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Potential Gene Flow Between *Gossypium hirsitum* and *Abelmoschus esculentus* Through Interspecific Hybridization

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Abstract: The introduction of transgenic varieties of cotton (*Gossypium hirsitum*) in Cameroon has posed numerous concerns. One of the most concern was the horizontal transfer of the *Bt* gene to related species such as *Abelmoschus esculentus*. The current study was conducted to assess the occurrence *Bt* gene transfer from *Gossypium hirsitum* to *Abelmoschus esculentus*. To achieve this, the stigma receptivity periods and the viability of pollen grains were evaluated. The fruit set rates of 675 controlled intra- and interspecific crosses were assessed. The results showed that stigma receptivity and pollen grain viability are optimal at anthesis in both species, with a longer duration over 24 hours in *G. hirsitum*. Intra-specific crosses within the two species have shown fruit set rates of nearly 60-80%. Interspecific crosses gave a fruit set rate close to 2%, especially when *A. esculentus* is used as the female parent, and zero results in reciprocal crosses. The finding of the study pointed out that the possibility of horizontal transfer of a transgene to cotton relatives. In future studies, it would be useful to monitor the fate of seeds from interspecific crosses between both species and assess the agro-morphological and molecular characteristics of these possible hybrids.

Keywords: Interspecific Hybridization, *G. hirsitum*, *A. esculentus*, Gene Flow, Trangenic Varieties

1. Introduction

The African cotton-producing countries of the CFA franc zone include one of the world's greatest cotton-producing territories [1]. They contribute for 12 to 15% of fiber exports, according to the International Cotton Advisory Committee [2]. Cotton farming is thus a driver of growth in several West and Central African countries. Cotton does, in fact, account for between 3 and 15% of the gross domestic product of these countries [3]. This crop is the economic lifeblood of Cameroon's northern region, occupying about 89% of farms and contributing more than 60% of the region's income [4]. However, the cotton business in Cameroon is beset by several issues, the most serious of which is a lack of trust between growers and cotton firms [5]. Additionally, there is the critical issue of controlling diseases, pests, and weeds, which leaves something to be desired due to the lack of effective herbicides [6]. Controlling all of these parameters to improve yields has led Cameroon to explore *Bt* cotton since 2012. However, it should be noted that the production of

transgenic plants is not a panacea, as it poses several environmental risks [7]. These include non-target insects, pesticide resistance, biodiversity loss, and food safety risks [8, 9]. The danger of gene flow via pollen between cotton and other related species is extremely concerning. Indeed, cotton's coexistence with other Malvaceae species raises concerns about the risks of horizontal pollination. Direct transgene flow into wild populations can proceed during hybridization between related species. These kinds of transfer have already been reported in cotton [10]. The hybridization of *G. hirsitum* (AADD genome) and *G. herbaceum* resulted in three types of hybrids, two of which were triploid (AAD) and the third was tetraploid (AAAD) (AA genome). Similarly, substantial effects were observed from hybridizations between cotton and other Malvaceae [11]. Based on the findings mentioned above, there is a risk of genetic material exchange between cotton and its relatives. Such a result raises serious concerns about the future of transgenic cotton if it is introduced in Cameroon. Therefore, the present work aims at assessing the risks of gene flow between *G. hirsitum* and *A. esculentus*.

2. Materials and Methods

2.1. Experimental Site

The field experimentation was conducted in one of the sites of the Genetics and Plant Breeding Unit (UGAP) located at the University of Yaoundé 1, in Yaoundé, republic of Cameroon from the year 2020 to 2021.

2.2. Plant Material

The plant material consisted of cotton varieties IRMA L484 and Q302 obtained at the Institute of Agricultural Research for Development (IRAD) of Ngaoundéré and *A. esculentus* local (VL) variety conserved within UGAP and locally collected.

2.3. Trial and Field Management

G. hirsitum varieties were sowed with three seeds per plot using a 1m x 1m sowing pattern at a depth of 30 cm over a 22 m 18 m area. Thinning was done after four true leaves emerged, with the aim of keeping one seedling per cluster. To synchronize flowering between the two species, *A. esculentus* was seeded under the same conditions as cotton but 22 days later. Watering, weeding, and hoeing were used to keep the plots in good conditions. Fertilizer (urea) was applied 30 days after sowing (DAS), and the insecticide cypermulk was applied every 2 weeks.

3. Methodology

3.1. Study of Stigma Receptivity and Pollen Viability

According to the fact that IRMA L484 and Q302 belong to the same species, Stigma receptivity as well as pollen grain viability were carried out on IRMA L484 and the VL.

Stigma receptivity was assessed by the deposition of a drop of oxygenated water at different stages of flower bud development [12]. These stages were 1, 9, 15, 17, and 18 days in cotton and 1, 8, 12, 20 and 21 days in *A. esculentus* according to the development of the flower bud in each species.

The viability of pollen grains was analyzed by the modified Alexander method from the anthesis of flowers of both species [13]. The counting of pollen grains was done under a light microscope (×10). Pollen viability expressed as a percentage was measured at anthesis, at 12 h, 16 h and 24 h after anthesis [14], according to the following formula $V\% = \frac{\text{NGPV}}{\text{NGPT}} \times 100$. With V%: viability rate, NGPV= number of viable pollen grains (NGPV), NGPT: number of total pollen grains.

3.2. Evaluation of the Fruit Set Rate of Intra- and Interspecific Crosses

Based on the stigma receptivity and pollen viability data, 675 controlled crosses were made, 600 interspecific and 75 intraspecific between the two species according to the combinations shown in Table 1. The number of capsules formed containing seeds after each cross was used to calculate the percentage of fruit set. To understand the possible failures during the crosses, 30 castrations without fertilization (10 for each species) were performed and the duration of the failure of the crosses was determined.

Table 1. *Combinations made between Irma L484 and Irma Q302, VL.*

♀ \ ♂	**L484**	**Q302**	**VL**
L484	L484 x L484	L484 x Q302	L484 x VL
Q302	Q302 x L484	Q302 x Q302	Q302 x VL
VL	VL x L484	VL x Q302	VL x VL

4. Results

4.1. Stigma Receptivity

The receptivity of the cotton stigma begins four days before anthesis, i.e. the 12th day after the appearance of the floral bud. It is maximal on the day of anthesis and continues 24 h later. On the other hand, in *A. esculentus* (VL) it starts two days before anthesis (20th day after the appearance of the floral bud), becomes maximum at anthesis and continues a few hours afterward before becoming zero 24 hours later (Table 2).

Table 2. *Receptivity of studies species stigma.*

Stage of flower bud development	Stigma receptivity	
	Gossypium hirsitum	***Abelmoschus esculentus***
Third stage (15 and 12 days respectively)	+	0
Fourth stage (17 and 20 days)	++	+
Fifth stage (anthesis, 19 and 22 days)	+++	+++
12 hours after anthesis	++	++
16 hours after anthesis	+	+
24 hours after anthesis	+	0

0: no receptivity +: low receptivity, ++: medium receptivity, +++: maximum receptivity

4.2. Viability of Pollen Grains

The viability of pollen grains in *G. hirsitum* is maximum at anthesis and gradually decreases until it is canceled after 24 hours. In *A. esculentus*, it is also maximum at anthesis and decreases progressively until it is cancelled. However, it decreases more in *A. esculentus* than in *G. hirsitum* (Table 3).

Table 3. *Viability of pollen grains of studies species.*

Stage of flower bud development	Pollen grain viability (%)	
	G. hirsitum	***A. esculentus***
Anthesis	90.5 ± 8.4	87.3 ± 34
12 hours	69 ± 11.5	66.5 ± 6
16 hours	0.45±85	0
24 hours after anthesis	0	0

4.3. Fruit Set Rate in Intra- and Interspecific Crosses

The fruit set rates of intraspecific crosses vary from 66.6 (VL × VL) to 80% (Q302 × Q302, L484 × Q302 and Q302 × L484), L484× L484 (Table 4).

In interspecific crosses, 4 out of 600 crosses resulted in fruiting at a fruit set rate of nearly 2%. These fruits were obtained whenever *A. esculentus* (VL) was used as a female parent. However, no fruit set was recorded when *A. esculentus* was used as a male parent (Table 4).

Castrations without fertilization in *A. esculentus* result in flower drop three days after emasculation whereas those in *G. hirsitum* drop within ten to twelve days as in interspecific crosses when *G. hirsitum* was used as the female parent. In contrast, the latter fail 3 days later when used as a male parent as in intraspecific crosses of this species (Table 4).

Table 4. *Fruit set rates of interspecific and intra-specific crosses.*

Crossings (♀×♂)	Number of pollinations	Number of Capsules formed	Number of capsules with well-formed seeds	Fruit set percentage (%)	Time to notice failure (day)
Intraspecific crosses					
VL × VL	15	12	10	66.6	3
L484 × L484	15	13	11	73.33	10 ± 2
Q302 × Q302	15	13	12	80	10 ± 2
L484 × Q302	15	12	12	80	10 ± 2
Q302 × L484	15	13	12	80	10 ± 2
Total	75	63	57		
Interspecific crosses					
VL x L484	150	2	2	1.33	10 ± 2
L484 × VL	150	0	0	0	2 ± 1
VL x Q302	150	2	1	0.667	10 ± 2
Q302 × VL	150	0	0	0	3
Total	600	4	3		

5. Discussion

Cotton stigma receptivity begins four days before anthesis (12th day after the appearance of the floral bud). It begins on the day of anthesis and lasts for 24 hours. In *A. esculentus*, it starts two days before anthesis (20th day following the appearance of the floral bud), peaks at anthesis, and continues for a few hours until becoming null 24 hours later. These results are in line with previous work [15], which examined stigma receptivity and pollination in *A. esculentus* at different stages of anthesis and reported that the best results for stigma receptivity were obtained on the day of anthesis and gradually decreased. These results confirmed those which showed that receptivity is maximal during anthesis and lasts for 12 hours [16].

The viability of pollen grains in both species was found to be maximum during anthesis and progressively decreases over time until it is cancelled. Similar findings were observed on *Cucumis melo* L, demonstrating that vitality declines over time [17].

All intraspecific crosses within the two species are successful. These were successful because there were no incompatibility hurdles.

Fruiting resulted at a fruit set rate of about 2% in interspecific crosses (4 out of 600 crosses). The stress of the flower buds during manual castration can explain *G. hirsitum's* failure as a female parent. This occur 10-12 days or hours? after the conception. However, castrated cotton buds that haven't been pollinated fall off within the same time interval, suggesting that it will be impossible to identify whether this failure is pre- or post-zygotic. However, according to a previous study, pollination to fertilization takes 20-30 hours [18]. As a result, the time required for the failure to occur suggests a zygote formation seems to be possible. Interspecific crosses have used *A. esculenthus* as the female parent resulted in a fruit set percentage of around 2%. Indeed, three fruits were obtained from a tetraploid (*G. hirsitum*) and a diploid (*A. esculenthus*), suggesting the possibility of genetic material exchange between the two species. Aneuploidy phenomena can explain these results. The hybridizations between *G. hirsitum* (AADD) and one of its ancestors, *G. herbaceum* (AA), have given rise to three hybrids, two of which were triploid (AAD) and the third tetraploid (AAAD) [10]. Other results further supported this position through an aneuploidy cotton plants obtained by crossing *G. hirsitum* and *A. esculentus* [11]. Crosses between cotton species and Malvaceae (*Malva sylvestris* L., *Hibiscus syriacus* L., and *Abelmoschus esculentus* Moench) have yielded some of the expected results. For example, the aneuploid hybrids obtained by combining the characters of the cotton species were obtained by crossing F1 cotton (*G. hirsutum* L., *G. barbadense* L.) with *A. esculenthus*.

6. Conclusion

The objective of this study was to evaluate the risk of gene flow between *G. hirsitum* and *A. esculentus*. According to the results obtained on the receptivity of *G. hirsitum* stigma, this starts four days before anthesis (12th day after the appearance of the floral bud). It starts on the day of anthesis nd lasts for 24 hours. Receptivity in *A. esculentus* occurs two days before anthesis and peaks at anthesis. The viability of pollen grains in both species was highest at anthesis, indicating that this was the best possible time for pollination. When *A esculentus* is the female parent, the percentage of fruit set is at 2%, which indicates the possibility of gene transfer between *G. hirsitum* to *A. esculentus*. Future studies would monitor the fate of seeds from interspecific crosses between the two species and assess the agro-morphological and molecular characteristics of these possible hybrids.

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