OVERCOMING CROSS- AND SELF-INCOMPATIBILITY IN *IPOMOEA BATATAS* (L) LAM AND *IPOMOEA TRICHOCARPA* ELLIOT

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SUMMARY

Pollen germination in the wild species *I. trichocarpa* is inhibited on the stigma of incompatible genotypes. Removal of the stigmatic lobes allowed successful germination. No similar inhibition mechanism was found on the stigma of *I. batatas* in which however short flower life, coupled with slow rate of pollen tube penetration, limited fertilization in incompatible pollinations. Suppression of abscission by the application of 2,4—D (conc. 100 ppm) to the pedicels provided adequate time for pollen tube penetration to occur in incompatible styles of sweet potato and resulted in fertilization and seed-set. Seedling development could be accomplished by embryo culture before fruits matured. The practical techniques developed allow a measure of sifting to be introduced into the breeding programme and for crosses to be made between pairs of desirable but normally incompatible parental genotypes.

RESUME

La germination pollinique dans les espèces *I. trichocarpa* sauvages est contrariée sur le stigmate des génotypes incompatibles. La germination se produit normalement quand on retire les lobes stigmatiques. On ne rencontre pas un phénomène d'inhibition semblable sur le stigmate de *I. batatas* dans lequel, toutefois, la courte durée de floraison et le taux lent de pénétration du tube pollinique limitent la fertilization dans les pollinisations incompatibles. La suppression de l'abscission lorsqu'on applique 2,4—D (con. 100 ppm) aux pédicelles assure le temps nécessaire pour que la pénétration due tube pollinique se réalise dans les styles incompatibles de la patate douce et aboutit à la fertilisatio et à la formation des graines. On peut obtenir le développement des plantules par la culture d'embryon avant la maturité des fruits. Les méthodes pratiques mises au point permettent une mesure de vannage à introduire dans le programme de sélection et des croisements à opérer entre des paires de génotypes parentaux aux caractéristiques désirables mais normalement incompatibles.

RESUMEN

La germinacion de polen en las especies silvestres de *I. trichocarpa* se inhibe en el estigma de genotipos incompatibles. La remoción de los lóbulos estigmáticos permitió una germanación exitosa. No se encontró un mecanismo inhibidor similar en el estigma de *I. batatas* en el cual, sin embargo, la reducida vida de la flor, apareada con el bajo grado de penetración de los tubos polínicos, limitó la fertilización en las polinizaciones incompatibles. La supresión de la abscición con la aplicación de 2,4—D (conc. 100 ppm) a los pedicelos, proveyó de un tiempo edecuado para que la penetración de los tubos polínicos ocurriera, obteniéndose como resultado fertilización, y produccion de simillа. El desarrollo de las plantas se logró mediante cultivo de embriones antes de que los frutos estuviesen maduros. Las técnicas prácticas que se desarrollaron permiten introducir una forma de selección en los programas de mejoramiento genético y en las cruzas que se hagan entre pares de tipos deseables, con genotípos parentales normalmente incompatibles.

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INTRODUCTION

Cross- and self- incompatibility in West Indian cultivars of sweet potato has been a problem in breeding, since many of desirable parents are in the same incompatible group.

Self-incompatibility in the sweet potato was first reported by Mendiola in 1921 (in 16). Cultivar variation in self-incompatibility occurs[9].

Several methods have been employed to overcome the fertilization barriers. Togari and Kawahara[15] and Fujisie[3] were unsuccessful when they tried to self-fertilize sweet potato cultivars by bud pollination. Van Schreven[16] however reported an increase in pollen tube development after bud pollination with self pollen. Mangelsdorf and Reeves[7] obtained hybrid seed from a cross between the two genera *Zea* and *Tripsacum* by cutting off part of the style and Davies[2] used the same method successfully or crossing two species of *Lathyrus*. Chaudhuri[1] lengthened the life of flowers in arrowroot by applying p-chlorophenoxy acetic acid (conc. 100 ppm) to pedicels and was able to attain successful seed-set in crosses. Laibach[5,6] was the first to demonstrate the possibility of using embryo culture technique to attain seedling development from inter-specific crosses in *Linum* where normal seed production fails on account of premature abscission of young fruits.

The purpose of this study was to investigate the nature of the barriers to self-fertilization in *I. trichocarpa* and in *I. batatas* cultivars of West Indian sweet potato, and attempt to overcome it.

MATERIALS AND METHODS

Eight cultivars of *Ipomoea batatas* (L.) Lam., and two strains of a wild species, *Ipomoea trichocarpa* Elliot were studied (Table 1). Experiments were conducted in an insect proof greenhouse and an additional set of plants was grown in the open for comparison.

Flowers were emasculated between 1.00 and 3.00 p.m. the day prior to anthesis and paper clips were placed over the end of the corolla to keep the flower closed until the crosses of selfs were made. The corollas of flowers to be used as pollen sources were also clipped. Pollinations were made on the day following emasculation between 8.00 – 10.00 a.m. when stigmatic surface of pistils appeared to be most receptive. Where capsules developed they were harvested within a month of pollination. Pollen germination and tuber penetration in self- and cross-pollinated flowers were examined *in vivo* using excised styles, stained with aniline blue and fluorescent pollen bands examined under UV light of 365 *mu* wavelength according to the method of Martin[8].

Experiment 1. The cause of the incompatible reaction of pollen in *I. trichocarps* was investigated in styles following self- and cross-pollinations. Eight styles of each treatment were examined after 2,4,6 and 12 hrs. (Table 2).

Experiment 2. The following techniques were employed to investigate the inhibition to pollen germination after self-pollination in *I. trichocarpa:* 1. the stigma lobes were scraped superficially, 2. the lobes were excised, 3. the style was trimmed to half its length and 4. the style was severed at the style-ovary junction. Sixteen flowers of strain 3 were used for each treatment and styles were examined after 6 and 24 hours (Table 3).

Experiment 3. The self-incompatibility reaction of pollen was investigated in styles of *I. batatas* with stigma lobes removed and retained. Eight flowers of each of five cultivars were examined for pollen germination and depth of tube development at 3,6,24 and 30 hrs. An ocular micrometer was used to measure tube length and depth of penetration was expressed as a percentage of the entire length of the style (Table 4).

Experiment 4. Observations were made on floral life following anthesis in both species in the open. In an attempt to induce seed set, flowers were self-pollinated, 1. after excision of stigma lobes and, 2. with lobes retained. Eight flowers of each treatment were used in each species.

Experiment 5. Investigations of an exploratory nature were done to prolong floral life by using growth substances to suppress the formation of the abscission layer in pedicels. Indole acetic acid, naphthalene acetic acid, 2,4—Dichlorophenoxy acetic acid and 2,4,5—trichlorophenoxy acetic acid were dissolved separately in an anhydrous lanolin base to give concentrations of 50 and 100 ppm. The pedicel and ovary base of eight flowers of the cultivar 30/57 of *I. batatas* and strain 3 of *I. trichocarpa* were treated with each level of four growth substances immediately after self-pollination. The stigma lobes were removed only in *I. trichocarpa* (Table 5).

Experiment 6. The frequency of 2,4—D (100 ppm) application was increased to determine its effect on

further ovary development. Two applications were applied to eight flowers of each species at a 16-day interval; three applications at an 8-day interval and five applications at a 4-day interval (Table 6).

Experiment 7. Embryos were cultured from immature seeds of *I. batatas* resulting from compatible and incompatible pollinations. Seeds were surface sterilized with 0.1% mercuric chloride for 1 minute and washed with distilled water. Embryos were then excised using a needle and forceps sterilized in 80% ethanol and flamed. They were then cultured inside screw cap bottles with a mineral nutrient medium containing 0.8% agar and 2.0% dextrose[4]. Fungal and bacterial contaminations were reduced by flaming the mouth of bottles before making transfers in an isolation chamber. Cultures were developed at room temperature under ordinary daylight.

Thirty five flowers of A26/74 were pollinated with compatible pollen from R223. Sixty three flowers of A26/74 were pollinated with incompatible pollen from 30/57. A total of ninety-six flowers were self-pollinated in R223. Seed-set was induced artificially by the application of 2,4—D (100 ppm) after pollination (Table 7).

RESULTS

Pollen retention and germination on the stigma differed between self- and cross-pollinations in *I. trichocarpa* (Table 2). Pollen failed to germinate on stigmas following self-pollination; the mean number of grains retained on the stigma was considerably less than those found in the cross-compatible pollinations. Tube penetration into the styles of compatible pollinations was central and completed within two hours.

No germination was visible on any of the carpels in *I. trichocarpa* when the stigma lobes were superficially scraped nor when styles were severed at their bases before self-pollination (Table 3).

Successful germination was observed on all carpels with stigma lobes removed and on 25-50 percent of the styles trimmed to half their lengths. Further examination in treatment 2 showed that pollen development was peripheral to the central core of the style and the depth of penetration was ¾ the total length of the style at 6 hours. Complete penetration into the style and ovaries was accomplished after 24 hours.

Pollen germination on stigmatic surfaces was visible in all five cultivars of *I. batatas* irrespective of whether the stigma lobes were removed or retained. The mean percentage depth of tube extension in the styles differed in the early stages of development among cultivars (Table 4). With stigma retained, no tube penetration was observed in 12/56, 22/56 and 30/57 after 3 hours and after 6 hours tube penetration was inhibited only in 12/56. When the stigma was excised no delay in tube penetration was observed at 3 and 6 hours in any cultivar. However, considerable variation in tube depth was observed among cultivars at 3 hours. The tube depths in styles of 12/56 and 22/56 were very much shorter than in any of the other cultivars. These differences were not apparent after 6 hours of pollination. After 24 and 30 hours no difference in tube depth was observed in ovules of carpels examined when stigmas were either excised or retained.

It was observed that flowers in both species opened in the morning and closed during the hotter periods of the day (Experiment 4). Within 3 hours of flower opening, anthers showed signs of withering; by evening styles began to shrivel up and flowers showed signs of wilting. Complete floral abscission occurred within 1—3 days after anthesis. Abscission was earlier by one day in *I. trichocarpa* than in *I. batatas*. There were signs of ovary enlargement in incompatible crosses of *I. batatas* only when stigmas were excised but nevertheless no seed-set was accomplished.

The application 2,4—D at a concentration of 100 ppm prolonged floral life more in both species than any of the other treatment combinations (Table 5). In *I. trichocarpa* and *I. batatas* flower life was extended by 15 and 10 days respectively beyond that of the controls. Although ovary enlargement was observed, ovary abortion however took place.

When the frequency of 2,4—D (100 ppm) application was increased, ovary development resulted in seed-set in both species (Table 6). In *I. trichocarpa* no differences were found between the three frequencies of application in the percentages of flowers which set seed. However, in *I. batatas* the percentage of flowers that set seed increased from 0 to 75 percent with increasing frequency of application and optimum response was obtained with 5 applications at 4-day intervals. No germination was obtained from seeds collected from dried capsules in either species. Further examination revealed that the embryos had completely aborted and disintegrated in mature seeds.

The highest percentage of capsules was obtained in the compatible cross A 26/74 x R 223 (Table 7). The percentage capsules developed in the incompatible cross A 26/74 x 30/57 was considerably greater than that resulting from self-pollination in R 223. No embryo abortion was found in immature seeds in the compatible cross. Embryo abortion was 30.4 percent in the incompatible cross and 61.2 percent in the selfing of R 223. Despite precaution taken to prevent contamination, approximately 20 percent of the embryos excised in treatment 1 failed to develop on account of fungal and bacterial infections. Contamination accounted for 53.1 percent embryo loss in culture in treatment 2 and 66.6 percent in treatment 3. Excised

embryos from the compatible cross germinated and developed relatively faster than those from the incompatible pollination. The rate of embryo development from R 223 after self-pollination was very much slower than in embryos from the incompatible cross. Over sixty-six percent of the immature seeds from selfing R223 failed to germinate. From the compatible cross, 79.3 percent of the embryos excised developed and produced 23 seedlings. From the incompatible cross 46.8 percent of the excised embryos germinated in culture and 15 seedlings were developed. Only four seedlings developed representing 33.3 percent of the number of embryos excised from the self pollination of R 223.

DISCUSSION

The self-incompatibility reaction was stronger in the wild species, *I. trichocarpa* than in the cultivated forms of *I. batatas* studied.

The primary site of the self-incompatibility mechanism in *I. trichocarpa* was the stigmatic surface which inhibited wrong pollen germination. This is similar to the mechanism reported for *I. batatas*[10,12,15,16] Fujisie[3] attributed the failure of pollen germination to lack of stylar growth substances necessary to stimulate germination of incompatible pollen.Complete pollen germination failure was ascribed by Martin and Cabanillas[10] to a strong incompatible reaction resulting from the interaction of substances diffusing from pollen or stigma or both. Martin [14] reported disorientation of pollen tube development in incompatible styles in *I. batatas* and confirmed similar observations made in *I. trichocarpa* when the stigma lobes were removed before self-pollination. The peripheral pathway taken by pollen tubes allowed the evasion of substances inhibitory to tube development present in the central core of the style. Mechanical retardation in tube growth could have resulted from the nature and arrangement of cells in the region of tube penetration as reported by Martin and Ortiz[13].

Williams and Cope[19] found that fewer pollen grains were retained on incompatible stigmas than on compatible ones in *I. trichocarpa* and explained this as due to lack of mechanical anchorage by pollen tubes penetrating the stigma of compatible styles. Similar observations have been made in this study. The technique used by Mangelsdorf and Reeves[7] and Davies[2] of truncating the styles before pollination gave greater reduction in germination than when only stigma lobes were removed. This suggests that the physiological reaction for pollen incompatibility may increase with nearness to the ovary. Complete failure of pollen germination for pollen placed at the style-ovary junction confirmed results obtained by Martin and Cabanillas[10]. The rate of pollen tube extension in incompatible styles when the stigma lobes were removed was slower by 22 hours than in compatible styles.

No instance of complete germination failure on an incompatible stigma occurred in cultivars of *I. batatas*. The principal self-incompatibility mechanism appeared to involve failure of the pollen tubes to grow normally after germination. The mechanism for this is not yet understood. The relative levels of self-incompatibility between cultivars could be assessed during the initial phase of tube penetration within 3—6 hours after germination. Under field conditions a temporary arrest in the early phase of tube penetration by incompatible pollen may effectively prevent fertilization by wrong-pollen since styles tend to shrivel shortly after flowers are opened on the same day. The possibility of further delay occurring at the style-ovary junction as suggested by Martin and Cabanillas[11] cannot be ruled out, although no evidence of this was visible after 24 hours of tube penetration in our materials.

Wang[17] reported that in addition to the incompatibility factor, temperature, pollen viability and meiotic abnormalities are important factors influencing seed-setting ability of different cultivars. Warmke and Cruzado[18] suggested that non-disjunction and other irregularities are common and can account for a high percentage of poorly formed pollen grains in commercial varieties of sweet potato. Martin[14] suggested that seedset failure may not only be due to the incompatibility reaction of pollen but to certain sterility factors associated with gametic imbalance. We have not investigated these other factors in our material but our findings suggest a physiological rather than a genetic mechanism.

The production of non-viable seed from self- and cross-incompatible pollinations but the successful development of seedlings from such crosses by embryo culture are similar to the results obtained for *Linum* by Laibach[5,6]. It seems that there may be an incompatible reaction between tissue of the maternal parent and the developing embryo as the seed matures.

Current practical results have made it possible to establish an improvement programme employing selfing as a step in a breeding method. At least partially homozygous forms can be tested for combining ability for certain desirable characters. Also benefit can now be derived from hybridization among cross-incompatible groups having good commercial characteristics. The fact that the sweet potato is an asexually reproduced crop allows improved genotypes to be perpetuated.

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TABLE 1

Description of plant material

Species	Accession No.	Source	Description
I. batatas	09/56	Trinidad	
	22/56	"	
	12/56	Barbados	Indigenous West Indian cultivars
	13/56	"	
	30/57	St. Vincent	
	02/59	Granada	
	C 9	Department of Crop Science, UWI Tinidad	Progenies from open-pollination of West Indian cultivars
	O 49		
	A26/74	"	
I. trichocarpa	01/64(strain 1)	Department of Botany,UWI, Trinidad.	Wild species
	03/64(strain 3)		

TABLE 2

Mean number of pollen grains retained on stigmas and pollen germination and development in styles of *I. trichocarpa* after self- and cross-pollinations

Pollinations within and between strains of *I. trichocarpa*	Mean number of grains on stigma				Pollen germination and tube development			
hours	2	4	6	12	2	4	6	12
3 x 3 (IC)	8	11	15	15	0	0	0	0
3 x 1 (C)	108	96	111	62	+	+	+	+
1 x 3 (C)	152	131	138	135	+	+	+	+

IC = incompatible pollination

C = compatible pollination

TABLE 3

Percentages of carpels on which pollen germination was observed

Treatments	ca. % of carpels on which pollen germinated	
	6 hr	24 hr
1. stigma 'cap' scraped	0	0
2. stigma 'cap' excised	100	100
3. style trimmed to half its length	50	25
4. style severed at the base	0	0

TABLE 4

What percentage depth of pollen tube penetration in styles of cultivars of *I. batatas* at 3, 6, 24 and 30 hrs., after self-cultivars of *I. batatas* at 3, 6, 24 and 30 hrs., after self-pollination

Cultivars	X percentage depth of tube penetration in styles							
	Stigma retained				Stigma excised			
Hours	3	6	24	30	3	6	24	30
09/56	58.0	100.0	100.0	100.0	69.0	100.0	100.0	100.0
12/56	0.0	0.0	100.0	100.0	12.5	100.0	100.0	100.0
22/56	0.0	100.0	100.0	100.0	12.5	100.0	100.0	100.0
20/57	0.0	63.0	100.0	100.0	53.0	100.0	100.0	100.0
02/57	100.0	100.0	100.0	100.0	88.0	94.0	100.0	100.0

TABLE 5

Effect of growth substances on the length of life of flowers in both species after incompatible pollination

Treatment	Conc. (ppm)	Average length of life of flowers (days after pollination) *I. trichocarpa* (strain 3)	*I. batatas* (30/57)
Control	-	3	4
IAA	50	7	4
IAA	100	6	5
NAA	50	6	5
NAA	100	7	6
2,4-D	50	13	6
2,4-D	100	18	14
2,4,5-T	50	12	7
2,4,5-T	100	17	12

TABLE 6

Percentage seed-set in both species after 2,4—D (100 ppm) application at 3 frequencies

Number of applications at 16, 8 and 4 day intervals respectively	ca. % of flowers which set seed *I. trichocarpa* (Strain 3)	*I. batatas* (30/57)
2	50	0
3	50	25
5	50	75

TABLE 7

Percentage seedlings developed from cross-compatible and self- and cross-incompatible pollinations following seed-set induction and embryo culture in *I. batatas*

Treatment	Pollinations ♀	♂	Number of capsules: Flowers	Number	%	Embryo excised: Number	A	D	Embryo loss: % aborts	% diseased	Seedlings: Number	%
1	A26/74 x R223 (C)		35	29	84.0	29	0	6	0.0	20.7	23	79.3
2	A26/74 x 30/57 IC		63	46	73.0	32	14	17	30.4	53.1	15	46.8
3	R223 selfed (IC)		96	31	32.3	12	19	12	61.2	66.6	4	33.3

C = Compatible IC = Incompatible A = number of aborts
D = number of diseased embryos