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6-Benzylaminopurine (BAP) and Methyl Jasmonate (MeJa) Affect Sex Expression, Flowering Time and Flowering Intensity in Cultivated Yam *Dioscorea rotundata* (Poir.)

Narcisse Denadi [1,2,3], Aline Vanhove [1], Jeanne Zoundjihékpon [2], Christophe Gandonou [3] and Muriel Quinet [1,*]

[1] Groupe de Recherche en Physiologie Végétale (GRPV), Earth and Life Institute—Agronomy (ELI-A), Université Catholique de Louvain, Croix du Sud 5 (Bte L7.07.13), 1348 Louvain-la-Neuve, Belgium; nar6denadi@gmail.com (N.D.)
[2] Laboratoire de Génétique Ecologique, Faculté des Sciences et Techniques (FAST/UAC), Université d'Abomey-Calavi, Cotonou 01BP4521, Benin; agboyinou@gmail.com
[3] Unité de Recherche en Physiologie Végétale et Adaptation des Plantes aux Stress Abiotiques, Laboratoire des Sciences du Végétal et Pharmacopée, Faculté des Sciences et Techniques (FAST/UAC), Université d'Abomey-Calavi, Cotonou 01BP526, Benin; ganchrist@gmail.com
* Correspondence: muriel.quinet@uclouvain.be; Tel.: +32-10-47-34-43

Abstract: Cultivated yam (*Dioscorea rotundata*) is a staple tuber crop in West Africa which is mainly vegetatively propagated. Although the majority of yam cultivars flower, the control of their sexual reproduction remains largely unknown despite its importance for plant-breeding programs. While described as a dioecious species, yam has several monoecious (mix) cultivars that are often subject to spontaneous sex changes. This study aims to evaluate the impact of phytohormones on sex expression and flower development in cultivated yams *D. rotundata*. Exogenous applications of 1 mM 6-benzylaminopurine (BAP) and 1 mM methyl jasmonate (MeJa) were performed on seedlings of female (Gnidou, Anago), male (Flou) and mix (Katala and Laboko) cultivars. The mix cultivars produced monoecious, male and female plants while the female and male cultivars had rather stable sex. Our results showed that MeJa exhibited a significant masculinising effect in mix cultivars and induced inflorescence and flower malformations in female cultivars (56% in Gnidou and 37% in Anago). Most malformations were inflorescence branching and sterile flowers (non-differentiated ovary) with extra cycles of sepals. Moreover, MeJa reduced flowering time in the cultivars of all sexes and increased the number of inflorescences per plant as well as the number of flowers per plant. Our results showed that BAP reduced the flowering time, synchronized flowering in female plants and increased the number of inflorescences per plant in monoecious plants. However, our results did not allow for strong conclusions regarding the effect of BAP on sex expression due to the high proportion of female flowering in both the control and BAP-sprayed plants. Nevertheless, we did not observe any masculinising effect for BAP. Further research that would highlight hormone and homeotic gene interactions in flowering could be of key interest in understanding the hormonal control of sex in cultivated yams *D. rotundata*.

Keywords: *Dioscorea rotundata*; phytohormones; sex expression; flower development

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1. Introduction

The control of the reproductive system of cultivated plants is a key element for their improvement. There is a great diversity in reproductive systems in flowering plants, ranging from hermaphrodism to monoecy and dioecy with intermediate forms [1]. The diversity of types and forms of sexual polymorphism is controlled by numerous mechanisms [2–4], including phytohormones [3,5]. Phytohormones are a class of internal signalling molecules that integrate external or internal signals to regulate plant growth and development processes [6] and they play important regulatory roles in plant physiology [7–9]. Much research has reported their involvement in floral induction, inflorescence and flower development

and sex identity in several plant species, including tuber and root crops such as taro, cassava and sweet potato [10–16].

Yam is one of the most widely grown edible tuber crops in the tropics. It is the second most important root and tuber crop after cassava [17]. Yam accounts for more than 600 species [18] but few are cultivated. Most yam species belong to the genus *Dioscorea*, including the species *D. rotundata*, *D. cayenensis*, *D. alata*, *D. esculanta*, *D. trifid*a and *D. pentaphylla* [19]. *Dioscorea rotundata* is indigenous to West Africa and is the most important yam species in terms of production volume [20]. Yam is mainly propagated vegetatively, although it does flower. Until now, yam cultivation in the field has been achieved using micro-tubers, sections of long tubers [21] or minisetts [22]. Yam flowering allows research programs to combine the potential of vegetative propagation with sexual reproduction to optimise varietal selection [23–26].

The species *D. rotundata* is essentially characterised by dioecy (separate sexes carried by different plants) with an often unbalanced sex ratio depending on the region [27,28]. However, rare monoecious plants bearing both sexes on the same plant or hermaphrodites bearing both sexes in the same flower can be found [29–31]. While the conservation of sexual reproduction offers opportunities for the genetic improvement of the species, many useful cultivars that could serve as parents in a hybridisation program have never flowered or are irregular and sparse in flowering, i.e., few flowering plants, few inflorescences per plant or few flowers per inflorescence [32,33]. Problems of sex instability have also been reported within the species [24,34,35], especially in cultivars producing monoecious plants, despite the existence of potential genes determining sex expression within the species [35,36]. Plants from monoecious seed tubers could completely change sex giving male and female offspring [37]. Tamiru et al. [36] identified a genomic region associated with female heterogametic sex determination in *D. rotundata* so that plants with a ZZ genotype are male while plants with a ZW genotype could be female, monoecious, male or non-flowering [36]. However, we previously observed that this locus did not allow for the clear discrimination of sex in Beninese cultivars of *D. rotundata* [35]. Agre et al. [38] suggested that sex variation in *D. rotundata* monoecious plants is related to the differential expression of the W locus depending on growth environment [36]. Moreover, through genome-wide association studies aiming to dissect the molecular basis of plant sex in *D. rotundata*, Asfaw et al. [39] identified that most candidate genes associated with sex were involved in the regulation of phytohormones. This suggests a possible involvement of phytohormones and environment in sex expression in *D. rotundata*.

Several studies have reported on the ability of hormones to reverse sex in plant species when applied in required concentrations alone or in combination. In hemp (*Cannabis sativa*)—characterised by a dioecious–monoecious system—or cucumber (*Cucumis sativa*), which is monoecious, short days as well as the exogenous supply of abscisic acid and auxin had a feminising effect whereas long days as well as the exogenous supply of gibberellins had a masculinising effect [40]. In cassava, an essentially monoecious species, trauma (pruning of branches) led to a generalized feminisation with a strong transformation of male flowers into hermaphrodite flowers, and an application of cytokinins to plants with pruned branches completely transformed these hermaphrodite flowers into females [15]. This treatment also reduced flowering time and increased the number of inflorescences and flowers [26]. In cultivated yams, studies have shown that sex expression and flowering were often influenced by growing conditions (initial tuber size, plant health, soil moisture, atmospheric humidity, soil fertility, temperature and photoperiod) and the parts of the propagules (seed or seed tuber) used for planting [19,33,36,41–45]. In addition, recent research has shown that pruning combined with the application of silver thiosulphate (STS)—an anti-ethylene growth regulator—can influence sex expression and flowering intensity in certain yam cultivars of *D. rotundata* [46].

Our previous work showed that the hormonal profile of tubers varied according to the sex of the plant [37] and suggested that jasmonic acid and salicylic acid might have a masculinising effect while abscisic acid, cytokinins and benzoic acid might have a feminising

effect in *D. rotundata*. This study aims to highlight the effect of phytohormones on flowering and sex expression in *D. rotundata* cultivars to identify whether phytohormone application could control sex determination. More specifically, we aim to investigate (1) whether methyl jasmonate (MeJa) could have a masculinising effect and 6-benzylaminopurine (BAP) could have a feminising effect on *D. rotundata*, and (2) whether these treatments could increase and synchronize flowering. The exogenous application of MeJa and BAP were performed on monoecious, male and female plants and flowering parameters and flower development were followed. Our results showed that MeJa and BAP affect flowering in *D. rotundata* and that MeJa had a masculinising effect in this species.

2. Materials and Methods

2.1. Plant Material

Five cultivars (Laboko, Katala, Flou, Gnidou and Anago) grouped in 3 categories were used in this study:

- Male (M): Flou; plants of this cultivar usually produce male flowers.
- Female (F): Gnidou and Anago; plants of these cultivars usually produce female flowers.
- Mix (Mx): Laboko and Katala; plants of these cultivars produce either male or female or monoecious plants (i.e., plants carrying both male and female flowers/inflorescences on the same individual).

Two experiments were performed. The cultivars Laboko and Katala were used in the first experiment aiming to identify possible masculinising or feminising effects of BAP and MeJa on mix cultivars (monoecious plants). The cultivars Gnidou, Anago, Flou and Katala were used in the second experiment to confirm the masculinising effect of MeJa.

2.2. Experimental Site

Field trials were performed at the 'Centre de Recherche, de Formation, d'Incubation et d'Innovation pour le Développement Agricole' (CREFIISDA). This center covers an area of 24 ha and is located in the commune of Zogbodomey (village of Lomey, 6°56′–7°08′ North, 1°58′–2°24′ East). This region has herbaceous vegetation on clay soil with good water retention, which is ideal for growing yams. The average annual temperature was 26.8 °C, the annual rainfall was 1244 mm and the average photoperiod was 12/12 h.

2.3. Growing Conditions and Phytohormone Application

2.3.1. Impact of BAP and MeJa on Monoecious Plants

In the first experiment, clones were obtained by the minisett technique from a tuber of a monoecious plant of Laboko and Katala cultivars. For each of the two cultivars, a long tuber (Figure 1a) was cut into 20–25 g slices. About 40 minisetts were obtained for each cultivar. The fragments were dried in the shade (Figure 1b) for about an hour and then germinated (Figure 1c) on 20 February 2019 in a shaded area and watered once a day. After germination (Figure 1d), 45 days after sowing, seedlings were transferred to mounds (Figure 1e) in two blocks, one block per cultivar. The tubers of the resulting plants were harvested in December 2019 and stored (Figure 1f) to be used for the experiment with hormonal treatment.

After 5 months of dormancy, the tubers were sown on 13 May 2020 in a Fisher block design with 3 blocks of 4 rows of 10 ridges each. Each block included 20 plants of the Katala cultivar and 20 plants of the Laboko cultivar (randomized distributed) and corresponded to a different hormonal treatment.

The three treatments were (1) control, plants sprayed with demineralised water containing 0.004% Tween 20; (2) BAP, plants sprayed with a solution of 1 mM BAP containing 0.004% Tween 20; (3) MeJa, plants sprayed with a solution of 1 mM MeJa containing 0.004% Tween 20. Tween 20 was added as surfactant. Treatment sprayings started on 15 June 2020 (at about two-leaf stage) at a rate of once a week until complete flower formation and differentiation. For each plant, 2–4 sprays (depending on the development of the plant) were applied to the top of the plant. Twenty plants were sprayed per cultivar and

treatment, i.e., 120 plants in total. The experiment ended on 10 October 2020 after 117 days of treatment.

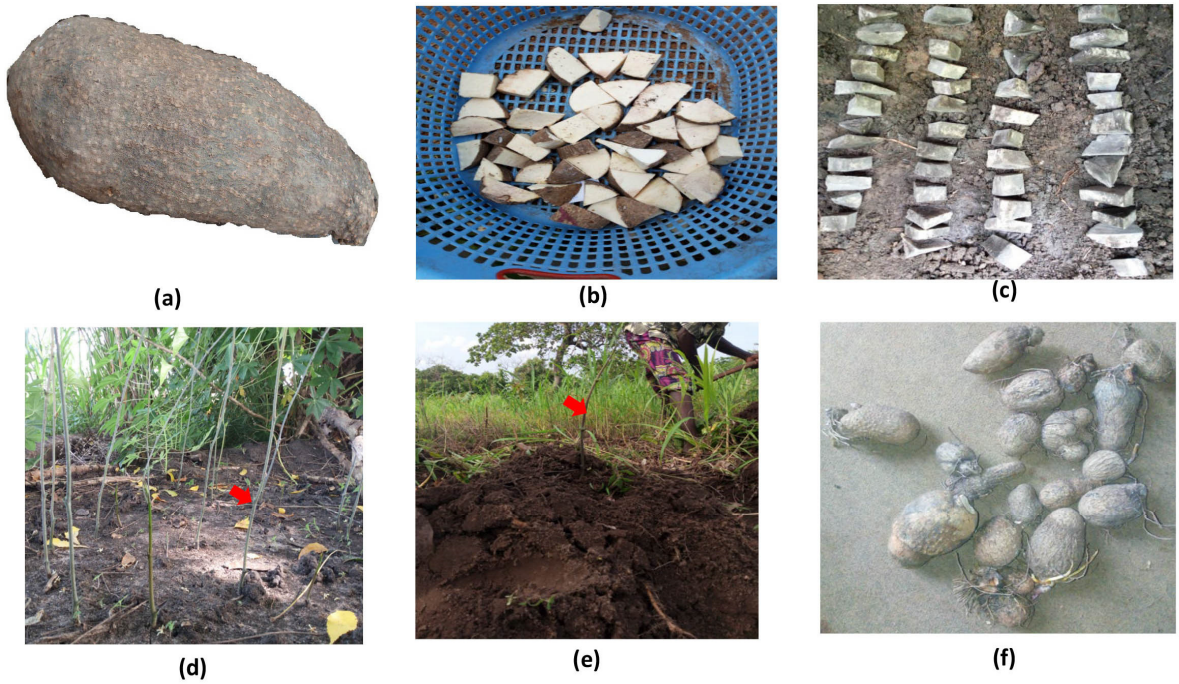

Figure 1. Production of clones by the minisett technique: (**a**) whole tuber; (**b**) dried tuber fragments; (**c**) pre-germination; (**d**) germinated seedlings; (**e**) transfer of seedlings into mounds; (**f**) micro-tubers (clones).

2.3.2. Impact of MeJa on Male, Female and Monoecious Plants

For the second experiment, the same set-up (Fisher block) was used with 2 blocks of 2 lines of 10 mounds each per cultivar. Each block corresponded to a treatment. In total, 30 plants of each cultivar (Gnidou, Anago, Flou and Katala) were used per treatment with 120 plants per block, i.e., 240 plants in total. Sowing took place on 13 March 2021. The first spraying started 46 days later (26 April 2021) when the germinated plants had at least two leaves. Two treatments were applied: (1) control, plants sprayed with demineralised water containing 0.004% Tween 20; (2) MeJa, plants sprayed with a solution of 1 mM MeJa containing 0.004% Tween 20. The application method, frequency and duration were as previously described. The experiment ended on 25 August 2021 after 120 days of treatment.

2.4. Measured Parameters

For both experiments, flowering time, sex of progeny, sex ratio, number of inflorescences per plant, inflorescence length and number of flowers per inflorescence were monitored.

The flowering time was calculated as the number of days between the germination date and the flowering date. The flowering date in the field was characterised by the appearance of the first inflorescence bud visible to the naked eye.

The sex ratio was calculated as the ratio between the number of plants of a given sex (male, female and monoecious) and the total number of flowering plants.

The number of inflorescences per plant was counted on all plants and the length of inflorescences and the number of flowers per inflorescence were counted on 10 inflorescences per plant.

The number of malformed flowers was also analysed (especially in the female cultivars Gnidou and Anago) on 10 inflorescences per plant. The rate of malformed flowers was calculated as the ratio between the number of malformed flowers and the total number of flowers per inflorescence, expressed as a percentage.

2.5. Histological Analysis

In order to evaluate the different types of malformations, we performed observations under stereomicroscope and histological sections of potentially malformed flowers. For this purpose, mature flowers were harvested and fixed in FAA (70% ethanol: acetic acid: formaldehyde (18:1:1, by vol.)). For histological analysis, samples were then dehydrated in a graded ethanol series, embedded in paraffin and 5 µm sections were made following the procedure of Quinet et al. [47,48]. Longitudinal sections were stained with haematoxylin-fast green and observed under a light microscope.

2.6. Statistical Analysis

Statistical analyses were performed with JMP Pro 16 (SAS Institute, New York, NY, USA, 2021) software. A logistic regression was performed on qualitative data and was thus used to measure the impact of treatment, parental sex and variety on the sex expression of the progeny (sex ratio). For quantitative data, ANOVAs were performed. For the first experiment, two-way ANOVA was used to measure the impact of treatment and variety on flowering parameters, namely, flowering time, number of flowers per inflorescence, inflorescence length and number of flowers per inflorescence. For the second experiment, one-way ANOVA was used to measure the impact of treatment on the flowering parameters inside each cultivar and sex. Before the ANOVAs, normality was checked based on graphs (box plot and qq plot) and homogeneity of variances based on Levene's test. Post hoc tests were performed via student's *t* tests for pairwise comparisons between treatments and between varieties. Principal component analysis (PCA) was performed using the 'FactoMineR' package in R (version 4.2.1, R Core Team, Vienna, Austria, 2022) to summarise and visualise graphically the effect of hormonal treatment on sex variation and flowering parameters according to the cultivars and the sex of the plants.

3. Results

3.1. Impact of BAP and MeJa on Mix Cultivars

The exogenous application of BAP and MeJa were compared in two mix cultivars, Katala and Laboko. Plants were clones from the same monoecious parent plant for each cultivar.

As shown in Figure 2, 80% and 82% of the control plants were female for Katala and Laboko, respectively, even though the seed tubers were from monoecious plants. Male and monoecious plants represented about 10% or less of the offspring whatever the cultivar. Similarly, plants sprayed with BAP showed strong female sex expression (85%) in both cultivars, the remaining plants being exclusively monoecious. In contrast, plants sprayed with MeJa were predominantly male (80%) in both cultivars with some monoecious and female plants.

Table S1 (Supplementary Material) summarised the data of flowering time, number of inflorescences per plant, inflorescence length and number of flowers per inflorescence after phytohormonal treatment according to the plant sex in both cultivars. However, due to the low percentage of male and monoecious plants observed under control conditions, statistical comparison among phytohormonal treatments was only possible for female plants between the control and BAP treatments (Figure 3).

Overall, male plants flowered before monoecious plants and female plants were the latest to flower whatever the treatment (Table S1). Plants treated with BAP flowered about 12 days earlier than the control plants ($F = 84.41$, $p < 0.0001$) and no differences were observed between cultivars ($F = 0.21$, $p = 0.64462$; Figure 3a). A reduction in flowering time with BAP was observed for both female and monoecious plants (Table S1). Flowering time was also reduced after MeJa treatment compared with the control plants whatever the sex of the plant and the cultivar (Table S1).

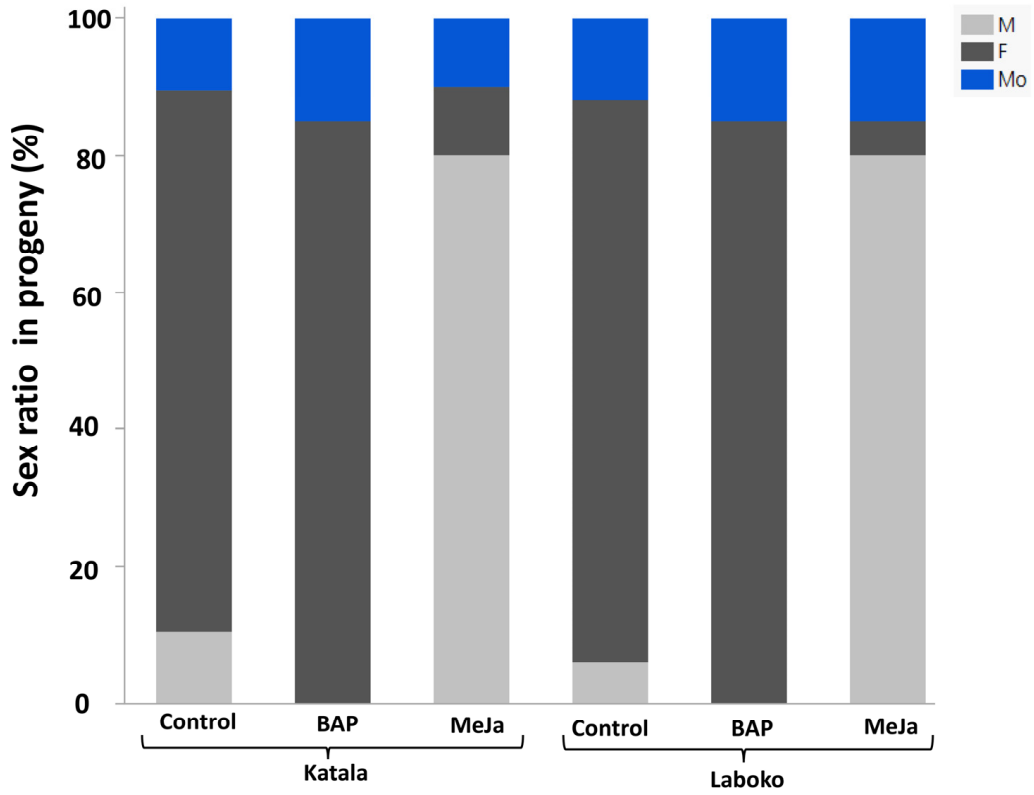

Figure 2. Sex ratio of the offspring of monoecious plants of *D. rotundata* cv. Katala and Laboko sprayed with exogenous application of water (Control), 1 mM benzylaminopurine (BAP) or 1 mM methyl jasmonate (MeJa). Plants were grown under field conditions and treatments were sprayed once a week to 20 plants per cultivar and treatment. For the same cultivar, plants are clones of the same monoecious parent. M = Male; F = Female and Mo = Monoecious.

Regarding inflorescence parameters, female plants usually produced less inflorescences per plant than male and monoecious plants while their inflorescences were longer (Table S1). BAP increased the number of inflorescences per plant (60 vs. 39; F = 314.06, $p < 0.0001$; Figure 3b), mainly in Katala, although the cultivar did not affect this number (F = 1.09, $p = 0.2990$). However, the inflorescences of Laboko were longer and contained more flowers than the ones of Katala (14.79 vs. 10.89 cm; F = 212.05, $p < 0.0001$; 43 vs. 18 flowers; F = 409.37, $p < 0.0001$; Figure 3b) but BAP affected neither the inflorescence length (F = 0.24, $p = 0.6219$) nor the number of flowers per inflorescence (F = 0.71, $p = 0.4005$). The number of inflorescences per plant also increased with MeJa but inflorescences were shorter with less flowers compared with the controls (Table S1).

PCA was performed to visualise the results of the first experiment (Figure 4). The first dimension explained 50.88% of the variability and was mainly explained by the flowering parameters with, on one side, the number of inflorescences per plant and, on the other side, the number of flowers per inflorescence, the inflorescence length and the flowering time. The second dimension explained 21.70% of the variability and was mainly explained by the sex ratio. The individuals were mainly grouped according to the sex of the plants with female plants flowering later and producing longer inflorescences with more flowers and male plants producing more inflorescences per plant. Differences between treatments were also visible mainly between MeJA on one side and the control and BAP on the other side.

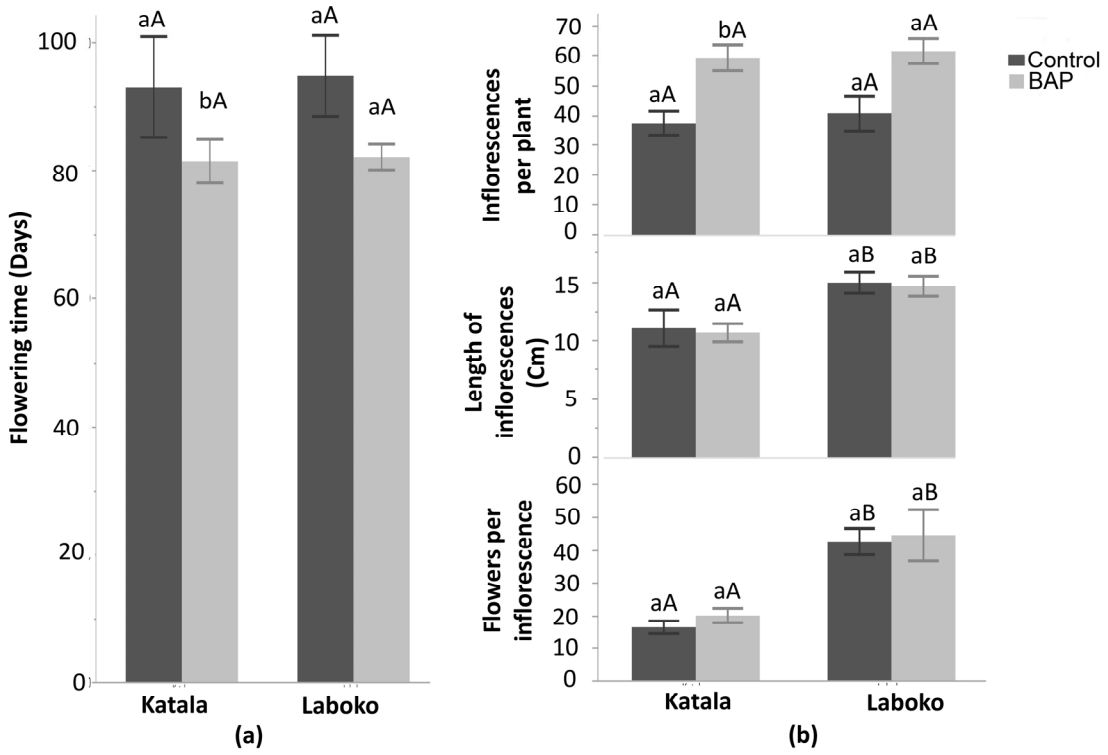

Figure 3. Flowering parameters of the female offspring of monoecious plants of *D. rotundata* cv. Katala and Laboko sprayed with exogenous application of water (control) or 1 mM benzylaminopurine (BAP). (**a**) flowering time, (**b**) number of inflorescences per plant, inflorescence length and number of flowers per inflorescence. Plants were grown under field conditions and treatments were sprayed once a week to 20 plants per cultivar and treatment. For the same cultivar, plants are clones of the same monoecious parent. Data are means ± SE. Treatments with the same lowercase letter for the same cultivar are not significantly different at the 5% level; cultivars with the same capital letter for the same treatment are not significantly different at the 5% level.

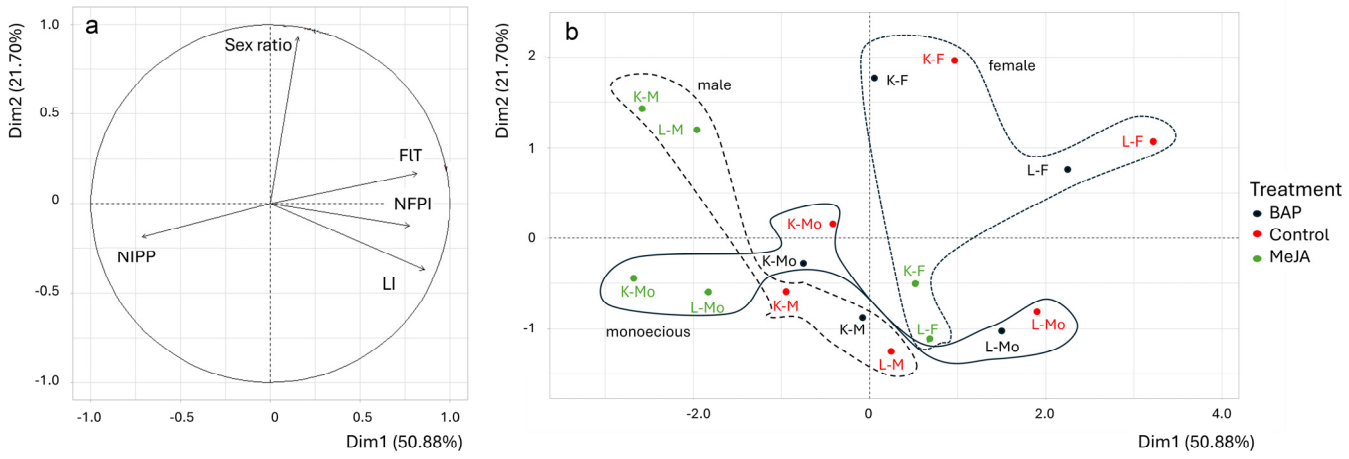

Figure 4. Principal component analysis (PCA) of sex ratio and flowering parameters of *D. rotundata* cv. Katala and Laboko sprayed with exogenous application of water (control), 1 mM benzylaminopurine (BAP) or 1 mM methyl jasmonate (MeJa). (**a**) Variable graph of PCA showing sex ratio and flowering parameters, (**b**) individual graph showing the mean individual for each cultivar and sex combination. K = Katala, L = Laboko, F = Female, M = Male, Mo = Monoecious, FlT = flowering time, NFPI = number of flowers per inflorescence, LI = inflorescence length, NIPP = number of inflorescences per plant.

3.2. Impact of MeJa on Male, Female and Mix Cultivars

To confirm the masculinising effect of MeJa, exogenous applications were performed on the offspring of male (Flou), female (Gnidou, Anago) and mix (Katala) cultivars.

Most of the plants of the female cultivars Gnidou and Anago kept their parental sex (Figure 5). Indeed, all the control plants and 92% of the MeJa-treated plants were female for these cultivars. The remaining 8% of the MeJa-treated plants were monoecious. Similarly, sex remained stable in the male cultivar Flou even after MeJa treatment since all plants of this cultivar were male. However, the sex ratio changed with the MeJa treatment in the Katala cultivar. The impact of MeJa was compared on the three sex types in this cultivar. For male plants, parental sex was retained in 73% of the control plants and 23% were monoecious, whereas all MeJa-treated plants were male (100%). In females, parental sex was retained in 56% of the control plants while 44% were monoecious. In MeJa-sprayed females, only 22% of plants retained parental sex; 64% were monoecious and 14% were male. However, the masculinising action of MeJa was not significant in female Katala plants ($\chi^2 = 3.5$, $p = 0.1736$). In monoecious plants, 46% of the control plants retained their parental sex; 23% and 31% of them were male and female, respectively. In monoecious plants treated with MeJa, only 20% of the plants retained the parental sex and 80% expressed the male sex. In monoecious plants, the masculinising effect of MeJa was highly significant ($\chi^2 = 10.31$, $p = 0.0058$).

This experiment also showed the impact of MeJa on the flowering time of male, female and mix cultivars (Figure 6a). Irrespective of the sex of the cultivars, MeJa advanced flowering time compared with the control plants ($F = 54.82$, $p < 0.0001$). In females, the difference in flowering time between the MeJa-treated and control plants was about 18 days in the cultivars Anago and Gnidou and 16 days in Katala. In the male plants of Flou and Katala, the difference was 9 and 11 days, respectively. In monoecious Katala plants, MeJa treatment advanced flowering by 17 days.

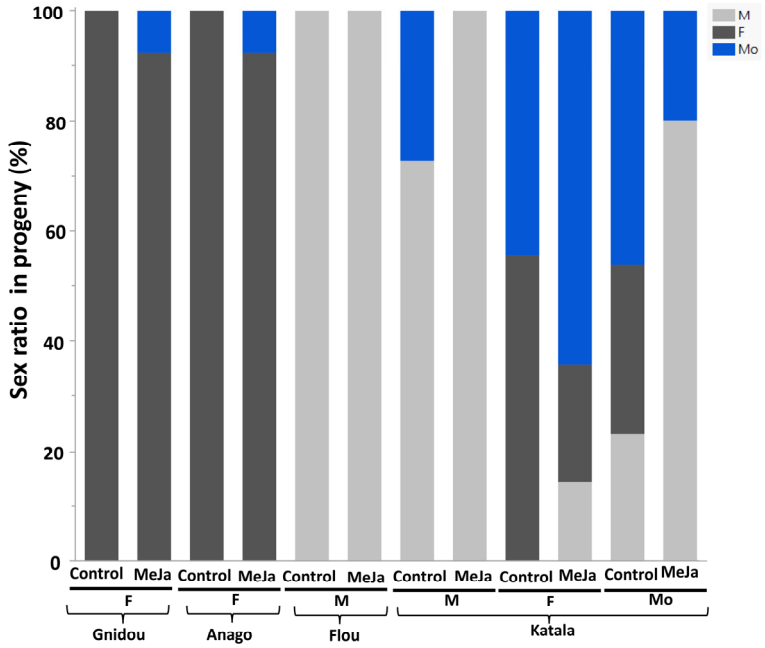

Figure 5. Sex ratio of the offspring of male, female and monoecious plants of *D. rotundata* cv. Gnidou, Anago, Flou and Katala sprayed with exogenous application of water (control) or 1 mM methyl jasmonate (MeJa). Plants were grown under field conditions and treatments were sprayed once a week to 30 plants per cultivar and treatment. Plants issued from the same cultivar and sex are clones. M = Male; F = Female and Mo = Monoecious.

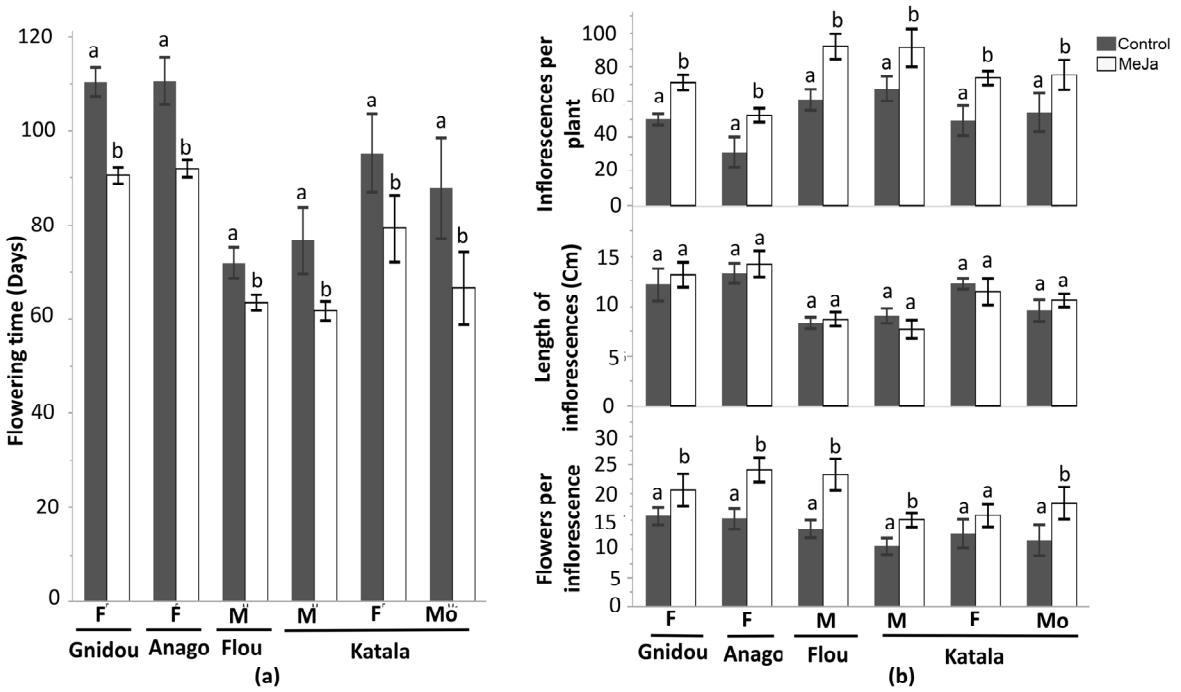

Figure 6. Flowering parameters of the offspring of male, female and monoecious plants of *D. rotundata* cv. Gnidou, Anago, Flou and Katala sprayed with exogenous application of water (control) or 1 mM methyl jasmonate (MeJa). (**a**) flowering time, (**b**) number of inflorescences per plant, inflorescence length and number of flowers per inflorescence. Plants were grown under field conditions and treatments were sprayed once a week to 30 plants per cultivar and treatment. Plants issued from the same cultivar and sex are clones. Data are means ± SE. Treatments with the same lowercase letter for the same cultivar and same sex are not significantly different at the 5% level. M = Male; F = Female and Mo = Monoecious.

Regarding inflorescence production, MeJa increased the number of inflorescences per plant compared with the control plants (80 vs. 52; F = 115.41, $p < 0.0001$) regardless of cultivar (Figure 6b). In contrast, MeJa had no impact on inflorescence length (F = 1.99, 0.1597) but increased the number of flowers per inflorescence (F = 103.15, $p < 0.0001$; Figure 6b).

Although exogenous MeJa increased the number of flowers per inflorescence, significant malformations were observed mainly in the female cultivars Gnidou and Anago. The malformation rate was about 56% in Gnidou and 37% in Anago. In the MeJa-sprayed plants, inflorescence branching was observed in contrast to the control plants (Figure 7a,b). While the female flowers showed a well-formed cycle of sepals and carpels (Figure 7d,e,i), some MeJa-sprayed plants showed flowers with additional cycles of sepals and undifferentiated carpels (Figure 7e,j,f,k). Some malformed female flowers also had an inflorescence-like appearance, sometimes with well-differentiated sepals, but the central cycles were not differentiated into stamens or carpels (Figure 7e,j). Other malformed female plants showed flowers with a similar structure to the male flowers except that, unlike the well-formed male flowers (Figure 7c,h), these had central cells that were not differentiated into stamens (Figure 7g,l).

PCA was performed to visualise the results of the second experiment (Figure 8). The first dimension explained 50.91% of the variability and was mainly explained by the flowering parameters with, on one side, the number of inflorescences per plant and, on the other side, the inflorescence length and the flowering time (Figure 8a). The second dimension explained 27.03% of the variability and was mainly explained by the number of flowers per inflorescence and the sex ratio. The individuals were mainly grouped according to the sex of the plants and the treatments, with the female plants flowering later and

producing longer inflorescences but less inflorescences per plant than the male plants and the MeJA-treated plants producing more flowers per inflorescence than the control plants.

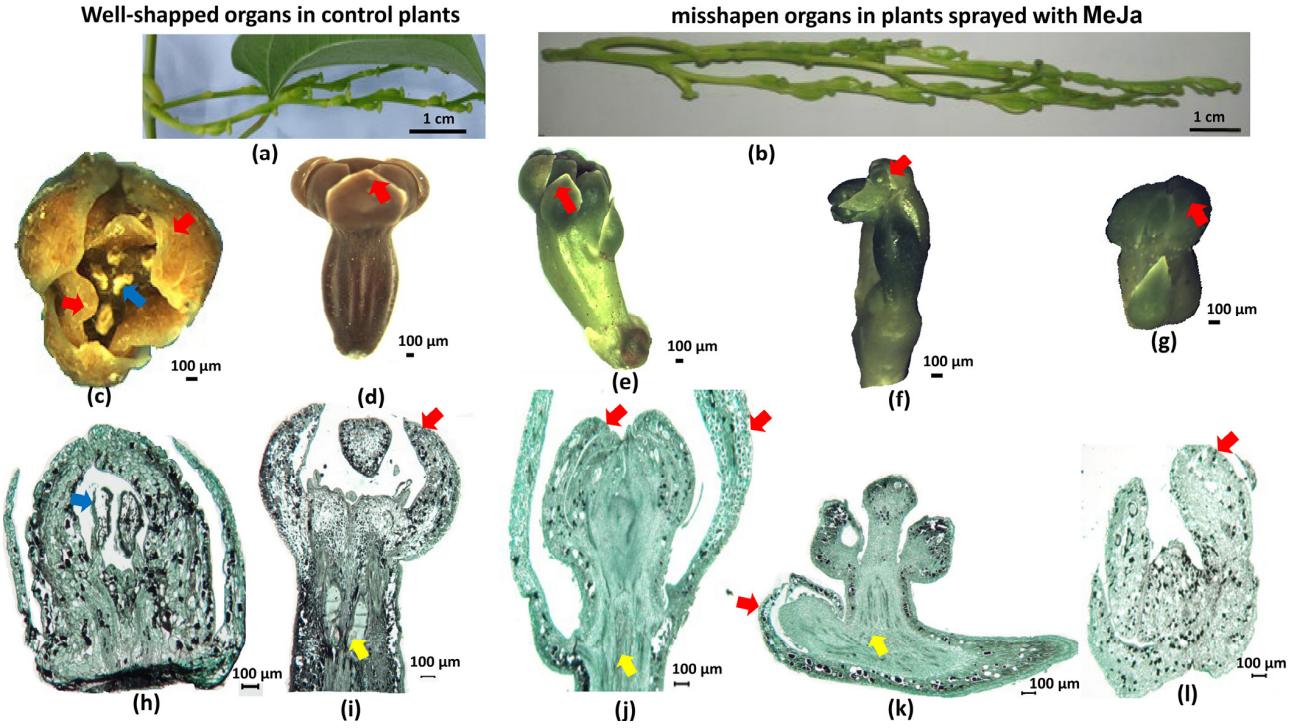

Figure 7. Impact of methyl jasmonate on flower development in female plants of Gnidou and Anago cultivars. (**a**,**b**): female inflorescence; (**c**): well-formed male flower; (**d**): well-formed female flower; (**e**–**g**): poorly formed female flowers; (**h**): longitudinal section of well-formed male flower; (**i**): longitudinal section of well-formed female flower; (**j**–**l**) longitudinal section of poorly formed female flower: (**j**–**l**) the ovaries were completely absent; (**k**) branched flowers were observed and (**i**) some male-looking flowers lacked stamens. Red, blue and yellow arrows indicate, respectively, sepal, stamen and ovary. MeJa: methyl jasmonate.

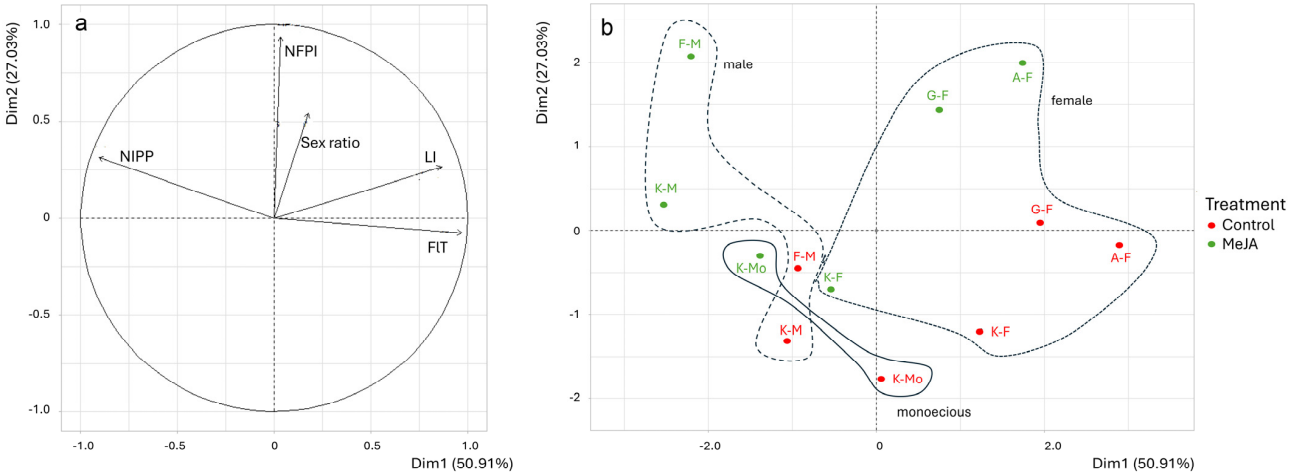

Figure 8. Principal component analysis (PCA) of sex ratio and flowering parameters of *D. rotundata* cv. Gnidou, Anago, Flou and Katala sprayed with exogenous application of water (control) or 1 mM methyl jasmonate (MeJa). (**a**) Variable graph of PCA showing sex ratio and flowering parameters, (**b**) individual graph showing the mean individual for each cultivar and sex combination. K = Katala, L = Laboko, F = Female, M = Male, Mo = Monoecious, FlT = flowering time, NFPI = number of flowers per inflorescence, LI = inflorescence length, NIPP = number of inflorescences per plant.

4. Discussion

4.1. Sex Variation in Monoecious Plants of D. rotundata

In the first experiment with the monoecious Katala and Laboko clones, a strong sex variation was observed in the progeny of the monoecious control plants, even though all plants were genetically identical. The progeny showed a strong female sex expression in both the Katala and Laboko cultivars. This sex variation was reconfirmed in the monoecious Katala control plants in the second year with progeny expressing both male and female sex. Sex variations were also observed in male and female Katala plants with a shift towards the monoecious trait. However, the sex of the progeny was stable in the male (Flou) and female (Gnidou and Anago) cultivars. These results confirm our earlier findings that (i) sex variation was mostly observed in mix cultivars and very little in strict male or female cultivars and that (ii) when sex variation was present, the progeny of monoecious plants gave mainly male or female plants while male and female plants gave mainly monoecious plants [37]. Spontaneous sex changes make the control of sex difficult in cultivated yams *D. rotundata*. We also observed that, during flower morphogenesis, both stamen and carpel primordia were initiated in *D. rotundata* and that in male and female flowers the floral organ development of the other sex was arrested earlier in cultivars with stable sex than in cultivars showing sex variability [37]. Genes involved in sex determination, floral organ identity and floral organ development most probably interact to determine sex stability. Basically, the initiation of floral organs in flowering plants is based on the differential expression of the MADS-box genes of the ABC model [47]. Class A genes determine the identity of sepals and petals, class B genes determine the identity of petals and stamens and class C genes determine the identity of stamens and carpels. In monoecious and dioecious plants, sex differentiation could occur prior to floral organ initiation or later during flower development depending on the species [48]. In *D. rotundata*, genes involved in sex differentiation most probably act after floral organ initiation and the control of floral organ identity by the ABC genes, as observed in *Zea mays* and *Cucumis sativus* [48]. This would explain the sex variability observed in *D. rotundata* and that hormonal and environmental factors could affect sex determination. According to the female heterogametic sex determination hypothesis of Tamiru et al. [36], the Z-suppressor effect would be under the influence of the environment and result in a female phenotype if the effect is complete, or in a monoecious, male or non-flowering phenotype if the effect is partial or nil. Plants showing the ZZ alleles for this locus would be male and plants showing the ZW alleles would be either female, monoecious, male or non-flowering. We indeed observed that the W allele was present in the mix cultivars Katala and Laboko [35], which could explain their sex variability. Golenberg and West [40] suggested that key sex-determining genes must exist in monoecious species, but that independent cues, either external environmental or internal physiological, must regulate their expression. It is indeed known that environmental conditions such as water stress, light intensity, shading, nutrient abundance, etc., or phytohormonal treatments may affect sex identity in several monoecious and dioecious species [49–53]. In certain selected mix cultivars of *D. rotundata* from Nigeria, it has recently been shown that plant pruning can promote male flowering [46] although this could have consequences for plant growth and development. Regarding phytohormonal control, auxins and cytokinins were reported to have rather a feminising effect [54–56] while gibberellins and jasmonic acid have rather a masculinising effect [57,58] even though differences could be observed among species.

4.2. MeJa Has a Masculinising Effect in D. rotundata

The results of this study showed that monoecious plants sprayed with MeJa had a strong male sex expression in their progeny. A possible masculinising effect of MeJa can be deduced, which is total in mix cultivars and partial in female cultivars. Other treatments such as pruning could also have a masculinising effect in *D. rotundata* [46]. The feminising effect of BAP could not be confirmed in our study due to the strong expression of the female sex in both the control and BAP-treated plants. Tests carried out with BAP

on selected mix *D. rotundata* cultivars in Nigeria did not show any effect on sex identity either [46]. However, the use of STS combined with tuber removal promoted female flowering in the mix cultivars [46]. Our results support the hypothesis of a masculinising effect of jasmonates in cultivated yams *D. rotundata*. We have previously observed a high concentration of jasmonic acid in tubers from male and monoecious plants in contrast to tubers from female plants in the cultivars Katala and Laboko [37].

Flowering and flower development is under the control of a crosstalk between genetic and hormonal regulation [59,60] and genes involved in flower development—such as the ABC model—interact with environmental and hormonal factors to ensure flower formation [61–66]. However, hormones do not control development via linear pathways but via complex interconnected webs of cross-regulation and crosstalk [60]. Hormones play also a key role in sex determination but an overall mechanism linking hormone signaling and sex determination has not been possible mainly due to the ambiguous action of hormones, which in many cases is species dependent [48]. In *D. rotundata*, Asfaw et al. [39] identified that most candidate genes associated with sex were involved in the regulation of phytohormones. However, how they interact with genes involved in flower development and sex identity remains to be investigated. Interactions between the genetic and hormonal regulation of sex determination and flower development has been investigated in other monoecious and dioecious plant species. In *Cucumis melon* and *C. sativus*, for example, the ethylene biosynthesis enzymes CmACS-7 and CmACS-11 are involved in sex determination [67,68] and are required for the epigenetic repression of the male sex promoter gene *CmWIP1*. It is possible that in *D. rotundata* yams, MeJa interacts with currently unknown genetic factors to affect sex expression. It is indeed known that jasmonic acid (JA) and its metabolites are involved in stamen and pollen maturation in several plant species [69,70]. For example, in Arabidopsis, the *AGAMOUS* (*AG*) floral organ identity gene controls late stamen development via JA biosynthesis by directly regulating the *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*) gene, which codes a catalytic enzyme of JA [71]. In rice, numerous JA-regulated genes have been shown to be involved in anther development [72].

While MeJa may have caused complete masculinisation in mix cultivars, this was not necessarily the case in typically female cultivars. In female cultivars (Gnidou and Anago) sprayed with MeJa, we observed malformations in reproductive structures with branched inflorescences and flowers, the suppression of the ovary in female flowers, and the transformation of female flowers into sterile male flowers (without stamens). This failure of masculinisation in female, compared with monoecious, plants may therefore be the result of a difference in their genetic status. Through a genome-wide association study, Asfaw et al. [39] identified 88 differently expressed genes (DEGs) between male, female and monoecious plants, and at least 5 of them were considered to be linked to sex expression or to the hormonal regulation of sex expression. Most of the malformed flowers had completely suppressed ovaries, so we hypothesise that MeJa could affect the expression of genes involved in ovary development in *D. rotundata*. It is known that phytohormones (gibberellins, jasmonates, auxins, brassinosteroids cytokinins. . .) play an important role in the regulation of floral morphogenesis and interact with floral organ identity genes (ABC model) and genes involved in flower development [60]. It is well known that appropriate hormonal balance is essential for the proper development of reproductive structures [73].

While cytokinins are known for their key roles in cell division and their involvement in carpel development and the suppression of stamen development [74,75], jasmonates mainly control late stages of stamen development such as filament elongation, the formation of viable pollen and anther dehiscence [76]. According to Marciniak and Przedniczek [76], jasmonates activate a complex of transcription factors crucial for normal stamen development. In *Arabidopsis thaliana*, exogenous applications of jasmonates could restore deficiencies in male fertility, anther dehiscence and filament elongation [70,77–80]. Given the strong involvement of jasmonates in the formation of male sex organs, it is obvious that the application of this hormone to female *D. rotundata* plants could lead to the masculinisation

of the flowers. It is known that in some species, hormonal treatment can influence sex expression. This is the case for cucumbers (*Cucumis sativus*), hemp (*Cannabis sativa*), spinach (*Spinachia oleracea*) or bitter melon (*Momordica charantia*), in which auxin is feminising and gibberellin masculinising [69,71,81–85]. According to Mondo et al. [46], gibberellin is not that effective in yam. In cassava, it has been shown that branch pruning leads to generalised feminisation with a strong transformation of male flowers into hermaphrodites and that an application of BAP to plants with pruned branches completely transforms male flowers into females [15]. Browse [86] has reported a masculinising effect of JA in the monoecious plant *Zea mays*, showing that JA has a masculinising effect in other plants than yam.

4.3. BAP and MeJa Accelerate Flowering and Increase the Number of Inflorescences and Flowers in D. rotundata

Our results showed that the exogenous application of BAP and MeJa accelerated flowering and reduced flowering time. In angiosperms, floral induction is under the control of exogenous and endogenous signals and depends on a complex interaction between genetic, environmental and hormonal factors [61–63]. For example, it is known that gibberellins are floral activators in *Arabidopsis thaliana* and that they act through the activation of flowering integrator genes [63,87–90]. Also, in potato, the exogenous foliar application of gibberellins could lead to early flowering [91]. Although gibberellins are the main hormones involved in floral transition in Arabidopsis, they interact with other phytohormones such as cytokinins, auxin, abscisic acid, ethylene, jasmonic acid and brassinosteroids [89]. In Arabidopsis, cytokinins could directly activate flowering genes [92,93]. Its involvement in floral induction has also been proven in *Sinapsis alba* [92,94,95]. In cassava, the exogenous application of BAP leads to early flowering [14] as we observed in *D. rotundata*. In line with our observations, some studies have reported that jasmonates promote flowering in certain species. In six oilseed rape cultivars, exogenous MeJa induced early flowering [96]. However, JA has been reported to have rather inhibitory effects on various plant growth and development processes [97–100] including flowering time [101]. In Arabidopsis, for example, the exogenous application of JA suppressed flowering [102]. In the short-day plant *Pharbitis nil*, the exogenous application of MeJa to cotyledons also significantly inhibits flowering [103,104]. A similar floral repressor effect of JA was observed in *Triticum aestivum* [105], *Chenopodium rubrum* [106] or *Zea mays* [107]. In other species, such as *Lemna minor*, JA regulated floral induction in a concentration-dependent manner: low concentrations of JA (0.475–47.5 nmol) favoured floral induction, while high concentrations (237.5 or 475 nmol) inhibited it [108]. In cucumber (*Cucumis sativus*), a reduction in endogenous jasmonate levels delayed the flowering of male flowers [109]. The effect of jasmonates on flowering time therefore differs between species and concentrations [101].

Furthermore, our results showed that BAP and MeJa also increased the number of inflorescences per plant and the number of flowers per inflorescence. In cassava, the application of BAP also strongly increased flowering intensity by promoting flower development and preventing inflorescences abortion [15]. Other phytohormones such as gibberellins, auxins, abscisic acid, ethylene or ascorbic acid could increase the number of inflorescences or flowers in root and tuber crops such as cassava (*Manihot esculenta*), taro (*Colocasia esculenta*) and potato (*Solanum tuberosum*) [11,14,15,110–112]. Moreover, the application of STS resulted in high production, longevity and reduced early flower abortion in cassava [15]. In *D. rotundata*, the pruning or application of STS increased the number of inflorescences and the flowering intensity [46]. Phytohormones thus play a key role in flowering and reproductive structure development even though their effect could differ among species. Controlling flowering time and flowering intensity could be relevant in root and tuber crops to synchronize flowering for breeding programs.

5. Conclusions

The results of this study showed that sex expression in cultivated yams *D. rotundata* can be modulated by exogenous hormone application. In monoecious and female plants,

MeJa induced masculinisation in flowers with early and abundant flowering. However, the application of MeJa caused malformations of floral organs in female plants, notably the formation of sterile flowers. Furthermore, our results did not allow us to draw any conclusions regarding the action of BAP on sex expression. Nevertheless, we found that BAP could also cause early and abundant flowering. BAP seems to be able to synchronise flowering in female plants. Then, for research or field management requiring synchronised, early and abundant male flowering in mix cultivars, we suggest using MeJa. When the target effect is synchronised, early and abundant flowering in female cultivars, we recommend the use of BAP. Controlling sex identity and flowering intensity in dioecious and monoecious species such as *D. rotundata* is of key importance for making crosses as a part of breeding programs. Understanding how hormones affect sex identity is therefore fundamental. It would be interesting to explore the mode of action of other hormones on sex expression and floral development in interaction with the cytogenetic status of the cultivars and environmental factors in cultivated yams *D. rotundata*. More particularly, complete masculinisation in female cultivars could be better achieved by combining other hormones with MeJa. A better understanding of the molecular and hormonal mechanisms underlying sex determination in *D. rotundata* will also enable better control of sex expression for breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applbiosci3040035/s1, Table S1: Effect of BAP and MeJa on flowering time and flowering intensity in *D. rotundata*.

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