423 19. Omolaja SS, Aikpokpodion P, Adedeji S, Vwioko DE. Rainfall and temperature effects on
424 flowering and pollen productions in cocoa. *African Crop Science Journal*. 2009; 17(1): p. 41–
425 48.
- 426 20. Raina M, Kaul V. Assessment of stigma receptivity via papillar integrity in *Kigelia pinnata*
427 (Bignoniaceae). *Proceedings of the National Academy of Sciences, India Section B:*
428 *Biological Sciences*. 2019; 89(3): p. 867–875.
- 429 21. Ramos Abril LN, Pineda LM, Wasek I, Wedzony M, Ceballos H. Reproductive biology in
430 cassava: stigma receptivity and pollen tube growth. *Communicative & Integrative*
431 *Biology*. 2019; 12(1): p. 96–111.
- 432 22. Salami AE. Performance of Hand-pollinated Maize Genotypes at Different Daytimes in a
433 Nigeria Forest Agro-ecosystem. *Journal of Experimental Agriculture International*. 2016; p.1–
434 9.