agriculture

MDPI

Review

Floral Biology and Pollination Efficiency in Yam (*Dioscorea* spp.)

Jean M. Mondo [1,2,3], Paterne A. Agre [1,*], Alex Edemodu [1], Patrick Adebola [4], Robert Asiedu [1], Malachy O. Akoroda [5] and Asrat Asfaw [1]

1 International Institute of Tropical Agriculture (IITA), Ibadan 5320, Nigeria; m.mubalama@cgiar.org (J.M.M.); a.edemodu@cgiar.org (A.E.); r.asiedu@cgiar.org (R.A.); a.amele@cgiar.org (A.A.)
2 Institute of Life and Earth Sciences, Pan African University, University of Ibadan, Ibadan 200284, Nigeria
3 Department of Crop Production, Université Evangélique en Afrique (UEA), Bukavu 3323, Congo
4 International Institute of Tropical Agriculture (IITA), Abuja 901101, Nigeria; p.adebola@cgiar.org
5 Department of Agronomy, University of Ibadan, Ibadan 200284, Nigeria; malachyoakoroda@gmail.com
* Correspondence: p.agre@cgiar.org

Received: 21 October 2020; Accepted: 12 November 2020; Published: 20 November 2020

Abstract: Yam (*Dioscorea* spp.) is a monocotyledonous herbaceous vine cultivated for its starchy underground or aerial tubers in the tropics and subtropics. It is an allogamous and polyploid species that reproduces by both sexual and asexual mechanisms. However, many of the landrace cultivars, including most of the popular varieties, reproduce exclusively by vegetative propagation (planting the tubers). These varieties are either sterile or produce sparse and irregular flowering with high flower abortion rate, low fruit and seed set. Production of crossbreed seeds for genetic improvement and for maintaining genetic diversity in yams is, therefore, mainly achieved through natural or managed pollination. Flowering in yam is mostly dioecious and, in some instances, monoecious. Flowering asynchrony, sticky nature of the pollen grains, and cross incompatibility are among the challenges in making genetic progress in yam breeding. There are many limitations in basic and applied knowledge of yam flower biology and pollination. This paper, therefore, reviews the flowering biology, pollination, and methods of improving pollination efficiency in yam breeding programs.

Keywords: profuse flowering; cross incompatibility breakage; pollen viability and storage; West Africa

1. Introduction

Yam is a common name for diverse species in the genus *Dioscorea*. This genus is composed of ~600 species, of which eleven are the main ones cultivated globally and eight of these are grown in Africa [1–4]. In Africa, yam is extensively cultivated in a region from West Cameroon to central Côte d'Ivoire, also referred to as the African "yam belt" [5,6]. The yam belt comprises six countries: Nigeria, Ghana, Côte d'Ivoire, Benin, Togo and Cameroon. This zone accounts for ~92% (67.1 million tons) of the annual global yam production [7]. Yam is an important source of dietary nutrients [8,9] with a low glycemic index, which gives better protection against diabetes and obesity [10,11]. Yam value chain is a direct income-generating activity for more than five million people in West Africa [12]. Thus, it provides an opportunity for poverty alleviation and food security.

Breeding is key to release the potential of yam for food security and poverty reduction. Yam breeding uses both sexual and asexual reproductive mechanisms. Sexual reproduction involves an artificial hand or natural open pollination during flowering to generate genetically variable offspring for selection [13]. This step starts with selecting parents with desirable traits. The mating design's choice is based on a breeding program's objective, technical and financial capacity. Progenies with desirable

traits such as yield stability, superior resistance to disease and pests, and higher tuber quality are then selected and advanced vegetatively for several generations and across locations before releasing as new varieties [3,13]. Therefore, plant breeding programs' success relies on genetic variation for traits of interest and successful transfer of the genetic allele of traits from source germplasm to the agronomically preferred background. The transfer of genes or alleles depends on flowering, pollination, fruit seed set, and seed germination.

Pollination is the movement of pollen from the male sexual parts of a plant to the receptive surface of a plant's female sexual parts [14]. Pollination is central to the transfer of genes in plant breeding. It involves three phases: the release of pollen, transfer of pollen, and placement of pollen (followed by germination) [14]. The pollination success is associated with many factors: pollen quantity and viability, stigma receptivity, cross compatibility, the efficiency of pollinator agents and the prevailing weather conditions [3,15] (Figure 1).

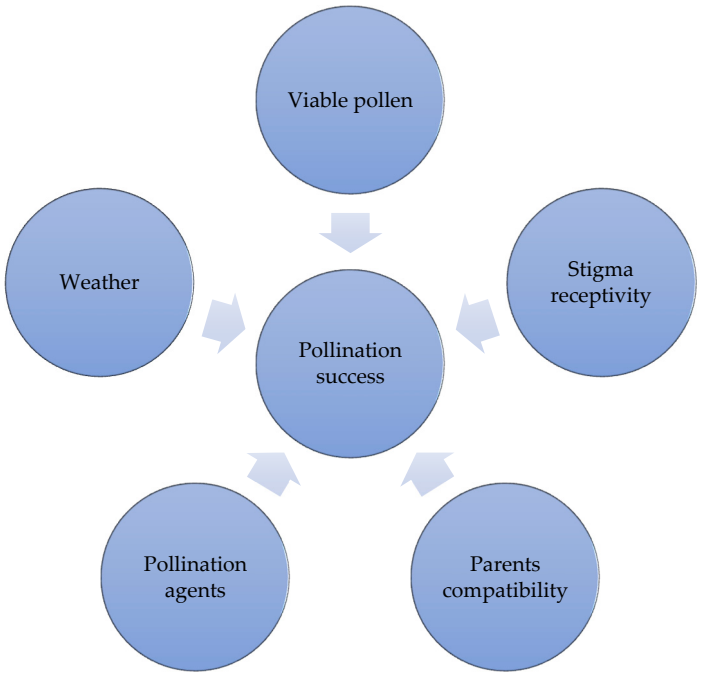

Figure 1. Factors linked to plant pollination.

Pollination efficiency at a breeding program level can be defined as the number of viable/germinable seeds over the number of ripe female flowers which received viable pollen. This ratio is low in yam plant and varies with studies from 1 to ~40% [3,15–17]. At the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, the percentage fruit and seed sets are estimated at 20.3 and 10.5%, respectively, for *D. rotundata* and 28.0 and 9.3%, respectively, for *D. alata* [3]. This low success rate is partly linked to yam's long-term vegetative propagation since its domestication [18]. Some of the domestication effects on yam reproduction are sparse, irregular, or absent flowering, high flower and ovule abortion rates, low fruit and seed set, and low seed viability [19]. Besides, the synchronized flowering time between male and female individuals is uncertain [3,15]. The sticky nature of the pollen grains renders wind pollination impossible. The inefficiency of insects intervening in pollen dispersal, cross incompatibility, and suboptimal weather conditions contribute to low pollination and seed set in yam under natural conditions [20,21]. Even though the pollination efficiency has remained low over time, the topic is currently neglected. Most relevant publications in yam floral biology and pollination are 30–50 years old. There is, therefore, a need to deepen knowledge on yam floral biology and update recommendations on practices linked to pollination efficiency to match with the current realities.

We hypothesized that yam pollination efficiency is improvable by integrating a range of practices discussed throughout this review. These include quality pollen collection and preservation, adequate pollination methods, identification of and rearing insect pollinators, agronomic and biotechnological manipulations. Therefore, this review explored available knowledge on yam floral biology, pollination practices, constraints limiting pollination efficiency, and provided insights for its improvement. Due to limited literature on the yam crop, this review analyzed some of the achievements with other related crops and their effective translation to improve pollination efficiency in yam.

2. Overview on Floral Biology and Pollination in Yam

The flowering pattern and intensity vary within and between the *Dioscorea* species. The majority of flowering yam genotypes is dioecious, with male (staminate) and female (pistillate) flowers borne on separate plants [1,19,22] but monoecious genotypes that produce staminate and pistillate flowers on the same plant exist [19,23] (Figures 2 and 3). Due to its predominantly dioecious nature, yam is mostly an outcrossing crop [13,23]. The rate of flowering is generally low among cultivated yams. The male flowering genotypes often outnumber the female flowering ones (Table 1). Studies on the flowering pattern revealed that male and female clones differ in flower initiation periods and the flowering duration. The difference between the male and female flowering peaks is 1–3 weeks, with male flowering earlier than the female counterpart [16,24,25]. Duration of flowering is mostly dependent on weather conditions within a season. It varies from 12–20 days for male genotypes and from 5–10 days among females of *D. alata* [26]. Monoecious plants often show variable flower sex expression over seasons and across locations. Propagules from the same monoecious plant produce plants with either male or female or both flowers over space and time. This sex-switch from year to year and across locations undermines experimental designs, particularly those involving cross-pollination. Tamiru et al. [23] hypothesized Z and W locus segregation in determining sex in *D. rotundata* yam. Accordingly, the homozygous locus state ZZ determines the male sex phenotype while the heterozygous (ZW) or hemizygous (Z-) locus state determines the female sex phenotype. This *D. rotundata* female heterogametic sex determination system (ZZ/ZW) is opposed to the male heterogametic system (XX/XY) described in other *Dioscorea* species such as *D. alata*, *D. floribunda* or *D. tokoro*. Agre et al. [19] showed that changes in sex with the growth environment are due to the W-allele's differential allelic expression in monoecious plants. Further investigations are required to elucidate the impact of the environment on the W allele for a deep understanding of mechanisms controlling the year to year spontaneous variations in sex in *D. rotundata*. It is also crucial to explore whether sex determination of *D. rotundata* could be partly under epigenetic control.

Figure 2. *Dioscorea rotundata* inflorescences showing (**a**) staminate and pistillate flowers, (**b**) staminate flowers, (**c**) pistillate flowers.

Figure 3. Monoecious plant of *Dioscorea rotundata* showing both pistillate and staminate flowers.

Table 1. Flowering intensity and flower sex distribution among genotypes of *Dioscorea* species.

Number of Observed Genotypes	% Non Flowering	% Flowering	% Male Flowers	% Female Flowers	% Monoecious	References
391	55.2	44.8	35.6	9.2	-	[20]
1938	44.7	55.3	43.5	11.8	2.8	[27]
2946	24.3	75.7	29.9	35.5	10.3	[16]
190	46.3	53.7	33.7	15.8	4.2	[19]
-	40.0	60.0	37.0	18.0	5.0	[19] *
367	43.0	57.0	35.0	12.0	10.0	[19] **
-	42.2	57.8	35.8	17.1	6.5	-

* segregating population, ** source population.

The male or female flowers are borne on axillary spikes (Figure 2). The male flowers are sessile, glabrous, and spherical and are borne axially or terminally. These flowers consist of a calyx of three sepals and a corolla of three petals, arranged regularly and almost similar in size and appearance, with three or six stamens [20,28]. The female flower is about 0.5 cm long; its ovary is trilocular with each locule having 2–3 ovules and located below the corolla [20,29]. The flowers of all *Dioscorea* spp. are entomophillous and are pollinated mainly by insects [2,5,21]. Pistillate and staminate flowers allow a three-way slit of 100–200 µm, restricting visitations to only small insects (thrips). Insects involved in yam pollination in Nigeria were identified by Segnou et al. [24]. These belong to *Coleoptera, Diptera, Hymenoptera*, *Hemiptera* and *Thysanoptera* genera. Yam pollen grains are highly vacuolated, small, and sticky. The sticky nature of the yam pollen grains, with strong adherence to anthers, makes pollination by wind impossible [20]. Akoroda [21] reported low viability of pollen from bisexual flowers on monoecious plants compared to pollen from unisexual flowers from dioecious plants of *D. rotundata*. The pollen remains viable for 4–5 h while the stigma is receptive up to 9–11 days after opening [26]. In open female flowers, stigma surfaces dry rapidly under direct sunlight, and flowers shaded by foliage usually set more fruits when pollinated than those in exposed positions. [21]. Fruit set is evidenced by an increase in ovary width to about 5 mm, 2–3 days after pollination, while fruit maturity is expected after 12 weeks [21,30].

The fruits are succeeded with the formation of dry, dehiscent, trilocular capsules (1 to 3 cm long) with each fruit bearing up to six seeds (Figure 4). The seed in each capsule is small, flat, light and has wings (freely moved by wind) that vary in shape within different species [5,28]. *D. rotundata* botanical seed is entirely encircled by the wing, whereas in *D. bulbifera* or other species, the wing is on one or both sides of the seeds, respectively. The yam seed consists of a small embryo surrounded by a relatively large endosperm (Figure 4d). Bisexual flowers usually produce deformed seedless fruits. The seed germination process was previously described by Lebot [31]. It is noteworthy that seeds from

some yam species such as *D. rotundata* are dormant for long period (3–4 months) after harvest. Others, such as *D. alata* seeds, have no dormancy and can germinate immediately after drying [30].

Figure 4. *D. rotundata* yam fruits and seeds: (**a**) Mature green fruits, (**b**) Dry fruits, (**c**) Dehisced dry fruit and released seed, (**d**) Dry winged seeds.

Yam species were domesticated independently (7000–5000 BP) across three continents (Africa, Asia and America) [6,31]. That domestication process was marked with significant evolutionary changes, characterized by a shift from sexual to vegetative propagation [6,18]. Consequently, flowering in cultivated yam species has become sparse, irregular, or absent, such that in some cases, the yam genotypes may not flower at all [27,32]. Even in flowering cultivars, seed production is often low due to a high degree of flower and ovule abortion [3,15,19]. Sexual determination is not exclusively under genetic control. Plant sex expression and flowering intensity and duration in yam crops are often influenced by growing conditions (initial tuber set size, plant health status, soil moisture, atmospheric humidity, and soil fertility, etc.) and parts of the propagules used for planting [15,16,21,23,24,33,34]. Intensity of flowering and fruiting is lower in plants from previous vegetative propagation compared to recent seedling-derived clones [16,35]. It is noteworthy that flowering is low or absent at the seedling (F_1) and first clonal generations following crosses [19,23]. Some yam varieties such as *Abe* in Nigeria flower, but their pollen grains are unviable [21].

According to Girma et al. [27,36], there is a correlation between the ploidy level and the flower type in *D. rotundata.* All triploids express either male or non-flowering behaviour as opposed to their diploid counterparts. Abraham and Nair [37] established a relationship between the ploidy status and the sexual fertility in *D. alata*; female plants with $2n = 40$ formed viable seeds and male clones with the same chromosome number ($2n = 40$) were highly fertile. Female clones with $2n = 60$ (triploids)

were mostly sterile. Lebot et al. [15] reported variation in pollination efficiency, seed set and seedling survival in crosses involving diploid × diploid, diploid × tetraploid, and the same pollen parent in different cross-combinations.

Staggering planting, proper staking, soil moisture and fertility management could improve plant vigor, profuse and long flowering window, necessary for high seed production in a crossing block. Also, marker prediction for cross-compatibility, flowering intensity and sex type in plants at the early growth stage could enhance the hybridization planning, and thus, increase the probability of high pollination success in a breeding program [19].

Interspecific crosses among yam species are often unsuccessful [16] and thus, there is a need for integration of direct in vitro pollination for overcoming incompatibility barriers. However, spontaneous interspecific hybrids of white Guinea yam with its wild relatives (*D. praehensilis*, *D. abyssinica* and *D. burkilliana*) are observed in West Africa and largely contribute to genetic diversity of cultivated yams [38,39]. Spontaneous and controlled crosses between *D. alata* and *D. nummularia* were reported by some studies [15,40]. In an interspecific hybridization study, Akoroda [21] recorded a high fruit set (46%) on *D. rotundata* × *D. praehensilis* crosses than *D. rotundata* × *D. cayenensis* crosses (2–8% and which were mostly seedless). More successful interspecific hybridizations between *D. rotundata* and *D. cayenensis* were reported at IITA [32]. *D. japonica* × *D. opposita* pollination was reported by Araki et al. [41]. All the attempts to cross Guinea yam species (*D. rotundata* and *D. cayenensis*) and *D. alata* by IITA were unsuccessful [3]. Most interspecific crosses' failures are associated with differences in ploidy levels among yam species [40].

3. Pollen Viability Assessment

Although flowering, pollen quantity and viability are of secondary importance for farmers in clonally propagated crops, they are essential in breeding programs to develop new varieties. Besides, pollen plays a role in incompatibility and fertility studies, genetics, gene bank maintenance, germplasm exchange without the threat of disease transfer, evaluation of dispersal and gene flow [42,43].

Based on the definition of Dafni and Firmage [42], the term "pollen viability" is an umbrella term that describes pollen's ability to live, grow, germinate, or develop. Several methods are used to assess pollen viability. These include the in vitro germination, the vital staining of pollen grains, in vivo fertilization and seed development, and the impedance or optical flow cytometry [43,44].

3.1. In Vitro Germination

This method consists of assessing the germination of pollen on a nutritive medium. The pollen grains are considered viable if their tubes elongate to a length that is at least their diameter [42,45]. The percentage of viability and germination is determined considering the number of viable and germinated grains with respect to the total number of grains found in each microscopic field, containing around 100 to 200 pollen grains. Although the manual count is common, there are automated and semi-automated counters which determine the viability of assessed pollen using morphometric software [43].

The optimum composition of culture medium must be determined for each crop for reliable in vitro germination tests [43,45,46]. The most commonly used medium is the Brewbaker and Kwack (BK)'s medium, with slight modifications for each crop. The initial BK medium advocated using distilled water with 10% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2{\cdot}4H_2O$, 200 ppm $MgSO_4{\cdot}7H_2O$, 100 ppm KNO_3 [47]. In most crops, there is a need to optimize the concentration of the boric acid, calcium nitrate, sucrose and the pH as requirements may vary with species [45]. The inclusion of polyethylene glycol in the medium has helped to improve germination percentages in several species [46]. Although varying with species, optimum pollen germination temperature for most crops is 25 °C [43].

In vitro germination percentage of yam pollen is usually low, although there is high variability among
species and cultivars. Viability ranges from 0.3–85% for composite pollen of *D. rotundata* [21] and

20–98% for *D. alata* [33]. In previous works on yam, BK medium was used in its initial recommendation without optimizing any of the elements which are known to change with species [20,48,49]. Therefore, a more suitable medium during in vitro pollen germination tests is needed for more accurate and reliable quality testing of pollen viability in yam breeding programs.

3.2. Staining Methods

Staining is one of the most commonly used methods to assess pollen viability in different crops. It is less demanding in labor (no preparation of culture medium) and time (30 min vs. 3–24 h) compared to the in vitro germination method. This method is based on metabolic activity (usually respiration rate) and membrane semi-permeability of pollen grains [46]. It uses staining dyes to discriminate among viable and non-viable/aborted/dead pollens. Main staining dyes include the fluorescein diacetate (FDA), Alexander, 2,3,5-triphenyl tetrazolium chloride (TTC), 2,5-diphenyl monotetrazolium bromide (MTT), acetic carmine, benzidine-H_2O_2, blue ink dyeing, methylene blue, I_2-KI, inorganic acid, and peroxide staining methods [50]. The effectiveness and suitability of these staining methods depend on the species. Therefore, an optimization assay is always necessary before deciding on adopting these methods in a crop breeding program. Acetocarmine was previously used by default to assess the yam pollen viability without prior testing [48,49]. Hence, there is a need to assess and identify the most effective or suitable staining dyes for estimating yam pollen viability.

The percentage viability of a pollen sample is determined, considering the number of viable grains with respect to the total number of grains found in each microscopic field. This counting can either be manual, semi-automated or automated [51]. Manual counting consists of counting stained and unstained pollens observed in fields, under compound microscopes. Automated and semi-automated devices for pollen count and viability assessment use software to process a series of images captured by the camera associated with the microscope [51]. Features of this automated pollen counter, its advantages and the prerequisites for its use in yam breeding programs are described in Section 3.5.

Although the staining test is most rapid, correlations with germination are not always high. Towill and Walters [46] explained that a pollen grain can be viable in the sense of being alive, retaining membrane semi-permeability and metabolizing, but may have lost the ability to germinate, or the ability to fertilize an egg. Unfortunately, this cannot be determined using staining methods. Besides, non-viable grains often absorb stain and lead to unreliable conclusions [45,52]. There is, therefore, the necessity of validating results provided by this method.

3.3. In Vivo Germination

In vivo pollen germination is time-consuming but remains the most suitable testing method for pollen grain viability. The pollen tube length is measured after a pre-determined time interval or by assessing the development of fruits and seed production [42,43]. A high viability level is not often required for this method as the fruit set occurs when the stigma is treated with only 5% viable pollen [43]. This method seems more practical for yam as in a pollen sample, only a few viable pollen grains are needed to fertilize the six ovules of a female flower. Akoroda [21,48] reported no significant correlation between the degree of fruit set and in vitro germination percent as pollen samples with low percent germination gave satisfactory fruit set.

In vitro germination is often enough in testing pollen viability as its correlation with the in vivo pollination is high for most crops. On white Guinea yam, Ng and Daniel [53] established a positive relationship between in vitro germination and fruit set after hand pollination. That positive relationship was also confirmed by Volk [43]. However, it is important to note that pollen could be viable but fail to germinate in field conditions due to stigma non-receptivity and/or unfavorable environmental conditions [42].

3.4. Impedance and Optical Flow Cytometry

Impe et al. [44] showed that impedance flow cytometry could allow more refined pollen viability estimations. This method measures the electrical cell properties of single cells using microfluidic chips. Pollen grains are stimulated by radio frequencies from 0.1 to 30 MHz with alternating current and resulting data are related to cell size, membrane capacitance, cytoplasmic conductivity of single cells, and cell concentration. A high correlation was found between this method's results and vital staining using the FDA on several crops such as cucumber, sweet pepper, and tomato [54]. This method is much faster, non-destructive, non-invasive and a high number of cells can be analyzed quickly. It does not require staining dye for analysis, sophisticated laboratory or trained labor and can be used for all species. Its accuracy is much higher as it is not influenced by the pollen's developmental stage, environmental conditions, or growing medium [54]. Luria et al. [52] demonstrated a much higher accuracy when optical flow cytometry was associated with a reactive oxygen species (ROS) probe dichlorodihydrofluorescein diacetate (i.e., H2DCFDA-staining). Luria et al. [52]'s method presents two major advantages over the classical impedance flow cytometry by Heidmann et al. [54] in studying pollen physiology. It allows the incorporation of labeling techniques and pollen sorted can be used after the test. There is no published report on the use of the flow cytometry for pollen viability estimate in yam. It should be tested to ascertain its effectiveness and correlation with other testing methods for pollen viability.

For reliable recommendations on pollen viability testing for yam breeding programs, the above-described methods should be assessed to determine the most convenient for time and resource requirements and correlations with successful fruit and seed sets using both fresh and stored pollen.

3.5. Imagery in Pollen Quality Assessment

Phenotyping of pollen traits is expensive, labor-intensive and time-consuming for plant breeding programs. Pollen counting is incredibly cumbersome due to the tiny size of the pollen grains. The manual pollen counting method used in previous yam studies is laborious, time-consuming and limits the number of accessions and samples that can be evaluated [26,48,49]. It is also prone to various errors, including colours' appreciation, counting, and data recording errors [51]. An alternative method for pollen counting and viability assessment is, therefore, necessary. In response to that challenge, automated and semi-automated devices for pollen count have been developed and used in several crops. Automated counters consider both the size and/or reaction of the pollen membrane to the staining dye. Viable and unviable pollens stain differently, facilitating the counter to dissociate the two groups [55]. The size of viable pollen is significantly larger than the dead pollen. Thus, the morphometric software easily distinguishes between viable and dead pollen grains based on the size [56].

An automated pollen count and viability assessment are commonly performed in two steps; (1) acquisition of microscopic images (using microscope equipped with a camera), and (2) the analysis of those images using software to count and discriminate pollen grains based on their viability status. ImageJ from the Fiji platform is the most commonly used software for biological image analysis, including pollen. The pollen counter can determine the pollen number in less than 60 s compared to 5–68 min required for a manual count [57]. A similar trend was reported by Ayenan et al. [51] as the semi-automated pollen counter was 32-fold faster than manual counting. The counting method by Kakui et al. [58] allowed shortening the required time to 3 min per flower (more than five times faster than the counting chamber method). Automation of pollen count is, therefore, time and labor-saver and thus allows simultaneous screening of multiple genotypes. Specific information on optimizing image-based software depends on the crop (its pollen size and circularity). The association between manual count and this morphometric software is high ($r > 0.75$) if the optimization is done correctly [51,55].

4. Pollen Storage and Longevity

In addition to standardizing the pollen viability testing methods, the establishment of short-, medium- and long-term pollen preservation methods is necessary to allow timely pollination when possible. Furthermore, pollen storage is useful for exchanging germplasm, diversity conservation, studies in basic physiology, biochemistry, in vitro fertilization, and transformation [46]. Other reports showed that the use of controlled pollen might reduce the spread of pathogens carried by pollinators and eliminate the need for isolation as male plants are not continuously grown [49,59].

4.1. Pollen Collection

Fresh yam pollen is collected in vials from 10 a.m.–2 p.m. from recently opened male flowers [48,49]. For harvesting pollen with reduced moisture content, 12–2 p.m. is recommended unless wet-cold storage is planned or prior dehydration before storage. Pollen grains are tiny (15–20 µm) and, thus, difficult to dissociate them from dehisced anthers' thecal walls. Therefore, excised anthers [48], a full flower with mature anthers [49] or flower buds [53], are tightly closed in vials and sealed with film tape for storage.

4.2. Storage Methods

Protocols for preserving yam pollen viability up to two year-period have been suggested using dry- and wet-cold preservation methods [48,49,53]. Using the dry-cold preservation method (in which pollen is desiccated to specific moisture content), Akoroda [48] found that pollen viability could be preserved for a year with low relative humidity (0%) at the temperature of −5 °C. Wet-cold preservation consists of hermetic cold storage without prior drying of pollen grains. Using this method, Daniel et al. [49] reported that pollen frozen at −80 and −20 °C conserved both viability and germination capacity for two years. In both studies, temperatures above 0 °C were not suitable for pollen preservation as the pollen viability and germination were lost shortly. However, the implementation of protocols from these studies in yam breeding programs in West Africa has been slow. This slow uptake could be attributed to the low reproducibility of protocols developed by Akoroda [48] and Daniel et al. [49]. Minimal factors were studied and controlled in their studies. In addition to standardizing these protocols, improvements need to be suggested to ensure optimum results and their full uptake by breeding programs, even for those with limited logistics in West Africa.

For the long-term storage of pollen, cryopreservation is another option for yam breeding programs. Cryopreservation is a biological technology that allows the safe storage of living material in liquid nitrogen (−196 °C) or the vapor phase of liquid nitrogen at −135 °C [60]. It is a multi-step process, involving (i) the initial excision of the germplasm such as seed embryos or shoot tips to obtain sufficiently small material; (ii) desiccation or preculture on osmotic media to reduce water content; (iii) cryoprotection through exposure to cryoprotective agents (CPAs) to promote vitrification; (iv) cryopreservation in liquid nitrogen; (v) rewarming; and finally (vi) the washing (unloading of CPA solutions) and recovery of the germplasm after cryopreservation [61]. It presents several advantages: the need for limited space for storage, minimal upkeep, and low risk of contamination. It is applicable to a range of plant tissues such as pollen, seed, shoot tips, somatic and zygotic embryos, cell suspensions, dormant buds, etc. [62,63]. High-quality pollen, dehydrated to optimal moisture content and stored at liquid nitrogen temperatures, can be stored for over 10 years [43,59]. However, several reports showed decreased pollen viability after cryopreservation in most crop species [46,60]. Intrinsic characteristics of species and inadequate ante- and post-storage handling procedures are critical factors for loss of viability. Among these factors, the inadequate determination of pollen moisture content, failure of selecting appropriate cryoprotectants and thawing methods are the most detrimental. These can be seen as results of poor understanding of the pollen biology and physiology. At the time of writing this review, no attempt has yet been made to determine the effectiveness of cryopreservation in conserving yam pollen's viability.

4.3. Factors Affecting Stored Pollen Longevity

Past efforts in pollen storage for yam are simply observations made after storing pollen under what may be considered logical conditions without exploring factors affecting pollen longevity. As it has been the case on other crops, investigations should be conducted to determine factors linked to the longevity of stored pollen of yam. Several studies agree on the most critical factors or parameters affecting pollen longevity regardless of the species. These are either linked to the species' intrinsic characteristics or environmental and handling factors [42] (Figure 5). Basic parameters include the cultivar/species, initial water content, equilibrated (desiccated) water content, rehydration methods, and handling procedures [46]. Other factors include the presence or absence of sucrose and polysaccharide; these two confer greater longevity to pollen by protecting the membrane from desiccation or temperature stress [42,43]. The storage temperature is also a critical factor; low temperatures increase pollen longevity. Other factors to consider are the age or developmental stage at which the pollen is collected and levels of unsaturated fatty acids, these two factors shorten pollen shelf life. Brief, storing desiccated pollen under lower temperatures in an oxygen-free atmosphere improves longevity [42,43].

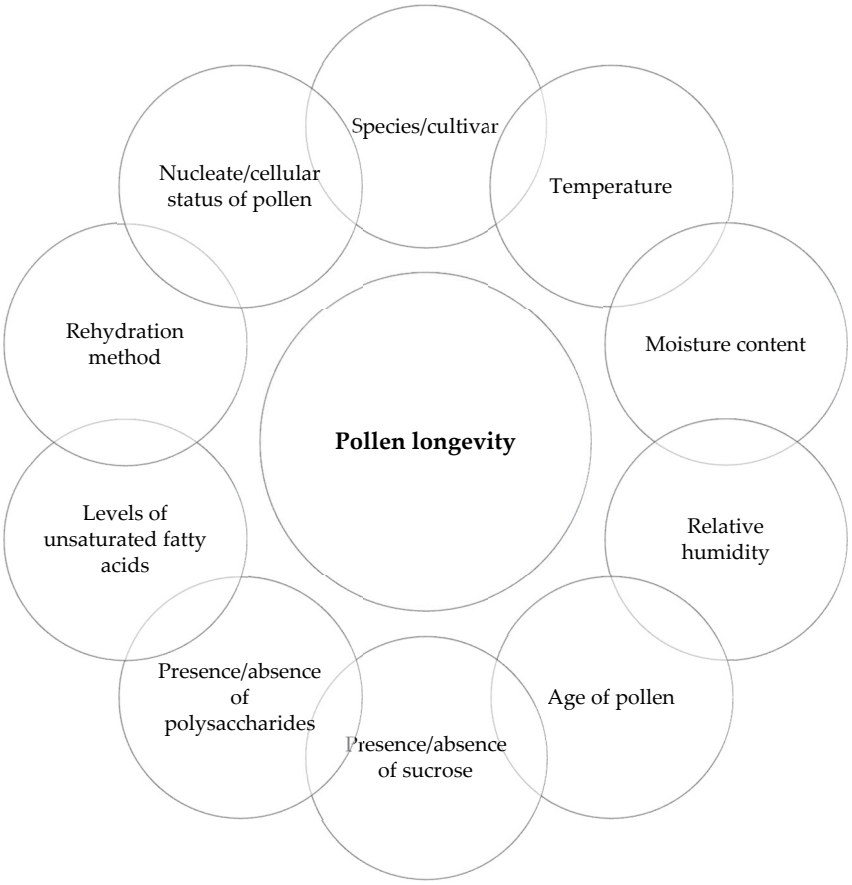

Figure 5. Factors affecting longevity of stored pollen.

Pollen desiccation can be achieved using different methods such as silica gel for 48 h at room temperature (22 °C), at 15 to 30% relative humidity (R.H.) [43]. If the ambient humidity is low, Towill and Walters [46] suggested that desiccation can be achieved by simply leaving pollen or anther on a laboratory bench. Exposure to saturated solutions of various salts (with well-defined R.H.), treatment with vitrification solutions and vacuum desiccation at 5 kPa for 40 min are other alternatives retrieved from the literature on pollen drying [59]. Although species-specific, the optimum moisture content of 15% is acceptable for several species before cold storage. Much lower moisture content (8–10%) is recommended when the pollen is cryopreserved to avoid the formation of ice crystals that

could damage the cell membrane. Some desiccation sensitive species lose their viability when dried below 25%, while most desiccation-tolerant may survive moisture content below 5% [46].

For yam, Akoroda [48] used silica gel for drying pollen and suggested that harvesting pollen from 12–2 p.m. at Ibadan (Nigeria) when the weather is dry could allow collecting pollen with reduced moisture content and thus omit the dehydration step. That study reported that pollen desiccation had more positive effects on yam pollen longevity than reduced temperature. Daniel et al. [49] held yam pollen in uncapped vials (2 days, 5 °C, 35% R.H.) to reduce the initial moisture content. Poor results achieved by Daniel et al. [49] were attributed to species desiccation sensitivity. Still, more refined studies needed to confirm this conclusion to ensure their observations were not merely a consequence of inappropriate pollen handling.

On the other hand, the choice of storage temperature is based mainly on storage objectives; long-term storage requires the lowest temperatures compared to short and medium-term repositories. As highlighted previously, −20 to −80 °C allowed extending yam pollen viability for up to two years [49]. Much lower temperatures should then be investigated in case longer conservation periods are desired.

There is an established relationship between the nucleate or cellular status at which the pollen is released by species and their tolerance to desiccation and, consequently, their storage longevity potential. Species with binucleate/bicellular pollen are classified as desiccation-tolerant while those with trinucleate/tricellular pollen are often sensitive to drying (i.e., desiccation sensitive), even though many exceptions have been reported [46,59]. No study has reported on the nucleate or cellular status of the yam pollen and, therefore, its determination would be crucial to better plan its optimum storage. The cellular nature of pollen can be identified using DAPI (4',6-diamidino-2 phenylindole) staining and imaged using an inverted fluorescent microscope [64].

4.4. Post-Storage Practices, Pollen Retrieval and Usage

We have seen from previous sections that pollen stores well for several months but can lose viability depending on post-storage practices. This is mainly the case when the pollen was stored at lower temperatures. Quick warming of pollen can result in serious injury (referred to as imbibitional injury) and or death of pollen grains [46].

Commonly for storage in refrigerator and freezer above −20 °C, vials containing pollen are open and placed in a 100% R.H. environment for 1 to 4 h at room temperature [43]. For pollen from low temperatures in the freezer (<−20 °C) and cryostorage (−196 °C to −135 °C), rewarming or thawing can either follow the slow or the short pathway. The slow path involves thawing the pollen grains for 30 min in the freezer (−20 °C), then 30 min in the refrigerator (4 °C) and then ambient conditions (25 °C) for the same period or leaving the cryotubes in running water at 25 °C for 15 min [59]. For the quick thawing pathway, tubes with pollen grains are put into a water bath at 37 °C for 5 min. The response to these thawing methods is species-specific and thus, calls for prior testing before adopting the optimum pathway for our target crop (yam).

5. Pollination Practices, Constraints and Tips for Improving Efficiency

5.1. Yam Crossing

Many genotypes of yam species are dioecious and an obligate out-crosser. Hence, reproductive success depends on pollinators, either by an insect intervention or hand transfer of pollen grains from the male to female flowering plants. In case of artificial hand pollination, multiple planting dates are recommended to control asynchrony in flowering or simply planting the female crossing block 2 to 4 weeks before the male counterpart. Male and female crossing blocks are space planted ~500 to 1000 m apart to avoid unintended pollination by insects [3]. When targeting natural pollination, male and female individual plants are grown closer to each other and their vines staked to the same supports to facilitate insect pollination, mostly by thrips [21,40]. Open pollination is cost-effective and

convenient, especially when using profuse flowering, fertile, and compatible parents; although the progenies' male parent usually needs verification [13,40].

In controlled pollination blocks, the female spikes containing mature flowers are carefully bagged (ideally using thrip-proof cloth-bags) 2 to 7 days before they open to prevent pollination from unwanted pollen sources [24]. Male flower viability is very short (~4–5 h) and usually open at noon. Thus the suitable time for pollination is from 12 noon to 3 p.m., after which pollen viability decreases significantly [16,26]. Pollination time can be enhanced by opening the male flowers 1 h before their natural opening to take out the anther and deposit it on the surface of the stigma. The pollinated flowers are then kept bagged for two weeks to keep off contamination from unwanted pollen and ensure the offspring's purity [40]. Abraham and Nair [26] recommend using female flowers at least one day after anthesis for better results.

5.2. Time of Pollination

Several attempts have been made to improve the pollination efficiency by determining the optimum time for crossing [16,26,65]. However, the optimum time for pollination is site-specific and seems to be dictated by local weather conditions in which the crop is grown (Table 2). Abraham and Nair [26] concluded that high relative humidity and atmospheric temperatures are the leading factors for successful pollination. Unfortunately, these factors are also among the most affected by climate change, and therefore, the trends recorded 30–40 years ago may have changed. There is a need for studies to ascertain whether the optimum time for pollination recommended is still viable or affected by the changes in climate over the 4 last decades in West Africa.

Table 2. Recommended pollination times for different yam growing regions.

Country	Species	Optimum Time for Pollination	% Pollination Success	References
India	*D. rotundata*	9.00–12.00 noon	-	[66]
India	*D. alata*	2.00 p.m.	-	[33]
Nigeria	*D. rotundata*	12.00–2.00 p.m.	28.0	[16]
Côte d'Ivoire	*D. rotundata* *D. cayenensis*	9.00 a.m.–1.00 p.m.	-	[25]
Guadeloupe	-	9 a.m.–1 p.m.	-	[67]
India	*D. alata*	12.00–3.00 p.m.	74.5	[26]
Côte d'Ivoire	*D. rotundata*	7.30–11.00 a.m.	8.3	[17]
Côte d'Ivoire	*D. alata*	7.30–11.00 a.m.	19.7	[68]
Nigeria	*D. rotundata*	12.00–2.00 p.m.	35.2	[21]

Notice: The difficulty of finding complete information on weather data during study periods makes it difficult to compare and understand optimal conditions for flower opening, closure, and pollination success for these regions.

Although 12 noon to 3 p.m. is theoretically most suitable for yam pollination in Nigeria, hand pollination is seldom practiced at mid-day. Predominant sunny conditions do not facilitate human work, as crossing blocks are in open fields. Technicians most conveniently operate from 8 a.m.–12 noon when the temperature is conducive for human labor. However, no study has focused on the efficiency of pollination in that period compared to the recommended time by the existing literature.

5.3. Pollination Tools

Hand pollination uses a brush, splinter or dropper [16,21,65]. Compared to the two others, the brush method gave constantly higher fruiting rate (41% vs. 16 and 3% for splinter and dropper, respectively) [21]. Good results were achieved by Abraham and Nair [26] using pencil method for excision, removal and deposit of anthers on the stigma. To overcome the challenges of handling tiny flowers, a magnifying glass can be worn (Figure 6).

Figure 6. Some tools for increasing pollination efficiency in yam breeding. (**a**) Use of magnifying glass to ease the difficulty of handling tiny flowers, (**b**) adapted pin for transfer of anthers to stigma, (**c**) thrip-proof cloth-bags allowing passage of oxygen, (**d**) ladder to reach all flowers in taller yam plants.

5.4. Mating Designs and Pollination in Yam Breeding

The mating design in plant breeding refers to a schematic system of crossing to create variability from which transgressive segregants are selected to make new cultivars. In yam breeding, both unsupervised (open-pollinated incomplete pedigree where only one parent is known) and supervised (complete pedigree with both parents known) mating designs are used [69,70]. Unsupervised mating is often achieved with natural open pollination using polycross blocks. Under this mating scheme, several clones are planted in a crossing block to facilitate random pollination by local insects. Planting arrangement is crucial for producing sufficient crossbreed seeds in polycross blocks. A completely randomized block design is suitable for less than 10 parents while the lattice design is recommended for more than 10 parents [71]. Random arrangement of genotypes ensured by these designs allows maximum recombinations in developed progenies. Many replications (20–30) are required to maximize suitable matching conditions for a high seed production in a crossing block. Akoroda [21] recommended an appropriate ratio between male and female plants (higher male-to-female ratio) for a successful open-pollination by insects. He also suggested that the distance between male and female parents be reduced to favor pollination by thrips. Based on the experience, IITA yam breeding team uses 1 m × 1 m as the optimal distance between a male and a female parent for insect pollination. Although easy and cheap, the open pollination makes some genetic analyses (specific combining ability, broad and narrow sense heritability) difficult as the male parent of a cross is not often known [69]. Its success is also uncertain for species with asynchronous flowering. Besides, natural pollination by insects has been found inefficient such that the absence of fruit sets

on many flowers is attributed to no pollination [21,26]. The unsupervised design can be improved using markers to accurately retrace parentage relationships in the half-sib progenies. The paternity of 56 to 100% progenies from polycross mating design was identified using markers by Norman et al. [13]. To avoid pollen from unknown parents, the polycross crossing block should be isolated from other yam fields or forests by at least 500–1000 m [3].

The hand pollination (also referred to as bi-parental mating design or full-sib breeding or paired crossing design) is used to control some of the drawbacks of open pollination. It is 2–3 times more efficient than natural pollination by insects [16,24]. It consists of crossing selected desirable males and females. Supervised paired mating design generates full-sib progenies with known pedigree. Thus, it facilitates all genetic analyses such as the estimation of narrow-sense heritability, general and specific combining abilities, additive and non-additive variances, etc. [69]. However, the tiny size of flowers, asynchrony in flowering, incompatibility among parents and the relatively short life of pollen (4–5 h) are challenges for the bi-parental mating design. It is also costly and time-consuming if a large number of progenies are to be generated [15,70]. Due to the expertise/skill required to transfer anthers from male flowers to the stigma, the technicians involved in pollination need to be carefully selected and trained to increase the pollination efficiency in a breeding program.

Norman et al. [70] suggested a complementarity between unsupervised polycross and supervised nested mating (North Carolina design I) designs for optimizing population improvement. They found that total fruit sets were lower under nested mating (26%) than the polycross design (44%). However, filled seeds per fruit were higher in nested mating than in polycross design.

Tamiru et al. [23] developed SNP (single nucleotide polymorphism) marker for yam plant sex identification at the early seedling stage. The sex phenotype prediction with markers facilitates male and female plants' identification for efficient crossing plan. The SNP marker sp16 is on white yam pseudo-chromosome 11 and tightly associated with femaleness within a female heterogametic sex-determination system. However, the sp16 marker only predicts the likelihood of femininity and may not be transferable to other species. Dinadi et al. [72] showed that although the absence of sp16 marker confirms the male sex of the individuals, its presence does not allow sex discrimination. From their study, it appeared that the best determinant of stable male cultivars is the absence of the sp16 marker and that the best determinant of stable female cultivars is the AA genotype of the sp1 marker. Further investigations are encouraged as sex determination in white Guinea yam would be controlled by more than one locus [72]. Electrochemical sex determination of *D. zingiberensis* and *D. bulbifera* plants using polydopamine-functionalized graphene sheets has been successful [73]. SuperSAGE transcriptome profiling allowed identifying 88 tags associated with male, female and monoecious sex expression, and among which, DnaJ-like protein and Transketolase were specific for sex determination [27].

Furthermore, Norman et al. [13] demonstrated the SNP markers' potential in the accurate identification of parentage relationships in the half-sib progenies of the white Guinea yam. Plant sex prediction combined with pedigree reconstruction would help ascertain the genetic progress in yam breeding.

6. Manipulation of Flowering: Lessons from Other Root and Tuber Crops

Table 3 presents practices applied in other root and tuber crops to improve flowering and pollination efficiency. These practices range from agronomic to biotechnological and hold potential to improve the pollination efficiency in yam. These are grafting, proper soil fertility management, choice of most suitable sites, treatment with growth regulators, photoperiod manipulation, gene editing, and genetic transformation. There are no reports on the application of such practices to improve flowering efficiency in yam. Root and tuber crops share, at varying degrees, similar self, and cross incompatibility problems. Thus, biotechnology tools such as ploidy manipulation, in vitro androgenesis or gynogenesis induction, embryo rescue, mentor pollination, somatic and protoplast fusion should be encouraged.

Table 3. Manipulation of flowering and pollination in root and tuber crops with potential application in yam.

Practices	Objectives	Species	References
Manipulation of ploidy with due regard to endosperm balance number (EBN)	To reduce cross-incompatibility among species	*Solanum tuberosum* L.	[74,75]
Double haploid technology through in vitro androgenesis or gynogenesis induction	To reduce cross-incompatibility among species. To develop inbred or homozygous lines in one step.	*Solanum tuberosum* L.; *Manihot esculenta* Crantz	[75–77]
Embryo rescue	To secure a hybrid where embryo abortion is due to a defective endosperm	*Solanum tuberosum* L.; *Manihot esculenta* Crantz	[75,78,79]
Mentor pollination	To achieve fertilization through a second pollination with compatible pollen to help incompatible pollen	*Solanum tuberosum* L.	[75,79]
Somatic (protoplast) fusion	To achieve difficult or impossible sexual hybridizations	*Solanum tuberosum* L.; *Ipomoea batatas* (L.) Lam; *Manihot esculenta* Crantz	[75,80,81]
In ploidy manipulation	To achieve maximum heterozygosity	*Solanum tuberosum* L.	[75]
Grafting	To induce intense flowering	*Ipomoea batatas* (L.) Lam.; *Manihot esculenta* Crantz; *Solanum tuberosum* L.	[79,80,82–85]
Reduced nitrogen fertilizer	To favor the flowering over lush and leafy vines	*Ipomoea batatas* (L.) Lam; *Manihot esculenta* Crantz	[80,86]
Gene editing using CRISPR–Cas9 system	To knock out the self-incompatibility gene *S-RNase* for potato. To incorporate PTST1 gene for earlier flowering in cassava.	*Solanum tuberosum* L.; *Manihot esculenta* Crantz	[84,87]
Treatment with growth regulators such as gibberellic acid, auxins, abscisic acid, ethylene, ascorbic acid, etc.	To induce earlier, profuse and long flowering	*Colocasia esculenta* L.; *Manihot esculenta* Crantz; *Solanum tuberosum* L.	[62,82,83,85,88,89]
Leaf removal	To induce artificial flowering	*Colocasia esculenta* L.	[88]
Drought and thermal stress induction	To induce artificial flowering	*Colocasia esculenta* L.; *Manihot esculenta* Crantz	[83,86,88]
Extended photoperiod using red light treatments at night	To induce early and profuse flowering	*Manihot esculenta* Crantz	[84,86]
Genetic transformation	To increase the level of *FT* genes; FLOWERING LOCUS T-like gene *MeFT1* to induce early flowering	*Manihot esculenta* Crantz	[83,90–92]
Choice of location with longer photoperiods and cooler temperatures	To induce early and profuse flowering	*Manihot esculenta* Crantz; *Solanum tuberosum* L.	[79,86,92]
Application of the anti-ethylene growth regulator silver thiosulfate (STS)	To increase flower production and longevity; to reduce early abortion	*Manihot esculenta* Crantz	[89]
Pruning young branches soon after flowering	To prevent the common abortion of first inflorescences	*Manihot esculenta* Crantz	[93]
Spraying of benzyladenine in pruned plants for cassava or foliar spray with GA_3 for potato	To promote flower development and feminization of male flowers	*Manihot esculenta* Crantz; *Solanum tuberosum* L.	[82,93]
Vibratome sectioning and clearing	To develop and optimize a methodology to induce doubled haploids via gynogenesis or from ovules pollinated with irradiated pollen	*Manihot esculenta* Crantz	[94]
Use of pollen mixture	To reduce cross incompatibility barriers among cultivars or species	*Solanum tuberosum* L.	[79]
Identification of matching parental groups	To reduce cross incompatibility barriers among cultivars or species	*Solanum tuberosum* L.	[79]
Increased plant density	To increase flowering intensity and seed weight	*Solanum tuberosum* L.	[82]
Shading of glasshouse to reduce temperature	To increase flowering intensity	*Solanum tuberosum* L.	[82]
Girdling or constriction of the stem	To increase flowering intensity	*Solanum tuberosum* L.	[79,82]

7. Insect Pollinators' Identification and Management

Insects play a crucial role in yam pollination. Segnou et al. [24] identified the most predominant insects visiting white Guinea yam in Nigeria. These are small insects (thrips) belonging to *Larothrips, Thysanoptera,* and *Thripidae* genera. Other pollinators are from the *Coleoptera, Diptera, Hemiptera* and *Hymenoptera* genera. Thanks to their small sizes (of less than 1 mm long and about 0.2 mm broad), they penetrate both staminate and pistillate flowers and play a role in yam pollination [20,24]. However, these insects' inefficiency is admitted as a major factor of low natural pollination success [20,21,24]. The inefficiency of insect pollination was attributed to low visitation rate, limited movements and selectivity [21,24,95]. For instance, Akoroda [21] concluded that low fruiting is rather a result of no visitation than of sterility per se. For this particular reason, this author advised that the distance between individual plants be reduced to increase chances of visitations. Besides, these pollinators are selective. Some species of yam such as *D. composita* and *D. floribunda* are seldom visited by insects. Their natural fruit sets are low: 0.9 and 4.5%, respectively [95]. These authors reported that flowers of species such as *D. spiculifora* attracted numerous flies and ants and had a fruit set above 90%. To achieve effective pollination, plants must produce food (high floral scent-effusion concentration) for insects to make visits worthwhile [24].

On the basis of the above facts, increasing insect population in a crossing block would result in improved pollination success. Use of hormones such as pheromones or plant volatiles, the selection of parental lines with abundant floral scent-effusion and commercial production of yam insect pollinators are to be explored. No attempt to increase pollinator populations on yam is reported.

The effort toward insect pollinators' rearing should start with collection. Segnou et al. [24] used a mouth aspirator to collect yam pollinators from 10 a.m. to 4 p.m. Potential yam pollinators' identification is based on three criteria that should be fulfilled simultaneously: (1) operate during the flowering blooming months; (2) being on- and around-yam fields at the day period (10 a.m. to 4 pm) when sweetly scented yam flowers are open to attract pollinators and; (3) smaller size as to be able to penetrate tiny yam flowers [24].

The next step for insect rearing will be to gather knowledge on factors likely to influence or constrain pollinators' growth and abundance. These factors include seasonality; habitat and distribution; lifecycle, generations, adult longevity, and fecundity; food requirements and climate [96]. Other factors such as pesticide sensitivity, presence of predators and parasitoids, inter-specific competition for resources and phenotypic variation among individuals should be considered [96]. Technical and safety considerations should not be overlooked in the choice of field-crop pollinators. These include the ability to mass rear insect pollinators at an appropriate scale; set measures for retaining numbers within targeted areas in the field; and to ensure their use does not significantly impact on non-target species and land-user interests [96]. All these considerations make protocols for pollinators' rearing species-specific. In general, protocols address aspects on the establishment of a captive colony, housing, laboratory rearing, release and population density regulation on fields [97,98].

8. Conclusions

Yam pollination is a complex process and requires that many factors are concurrently controlled for its success. This review on yam reproductive biology and pollination practices reveals that the yam pollination efficiency can be improved by an integrated management strategy based on:

(1) The establishment of highly reproducible and cost-effective protocols for quality pollen collection and storage, necessary for breaking the asynchrony and low flowering barriers. These storage efforts should be complemented by agronomic and/or hormonal manipulations to enhance the flowering intensity with long flowering window.
(2) Sites selection promoting optimum flowering, fruit and seed set.
(3) Markers prediction for flower sex's phenotype and intensity at early growth stage of the plant.

(4) Profiling of genotypes for ploidy status, cross-compatibility and flowering time to identify compatible groups.
(5) Use of biotechnological approaches such as ploidy manipulation, gene editing, androgenesis or gynogenesis induction, embryo rescue, somatic and protoplast fusion, etc. to break intra and inter-specific cross incompatibility.
(6) Proper choice of mating design, rearing insect pollinators, and a continuous training of technicians involved in pollination activities.

Author Contributions: A.A., J.M.M. and P.A.A. conceived the idea and drafted the manuscript. A.E., P.A., R.A. and M.O.A. contributed in writing up and revision. M.O.A., P.A.A. and A.A. did supervision, P.A.A. and J.M.M. did formatting. All the authors read the last version and approved its submission. All authors have read and agreed to the published version of the manuscript.

Funding: The funding support from the Bill and Melinda Gates Foundation (BMGF) through the AfricaYam project of the International Institute of Tropical Agriculture (IITA) (OPP1052998) is acknowledged. The first author is grateful for the scholarship granted by the African Union Commission for his Ph.D. studies at the Pan African University-Institute of Life and Earth Sciences (PAULESI).

Acknowledgments: We are grateful for the technical support and practical experiences shared by the IITA yam breeding staff which enriched the content of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wilkin, P.; Schols, P.; Chase, M.W.; Chayamarit, K.; Furness, C.A.; Huysmans, S.; Rakotonasolo, F.; Smets, E.; Thapyai, C. A Plastid Gene Phylogeny of the Yam Genus, *Dioscorea*: Roots, Fruits and Madagascar. *Syst. Bot.* **2005**, *30*, 736–749. [CrossRef]
2. Govaerts, R.; Wilkin, P.; Saunders, R.M.K. *World Checklist of Dioscoreales*; Yams and Their Allies: The Board of Trustees of the Royal Botanic Gardens: Kew, UK; London, UK, 2007; pp. 1–65.
3. Darkwa, K.; Olasanmi, B.; Asiedu, R.; Asfaw, A. Review of empirical and emerging breeding methods and tools for yam (*Dioscorea* spp.) improvement: Status and prospects. *Plant Breed.* **2019**, *139*, 474–497. [CrossRef]
4. Gatarira, C.; Paterne, A.A.; Matsumoto, R.; Edemodu, A.; Adetimirin, V.; Bhattacharjee, R.; Asiedu, R.; Asfaw, A. Genome-Wide Association Analysis for Tuber Dry Matter and Oxidative Browning in Water Yam (*Dioscorea alata* L.). *Plants* **2020**, *9*, 969. [CrossRef] [PubMed]
5. Coursey, D.G. *Yams. An Account of the Nature, Origins, Cultivation and Utilisation of the Useful Members of the Dioscoreaceae*; Longmans: London, UK, 1967; p. 230.
6. Scarcelli, N.; Cubry, P.; Akakpo, R.; Thuillet, A.-C.; Obidiegwu, J.E.; Baco, M.N.; Otoo, E.; Sonké, B.; Dansi, A.; Djedatin, G.; et al. Yam genomics supports West Africa as a major cradle of crop domestication. *Sci. Adv.* **2019**, *5*, e1947. [CrossRef]
7. FAO FAOSTAT. Available online: http://www.fao.org/faostat/en/#data/QL (accessed on 1 September 2020).
8. Obidiegwu, J.E.; Akpabio, E.M. The geography of yam cultivation in southern Nigeria: Exploring its social meanings and cultural functions. *J. Ethn. Foods* **2017**, *4*, 28–35. [CrossRef]
9. Omohimi, C.I.; Piccirillo, C.; Roriz, M.; Ferraro, V.; Vasconcelos, M.; Sanni, L.O.; Tomlins, K.; Pintado, M.M.; Abayomi, L.A. Study of the proximate and mineral composition of different Nigerian yam chips, flakes and flours. *J. Food Sci. Technol.* **2017**, *55*, 42–51. [CrossRef]
10. McKoy, M.-L.; Thomas, P.-G.; Asemota, H.N.; O Omoruyi, F.; Simon, O. Effects of Jamaican Bitter Yam (Dioscorea polygonoides) and Diosgenin on Blood and Fecal Cholesterol in Rats. *J. Med. Food* **2014**, *17*, 1183–1188. [CrossRef]
11. Ijato, J.; Tedela, P. Phytotoxic Potentials of Cold and Hot Aqueous Extracts of Chromolaena odorata against Fungal Deteriorating Agents of Yam Tubers (*Dioscorea rotundata*, Poir) after Harvest. *Am. J. Exp. Agric.* **2015**, *5*, 262–266. [CrossRef]
12. Mignouna, D.B.; Akinola, A.A.; Abdoulaye, T.; Alene, A.D.; Manyong, V.; Maroya, N.G.; Aighewi, B.A.; Kumar, L.P.; Balogun, M.; Lopez-Montes, A.; et al. Potential returns to yam research investment in sub-Saharan Africa and beyond. *Outlook Agric.* **2020**, *49*, 215–224. [CrossRef]

13. Norman, P.E.; Paterne, A.A.; Danquah, A.; Tongoona, P.; Danquah, E.Y.; De Koeyer, D.; Ikeogu, U.N.; Asiedu, R.; Asfaw, A. Paternity Assignment in White Guinea Yam (*Dioscorea rotundata*) Half-Sib Progenies from Polycross Mating Design Using SNP Markers. *Plants* **2020**, *9*, 527. [CrossRef]
14. Di-Giovanni, F.; Kevan, P.G. Factors affecting pollen dynamics and its importance to pollen contamination: A review. *Can. J. For. Res.* **1991**, *21*, 1155–1170. [CrossRef]
15. Lebot, V.; Abraham, K.; Kaoh, J.; Rogers, C.; Molisalé, T. Development of anthracnose resistant hybrids of the Greater Yam (Dioscorea alata L.) and interspecific hybrids with D. nummularia Lam. *Genet. Resour. Crop. Evol.* **2019**, *66*, 871–883. [CrossRef]
16. Akoroda, M.O. Floral biology in relation to hand pollination of white yam. *Euphytica* **1983**, *32*, 831–838. [CrossRef]
17. Bakayoko, Y.; Kouakou, A.M.; Dibi, K.E.B.; Ehounou, A.E.; Essis, B.S.; Nzue, B.; Kouassi, A.B.; Nguetta, A.S.; Adebola, P.O. Effect of the manual pollination period on the fruiting of yams (*Dioscorea rotundata* Poir; *Dioscoreaceae*) in Ivory Coast. *Eur. Sci. J.* **2019**, *15*, 448–464.
18. Akakpo, R.; Scarcelli, N.; Chaïr, H.; Dansi, A.; Djedatin, G.; Thuillet, A.-C.; Rhoné, B.; François, O.; Alix, K.; Vigouroux, Y. Molecular basis of African yam domestication: Analyses of selection point to root development, starch biosynthesis, and photosynthesis related genes. *BMC Genom.* **2017**, *18*, 782. [CrossRef] [PubMed]
19. Asfaw, A.; Paterne, A.A.; Nwachukwu, C.; Eolasanmi, B.; Obidiegwu, J.E.; Nwachukwu, E.; Adebola, P.; De Koeyer, D. Sample Preservation and Plant Sex Prediction in White Guinea Yam (*Dioscorea rotundata* Poir.) Seedlings. *J. Appl. Biot. Rep.* **2020**. [CrossRef]
20. Sadik, S.; Okereke, O.U. Flowering, Pollen Grain Germination, Fruiting, Seed Germination and Seedling Development of White Yam, *Dioscorea rotundata* Poir. *Ann. Bot.* **1975**, *39*, 597–604. [CrossRef]
21. Akoroda, M.O. Pollen management for controlled hybridization of white yam. *Sci. Hortic.* **1985**, *25*, 201–209. [CrossRef]
22. Schmid, R.; Kubitzki, K.; Kadereit, J.W. The Families and Genera of Vascular Plants. *TAXON* **2005**, *54*, 574. [CrossRef]
23. Tamiru, M.; Natsume, S.; Takagi, H.; White, B.; Yaegashi, H.; Shimizu, M.; Yoshida, K.; Uemura, A.; Oikawa, K.; Abe, A.; et al. Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biol.* **2017**, *15*, 1–20. [CrossRef]
24. Segnou; Fatokun, C.A.; Akoroda, M.O.; Hahn, S.K. Studies on the reproductive biology of white yam (*Dioscorea rotundata* Poir.). *Euphytica* **1992**, *64*, 197–203. [CrossRef]
25. Zoundjihékpon, J.; Hamon, P.; Noirot, M.; Tio-Touré, B.; Hamon, S. Flowering synchronisation between male and female West African cultivated yams (Dioscorea cayenensis-rotundata complex). *Euphytica* **1997**, *95*, 371–375. [CrossRef]
26. Abraham, K.; Nair, P.G. Floral biology and artificial pollination in *Dioscorea alata* L. *Euphytica* **1990**, *48*, 45–51.
27. Girma, G.; Natsume, S.; Carluccio, A.V.; Takagi, H.; Matsumura, H.; Uemura, A.; Muranaka, S.; Stavolone, L.; Gedil, M.; Spillane, C.; et al. Identification of candidate flowering and sex genes in white Guinea yam (*D. rotundata* Poir.) by SuperSAGE transcriptome profiling. *PLoS ONE* **2019**, *14*, e0216912. [CrossRef]
28. Wickens, G.E.; Onwueme, I.C. The Tropical Tuber Crops: Yams, Cassava, Sweet Potato and Cocoyams. *Kew Bull.* **1979**, *34*, 418. [CrossRef]
29. Sadik, S.; Okereke, O.U. A new approach to improvement of yam *Dioscorea rotundata*. *Nat. Cell Biol.* **1975**, *254*, 134–135. [CrossRef]
30. Abraham, K. Dormancy and viability of *Dioscorea alata* seeds. *J. Root Crop* **1992**, *18*, 142–143.
31. Lebot, V. *Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids*; CABI: Wallingford, UK, 2009; p. 413.
32. Lopez-Montes, A.; Bhattacharjee, R.; Tessema, G. Yam breeding at IITA: Achievements, challenges and prospects. *R4D Review Int. Inst. Trop. Agric.* **2012**, *8*, 1–3.
33. Bai, K.V.; Jos, J.S. Female fertility and seed set in *Dioscorea alata* L. *Trop. Agric.* **1986**, *63*, 7–10.
34. Hamadina, E.I.; Craufurd, P.Q.; Asiedu, R. Flowering intensity in white yam (*Dioscorea rotundata*). *J. Agric. Sci.* **2009**, *147*, 469–477. [CrossRef]
35. Akoroda, M.O.; Wilson, J.; Chheda, H.R. The association of sexuality with plant traits and tuber yield in white yam. *Euphytica* **1984**, *33*, 435–442. [CrossRef]

36. Girma, G.; Hyma, K.E.; Asiedu, R.; Mitchell, S.E.; Gedil, M.; Spillane, C. Next-generation sequencing based genotyping, cytometry and phenotyping for understanding diversity and evolution of guinea yams. *Theor. Appl. Genet.* **2014**, *127*, 1783–1794. [CrossRef] [PubMed]
37. Abraham, K.; Nair, P.G. Polyploidy and sterility in relation to sex in *Dioscorea alata* L. (*Dioscoreaceae*). *Genetica* **1991**, *83*, 93–97. [CrossRef]
38. Scarcelli, N.; Tostain, S.; Vigouroux, Y.; Agbangla, C.; Daïnou, O.; Pham, J.L. Farmers' use of wild relative and sexual reproduction in a vegetatively propagated crop. The case of yam in Benin. *Mol. Ecol.* **2006**, *15*, 2421–2431. [CrossRef]
39. Loko, Y.L.; Dansi, A.; Linsoussi, C.; Assogba, P.; Dansi, M.; Vodouhè, R.; Akoegninou, A.; Sanni, A. Current status and spatial analysis of Guinea yam (*Dioscorea cayenensis* Lam.-*D. rotundata* Poir. complex) diversity in Benin. Int. Res. *J. Agric. Sci. Soil Sci.* **2013**, *3*, 219–238.
40. Arnau, G.; Abraham, K.; Sheela, M.; Chaïr, H.; Sartie, A.; Asiedu, R. *Yams. Root and Tuber Crops*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2010; pp. 127–148.
41. Araki, H.; Harada, T.; Yakuwa, T. Some Characteristics of Interspecific Hybrids between *Dioscorea japonica* Thunb. and *Dioscorea opposita* Thunb. *J. Jpn. Soc. Hortic. Sci.* **1983**, *52*, 153–158. [CrossRef]
42. Dafni, A.; Firmage, D. Pollen viability and longevity: Practical, ecological and evolutionary implications. *Plant Syst. Evol.* **2000**, *222*, 113–132. [CrossRef]
43. Volk, G.M. Collecting Pollen for Genetic Resources Conservation. In *Collecting Plant Genetic Diversity: Technical Guidelines 2011 Update*; Guarino, L., Ramanatha, V.R., Goldberg, E., Eds.; Bioversity International: Rome, Italy, 2011; pp. 1–10.
44. Impe, D.; Reitz, J.; Köpnick, C.; Rolletschek, H.; Börner, A.; Senula, A.; Nagel, M. Assessment of Pollen Viability for Wheat. *Front. Plant Sci.* **2020**, *10*, 10. [CrossRef]
45. Alexander, L.W. Optimizing pollen germination and pollen viability estimates for Hydrangea macrophylla, Dichroa febrifuga, and their hybrids. *Sci. Hortic.* **2019**, *246*, 244–250. [CrossRef]
46. Towill, L.E.; Walters, C. Cryopreservation of Pollen. In *Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application*; Englemann, F., Tagaki, H., Eds.; JIRCAS/IPGRI Co-Publication: Tsukuba, Japan, 2000; pp. 115–129.
47. Brewbaker, J.L.; Kwack, B.H. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.* **1963**, *50*, 859–865. [CrossRef]
48. Akoroda, M. Long-term storage of yam pollen. *Sci. Hortic.* **1983**, *20*, 225–230. [CrossRef]
49. Daniel, I.O.; Ng, N.Q.; Tayo, T.O.; Togun, A.O. Wet–cold preservation of West African yam (*Dioscorea* spp.) pollen. *J. Agric. Sci.* **2002**, *138*, 57–62. [CrossRef]
50. Luo, S.; Zhang, K.; Zhong, W.-P.; Chen, P.; Fan, X.; Yuan, D. Optimization of in vitro pollen germination and pollen viability tests for Castanea mollissima and Castanea henryi. *Sci. Hortic.* **2020**, *271*, 109481. [CrossRef]
51. Ayenan, M.A.T.; Danquah, A.; Ampomah-Dwamena, C.; Hanson, P.; Asante, I.K.; Danquah, E.Y. Optimizing pollencounter for high throughput phenotyping of pollen quality in tomatoes. *MethodsX* **2020**, *7*, 100977. [CrossRef]
52. Luria, G.; Rutley, N.; Lazar, I.; Harper, J.F.; Miller, G. Direct analysis of pollen fitness by flow cytometry: Implications for pollen response to stress. *Plant J.* **2019**, *98*, 942–952. [CrossRef]
53. Ng, N.Q.; Daniel, I.O. Storage of pollens for long-term conservation of yam genetic resources. In *Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application*; Englemann, F., Tagaki, H., Eds.; JIRCAS/IPGRI Co-Publication: Tsukuba, Japan, 2000; pp. 136–139.
54. Heidmann, I.; Schade-Kampmann, G.; Lambalk, J.; Ottiger, M.; Di Berardino, M. Impedance Flow Cytometry: A Novel Technique in Pollen Analysis. *PLoS ONE* **2016**, *11*, e0165531. [CrossRef]
55. Tello, J.; Montemayor, M.I.; Forneck, A.; Ibáñez, J. A new image-based tool for the high throughput phenotyping of pollen viability: Evaluation of inter- and intra-cultivar diversity in grapevine. *Plant Methods* **2018**, *14*, 1–17. [CrossRef]
56. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An open-source platform for biological-image analysis. *Nat. Methods* **2012**, *9*, 676–682. [CrossRef]
57. Costa, C.M.; Yang, S. Counting pollen grains using readily available, free image processing and analysis software. *Ann. Bot.* **2009**, *104*, 1005–1010. [CrossRef]

58. Kakui, H.; Yamazaki, M.; Hamaya, N.-B.; Shimizu, K.K. Pollen Grain Counting Using a Cell Counter. In *Methods in Molecular Biology*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2020; Volume 2160, pp. 1–11.
59. Dinato, N.B.; Imaculada Santos, I.R.; Zanotto Vigna, B.B.; de Paula, A.F.; Fávero, A.P. Pollen Cryopreservation for Plant Breeding and Genetic Resources Conservation. *Cryoletters* **2020**, *41*, 115–127.
60. Ren, R.; Li, Z.; Zhou, H.; Zhang, L.; Jiang, X.; Liu, Y. Changes in apoptosis-like programmed cell death and viability during the cryopreservation of pollen from Paeonia suffruticosa. *Plant Cell Tissue Organ Cult. (PCTOC)* **2020**, *140*, 357–368. [CrossRef]
61. Funnekotter, B.; Colville, L.; Kaczmarczyk, A.; Turner, S.R.; Bunn, E.; Mancera, R.L. Monitoring of oxidative status in three native Australian species during cold acclimation and cryopreservation. *Plant Cell Rep.* **2017**, *36*, 1903–1916. [CrossRef]
62. Mukherjee, A.; George, J.; Pillai, R.; Chakrabarti, S.K.; Naskar, S.K.; Patro, R.; Nayak, S.; Lebot, V. Development of taro (*Colocasia esculenta* (L.) *Schott*) hybrids overcoming its asynchrony in flowering using cryostored pollen. *Euphytica* **2016**, *212*, 29–36. [CrossRef]
63. Streczynski, R.; Clark, H.; Whelehan, L.M.; Ang, S.-T.; Hardstaff, L.K.; Funnekotter, B.; Bunn, E.; Offord, C.A.; Sommerville, K.D.; Mancera, R.L. Current issues in plant cryopreservation and importance for ex situ conservation of threatened Australian native species. *Aust. J. Bot.* **2019**, *67*, 1–15. [CrossRef]
64. Gaudet, D.; Yadav, N.S.; Sorokin, A.; Bilichak, A.; Kovalchuk, I. Development and Optimization of a Germination Assay and Long-Term Storage for *Cannabis sativa* Pollen. *Plants* **2020**, *9*, 665. [CrossRef]
65. Akoroda, M.O.; Wilson, J.E.; Chheda, H.R. Artificial pollination, pollen viability, and storage in white yam. in Tropical Root Crops: Research strategies for the 1980s. In Proceedings of the 1st Triennial Root Crops Symposium of the International Society for Tropical Root Crops-Africa Branch, Ibadan, Nigeria, 8–12 September 1980; IDRC: Ottawa, ON, Canada, 1981.
66. Nair, S.G.; Abraham, K.; Sreekumari, M.T. Stigma receptivity in white yam (*Dioscorea rotundata*, Poir.). In Proceedings of the National Symposium on Production and Utilization of Tropical Tuber Crops, Thiruvananthapuram, Kerala, India, 27–29 November 1985; pp. 71–74.
67. Bulle-Legrand, M. Etude de la floraison de quatre espèces d'Ignames en vue d'une amélioration par la voie sexuée. Ph.D Thesis, Université de Paris-Sud, Orsay, France, 1983.
68. Ehounou, A.E.; Kouakou, A.M.; N'zi, J.C.; Dibi, K.B.E.; Bakayoko, Y.; Essis, B.S.; N'Zue, B.; Arnau, G.; Maledon, E.; Asfaw, A.; et al. Production of hybrid seeds by intraspecific crossing in yam *Dioscorea alata* L. *Int. J. Sci. Res.* **2019**, *8*, 1212–1221.
69. Otoo, E. Yam Breeding in Ghana. *J. Agric. Sci.* **2017**, *9*, 122. [CrossRef]
70. Norman, P.E.; Asfaw, A.; Tongoona, P.B.; Danquah, A.; Danquah, E.Y.; De Koeyer, D.; Asiedu, R. Pollination success in some white yam genotypes under polycross and nested mating designs. *Int. J. Biol. Sci. Appl.* **2018**, *5*, 19–28.
71. Acquaah, G. *Principles of Plant Genetics and Breeding*; Blackwell Publishing: Malden, MA, USA, 2012.
72. Denadi, N.; Gandonou, C.; Missihoun, A.A.; Zoundjihékpon, J.; Quinet, M. Plant Sex Prediction Using Genetic Markers in Cultivated Yams (*Dioscorea rotundata* Poir.) in Benin. *Agronomy* **2020**, *10*, 1521. [CrossRef]
73. Fu, L.; Wang, Q.; Zhang, M.; Zheng, Y.; Wu, M.; Lan, Z.; Pu, J.; Zhang, H.; Chen, F.; Su, W.; et al. Electrochemical Sex Determination of Dioecious Plants Using Polydopamine-Functionalized Graphene Sheets. *Front. Chem.* **2020**, *8*, 92. [CrossRef]
74. Carputo, D. Cytological and breeding behavior of pentaploids derived from 3x × 4x crosses in potato. *Theor. Appl. Genet.* **2003**, *106*, 883–888. [CrossRef] [PubMed]
75. Bradshaw, J.E.; Bonierbale, M. Potatoes. In *Root and Tuber Crops*; Springer: New York, NY, USA, 2010; pp. 1–52.
76. Seguí-Simarro, J.M. Androgenesis in Solanaceae. In *Methods in Molecular Biology*; Springer Science and Business Media LLC: Amsterdam, The Netherlands, 2016; Volume 1359, pp. 209–244.
77. Lentini, Z.; González, Á.; Tabares, E.; Buitrago, M.E.; Wêdzony, M. Studies on Gynogenesis Induction in Cassava (Manihot esculenta Crantz) Unpollinated Ovule Culture. *Front. Plant Sci.* **2020**, *11*, 365. [CrossRef] [PubMed]
78. Lentini, Z.; Restrepo, G.; Buitrago, M.E.; Tabares, E. Protocol for Rescuing Young Cassava Embryos. *Front. Plant Sci.* **2020**, *11*, 522. [CrossRef] [PubMed]

79. Luthra, S.K.; Gupta, V.K.; Tiwari, J.K.; Kumar, V.; Bhardwaj, V.; Sood, S.; Dalamu; Kaur, R.P.; Kumar, R.; Vanishree, G.; et al. *Potato Breeding in India*; Technical Bulletin No 74; ICAR-Central Potato Research Institute: Shimla, Himachal, India, 2020; p. 214.
80. Lebot, V. Sweet potato. In *Root and Tuber Crops*; Springer: New York, NY, USA, 2010; pp. 97–125.
81. Wen, F.; Su, W.-P.; Zheng, H.; Yu, B.-C.; Ma, Z.-F.; Zhang, P.; Guo, W.-W. Plant regeneration via protoplast electrofusion in cassava. *J. Integr. Agric.* **2020**, *19*, 632–642. [CrossRef]
82. Muthoni, J.; Shimelis, H.; Melis, R.; Kabira, J. Reproductive biology and early generation's selection in conventional potato breeding. *Austr. J. Crop Sci.* **2012**, *6*, 488–497.
83. Hernan, C.; Jaramillo, J.; Salazar, S.; Pineda, L.; Calle, F.; Setter, T. Induction of flowering in cassava through grafting. *J. Plant Breed. Crop. Sci.* **2017**, *9*, 19–29. [CrossRef]
84. Ceballos, H.; Rojanaridpiched, C.; Phumichai, C.; Becerra, L.A.; Kittipadakul, P.; Iglesias, C.; Gracen, V.E. Excellence in Cassava Breeding: Perspectives for the Future. *Crop. Breeding Genet. Genom.* **2020**, *2*, 200008. [CrossRef]
85. Souza, L.S.; Diniz, R.P.; Neves, R.D.J.; Alves, A.A.C.; De Oliveira, E.J. Grafting as a strategy to increase flowering of cassava. *Sci. Hortic.* **2018**, *240*, 544–551. [CrossRef]
86. Pineda, M.; Morante, N.; Salazar, S.; Cuásquer, J.; Hyde, P.T.; Setter, T.L.; Ceballos, H. Induction of Earlier Flowering in Cassava through Extended Photoperiod. *Agronomy* **2020**, *10*, 1273. [CrossRef]
87. Ye, M.; Peng, Z.; Tang, D.; Yang, Z.; Li, D.; Xu, Y.; Zhang, C.; Huang, S. Generation of self-compatible diploid potato by knockout of S-RNase. *Nat. Plants* **2018**, *4*, 651–654. [CrossRef]
88. Quero-García, J.; Ivancic, A.; Lebot, V. Taro and Cocoyam. In *Root and Tuber Crops*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2010; pp. 149–172.
89. Hyde, P.T.; Guan, X.; Abreu, V.; Setter, T.L. The anti-ethylene growth regulator silver thiosulfate (STS) increases flower production and longevity in cassava (Manihot esculenta Crantz). *Plant Growth Regul.* **2020**, *90*, 441–453. [CrossRef] [PubMed]
90. Adeyemo, O.S.; Chavarriaga, P.; Tohme, J.; Fregene, M.; Davis, S.J.; Setter, T.L. Overexpression of Arabidopsis FLOWERING LOCUS T (FT) gene improves floral development in cassava (Manihot esculenta, Crantz). *PLoS ONE* **2017**, *12*, e0181460. [CrossRef] [PubMed]
91. Odipio, J.; Getu, B.; Chauhan, R.D.; Alicai, T.; Bart, R.S.; Nusinow, D.A.; Taylor, N.J. Transgenic overexpression of endogenous FLOWERING LOCUS T-like gene MeFT1 produces early flowering in cassava. *PLoS ONE* **2020**, *15*, e0227199. [CrossRef] [PubMed]
92. Tokunaga, H.; Quynh, D.T.N.; Anh, N.H.; Nhan, P.T.; Matsui, A.; Takahashi, S.; Tanaka, M.; Anh, N.M.; Van Dong, N.; Ham, L.H.; et al. Field transcriptome analysis reveals a molecular mechanism for cassava-flowering in a mountainous environment in Southeast Asia. *Plant Mol. Biol.* **2020**, 1–16. [CrossRef] [PubMed]
93. Pineda, M.; Yu, B.; Tian, Y.; Morante, N.; Salazar, S.; Hyde, P.T.; Setter, T.L.; Ceballos, H. Effect of Pruning Young Branches on Fruit and Seed Set in Cassava. *Front. Plant Sci.* **2020**, *11*, 1107. [CrossRef]
94. Lentini, Z.; Tabares, E.; Buitrago, M.E. Vibratome Sectioning and Clearing for Easing Studies of Cassava Embryo Formation. *Front. Plant Sci.* **2020**, *11*, 1180. [CrossRef] [PubMed]
95. Martin, F.W.; Cabanillas, E.; Ortiz, S. Natural pollination, hand pollination and crossability of some Mexican species of *Dioscorea*. *Trop. Agric.* **1963**, *40*, 135–141.
96. Howlett, B.G.; Gee, M. The potential management of the drone fly (Eristalis tenax) as a crop pollinator in New Zealand. *N. Z. Plant Prot.* **2019**, *72*, 221–230. [CrossRef]
97. Nicholas, S.; Thyselius, M.; Holden, M.; Nordström, K. Rearing and Long-Term Maintenance of Eristalis tenax Hoverflies for Research Studies. *J. Vis. Exp.* **2018**, *135*, e57711. [CrossRef]
98. Doyle, T.; Hawkes, W.L.S.; Massy, R.; Powney, G.D.; Menz, M.H.M.; Wotton, K.R. Pollination by hoverflies in the Anthropocene. *Proc. R. Soc. B. Boil. Sci.* **2020**, *287*, 20200508. [CrossRef]