Cross Compatibility between Eggplant (*Solanum melongena* L.) and Wild Relatives

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Summary

Pollen tube growth behavior and success of interspecific crosses between eggplant and wild relatives were studied. *Solanum gilo*, *S. insanum* and *S. integrifolium* were found compatible to eggplant. *S. indicum*, as the male and female parent, produced mostly unfilled seeds with eggplant 'Senryo 2 gou'. This species as seed parent produced about 50 % filled seeds when it was pollinated by eggplant 'Uttara'. Only pollination by 'Senryo 2 gou' produced a few viable seeds on *S. surattense* and *S. xanthocarpum*. Pollen tubes of *S. mammosum* grew normally in the pistils of 'Senryo 2 gou' resulting 100 % parthenocarpic fruit development. The 'Uttara' produced no parthenocarpic fruit, while the former cultivar produced parthenocarpic fruit with 8 different species. Failure or poor germination of pollen, retarded growth of pollen tubes, termination of pollen tube growth before reaching the ovary, weak fluorescence of the pollen tubes, irregular deposition and size of callose plugs, swollen and/or branched pollen tube apex alone or in combination were observed in most of the incompatible crosses.

Introduction

Eggplant is susceptible to several soil borne diseases and insect pests. Some of the poor quality fruited cultivars and many related species of *Solanum* have partial to complete resistance against these hazardous pests (1, 5, 9, 10, 19). There are several promising *Solanum* species which can directly be used as root stock for eggplant cultivation or as breeding materials. The use of interspecific hybrid, having multiple disease resistance as root stock might have immense importance. Some sporadic attempts were made to establish species relationship and to transfer desired gene(s) from wild to cultivated *melongena* (2, 6, 9, 11, 13). In most of the earlier attempts, the number of species studied and/or the number of crosses made for each interspecific combination were poor. However, there are some arguable results of interspecific cross between eggplant and wild relatives (6, 12, 17). Reports on interspecific compatibility in relation to

pollen tube growth and seed set in eggplant relatives are meager. Moreover, pollen tube growth behavior in interspecific crosses has not yet been reported. Interspecific compatibility in *Solanum* species in relation to pollen tube growth behavior and seed setting ability was examined in this study aiming at producing hybrid plants.

Materials and Methods

Two cultivars of eggplant, 'Senryo 2 gou' (an F₁ hybrid from Japan) and 'Uttara' (a purebred from Bangladesh), and 17 wild relatives (Table 1) were grown in vinyl house during 1987 and '88 at the University farm, Kyushu University. Direct and reciprocal crosses between eggplant and wild relatives were made from April to July 1988 through bud pollination. Throughout the hybridization period the minimum temperature was maintained at 16°C and the maximum at 27°C. For the study of pollen tube growth behavior, pollinated pistils were collected after 3 days of pollination in acetic-alcohol (1:3 v/v) for 24 h, and transferred into 70 % ethanol for a day or long. Pistils of most of the species were softened at 60°C for 60 min in 1 N NaOH.

Received for publication August 26, 1989.

Part of this study was presented at the annual meeting of the Japanese Society for Horticultural Science, Tokyo, April, 1989.

Table 1. Pollen tube behavior in the pistils in self and interspecific crosses of *Solanum*.

Species	Self				As pistilate parent							
	1	2	3	4 ^z	'Senryo 2 gou'				'Uttara'			
					1	2	3	4	1	2	3	4
<i>S. melongena</i> L.												
'Senryo 2 gou'	M	N	N	N ^y								
'Uttara'	M	N	N	N								
<i>S. achrolcucum</i> Bast.	M	N	N	N	F	W	—	—	M	W	—	—
<i>S. ferox</i> L.	F	N	N	N+A	F	N	N+S	N+A	M+F	N	N+S	N+A
<i>S. gilo</i> Raddi.	M	N	N	N	M	N	N	N	M	N	N	N
<i>S. indicum</i> L.	F	N	N	N	M	N	N	N	M	N	N	N
<i>S. insanum</i> L.	M	N	N	N	M	N	N	N	M	N	N	N
<i>S. integrifolium</i> Poir.	M	N	N	N	M	N	N	N+A	M	N	N	N
<i>S. khasianum</i> Clarke.	M	N	N	N	M	N	N+BS	N+A	M	N	N+S	N+A
<i>S. mammosum</i> L.	M	N	N	N	M	N	N	N	M	N	N	N
<i>S. nigrum</i> L.	M	N	N	N	M	W	N+S	—	M	W	—	—
<i>S. nodiflorum</i> Jacq.	M	N	N	N	—	—	—	—	M	W	—	—
<i>S. sisymbriifolium</i> Lam.	M	N	N	N	M	N	N+BS	A	M	N	N+S	A
<i>S. surattense</i> Burm.	M	N	N	N	M	N	N+S	N+A	M	N	N+BS	N+A
<i>S. torvum</i> Swartz.	M	N	N	N	M	N	N	N	M	N	N+S	N
<i>S. toxicarium</i> Lam.	M	N	N	N+A	M	W	B	A	M	N	N+S	N+A
<i>S. verbascifolium</i> L.	M	N	N	N	F	W	—	A	M	W	—	—
<i>S. viarum</i> Dunal.	M	N	N	N	M	N	N+S	N+A	M	N	N	N+A
<i>S. xanthocarpum</i> S. et W.	M	N	N	N	M	N	S	A	M	N	N	N
									As pollen parent			
<i>S. achrolcucum</i>					F	N	—	—	M	N	N	N
<i>S. ferox</i>					F	N	—	—	F	N	—	A
<i>S. gilo</i>					M	N	N	N	M	N	N	N
<i>S. indicum</i>					M	N	N	A	M	N	N	N
<i>S. insanum</i>					M	N	N	N	M	N	N	N
<i>S. integrifolium</i>					F	N	N	N+A	M+F	N	N	N
<i>S. khasianum</i>					F	N	—	—	F	N	—	—
<i>S. mammosum</i>					F	N	—	—	M	N	N	N
<i>S. nigrum</i>					F	N	—	—	F	W	BS	—
<i>S. nodiflorum</i>					F	N	N+S	A	F	N	N+S	A
<i>S. sisymbriifolium</i>					M	N	—	—	M	N	N+S	—
<i>S. surattense</i>					M+F	N	N	N+A	M	N	N	N
<i>S. torvum</i>					F	N	—	—	F	N	N	N
<i>S. toxicarium</i>					M	N	—	—	M	N	—	—
<i>S. verbascifolium</i>					F	N	—	—	F	W	—	—
<i>S. viarum</i>					F	W	—	—	F	N	—	—
<i>S. xanthocarpum</i>					M	N	N	N	M	W	N	—

^z 1=Quantity of pollen germinated; 2=Fluorescent reaction; 3=Behavior of pollen tube apex; and 4=Behavior of callose plug.

^y A=abnormal. B=branched. F=few. M=many. N=normal. S=swollen. W=weak. —=unable to be ascertained.

They were stained by 0.1 % aniline blue(7) at least for 15 min, squashed and observed under a fluorescent microscope (wave length 365 nm). Quantity of pollen germinated and pollen tube growth through different parts of pistil, fluorescent reaction, pollen tube tip appearance and callose plug formation and dis-

tribution pattern were recorded. Small callose deposition with equidistant distribution of the plugs throughout the pollen tube were considered as normal. Where pollen tube growth was limited on the stigma and/or fluorescent reaction was weak appearance of pollen tube apex and behavior of callose plugs could not

Fig. 1. Relationship between pollen tube growth in the pistil and seed set per fruit in selfing and interspecific crosses of *Solanum*.

be recorded in most of the cases. Nine to 15 observations were made per combination.

Fruit were harvested at maturity after 50 to 60 days of crossing except *S. torvum* which took 90 days. Immature and readily recognizable flat seeds and the seeds which floated on water were considered to be unfilled. The fruits

which did not contain any seed were considered to be parthenocarpic. Number of filled and unfilled seeds per fruit were calculated on the total number of fruit set. Selfings were made as the control. Germination % of the F_1 seeds were evaluated in January '89. Where there was very poor seed set, some of the seeds

all the combinations but differential growth of pollen tube was observed (Fig. 1). 'Uttara', both as the pollen and pistillate parent, was found better than 'Senryo 2 gou' in respect of quantity of pollen germination. Stigmas of *S. melongena* were more favorable to germinate pollen of wild *Solanum* than those of other species studied. Poor pollen germination and/or weak fluorescence of pollen tubes were observed in the stigmas of *S. achrolocucum*, *S. ferox*, *S. integrifolium*, *S. khasianum*, *S. mammosum*, *S. nigrum*, *S. nodiflorum*, *S. torvum*, *S. toxicarium*, *S. verbascifolium* and *S. viarum* when they were pollinated by *S. melongena* or vice versa. Normal pollen tube tips(4), bright fluorescence of the tubes having normal collose plug formation and distribution (18) were observed in the crosses between eggplant and *S. gilo*, *S. insanum* and *S. indicum* irrespective of direction of the crosses. Pollen tube behavior of *S. mammosum* was normal in the pistils of *S. melongena* but in reciprocal direction cultivar difference was noticed. Pistils of 'Senryo 2 gou' was favorable for pollen tube growth behavior of *S. torvum*; *S. xanthocarpum* favored the pollen of 'Senryo 2 gou'. On the other hand pollen tubes of 'Uttara' exhibited normal growth behavior in the pistils of *S. integrifolium*, *S. surattense* and *S. torvum*, but as the pistillate parent, 'Uttara' was congenial for *S. integrifolium* and *S. xanthocarpum*. Swollen and branched pollen tube apex alone or in combination with normal tube tip in the same pistil were observed in about 50 % of the interspecific combinations when eggplant was the pistillate parent. Cultivar difference in *S. melongena* and difference due to direction of the interspecific crosses in relation to pollen tube growth behavior were also conspicuous in many cases. Random distribution of callose plugs and irregular deposition of callose in the pollen tubes were observed mostly in the tubes having abnormal growth. On selfing, pollen tube growth behavior was normal in most of the aspects studied in almost all the species.

Pollen tube growth and fruit set

Pollen tubes of 15 species grew down to the style and of 6 species to the ovary of 'Senryo 2 gou' (Fig. 1) while fruit set was observed

with 11 species combinations (Table 2). Pollen tubes of all the species entered into the style and of 4 species up to the ovary of 'Uttara' (Fig. 1), but fruiting was recorded with 3 species (Table 2) common to 'Senryo 2 gou' as well. In reciprocal crosses, both of the eggplant cultivars produced fruit on the same 7 species (Table 2). Eight species produced parthenocarpic fruit on 'Senryo 2 gou', while 'Uttara' produced none. In self pollination (Table 2) there was no parthenocarpic fruit development. *S. insanum*, as the male parent, produced bigger fruit on both the cultivars (Table 2) than that of respective self (Table 2).

Pollen tube growth and seed set

Successful pollen tube growth of *S. gilo*, *S. insanum* and *S. integrifolium* up to the ovary of *S. melongena* and vice versa resulted into production of good number of seeds per fruit (Fig. 1). The pollen tubes of *S. indicum* and *S. torvum* grew up to the ovary of 'Senryo 2 gou' but most of the seeds were unfilled. The former species as the seed parent showed cultivar difference in respect to seed set having about 50 % filled seed when was crossed with 'Uttara'. *S. integrifolium* as the seed parent produced 45 seeds per fruit when it was pollinated by 'Senryo 2 gou'; pollen tubes of which could not reach beyond the style base indicated rather slow rate of pollen tube growth. *S. surattense* and *S. xanthocarpum* as pistillate parents were found compatible to both of the cultivars. In case of self pollination pollen tubes grew down to the ovary in all species and seeds were produced except in *S. verbascifolium*. The highest number of seed per fruit was recorded in 'Uttara' (self) followed by the combinations of *S. melongena* and *S. insanum*.

Seed germination

More than 80 % germination of seeds was recorded in 12 different interspecific combinations (Table 3). Germination of *S. surattense* and *S. xanthocarpum* in combination with 'Senryo 2 gou' as the male was poor while with 'Uttara' it was nil. When *S. indicum* was pollinated by 'Senryo 2 gou' the seeds failed to germinate, while the seeds fertilized by 'Uttara' pollen had poor germination. Seeds of 'Senryo 2 gou' $\times$ *S. indicum* had 8 % germi-

Table 3. Seed germination (%) of interspecific hybrids between *S. melongena* ('Senryo 2 gou' and 'Uttara') and wild relatives.

Wild relatives	As seed parent		As pollen parent	
	'Senryo 2 gou'	'Uttara'	'Senryo 2 gou'	'Uttara'
<i>S. achrolocucum</i>	—	—	—	—
<i>S. ferox</i>	—	—	—	—
<i>S. gilo</i>	100 (40) ^z	99 (80)	88 (80)	86 (80)
<i>S. indicum</i>	8 (25)	—	0 (25)	9 (90)
<i>S. insanum</i>	98 (50)	90 (50)	98 (50)	100 (50)
<i>S. integrifolium</i>	99 (80)	98 (90)	100 (50)	96 (80)
<i>S. khasianum</i>	—	—	—	—
<i>S. mammosum</i>	—	—	—	—
<i>S. nigrum</i>	—	—	—	—
<i>S. nodiflorum</i>	—	—	—	—
<i>S. sisymbriifolium</i>	—	—	—	—
<i>S. surattense</i>	20 (10)	—	34 (50)	0 (25)
<i>S. torvum</i>	0 (25)	—	—	—
<i>S. toxicarium</i>	—	—	—	—
<i>S. verbascifolium</i>	—	—	—	—
<i>S. viarum</i>	—	—	—	—
<i>S. xanthocarpum</i>	—	—	12 (90)	0 (90)

^z Values in parenthesis are the number of seeds sown.

— No seed set in these combinations.

nation. The seeds produced in 'Senryo 2 gou' pollinated by *S. torvum* did not germinate.

Discussion

Interspecific incompatibility in conventional sexual process is resulted mostly either due to failure of pollen germination or anomalous pollen tube growth behavior (3, 4, 14). In our present observation it appeared that retarded pollen tube growth, branched or swollen pollen tube tips, irregular deposition and distribution of callose plugs in the pollen tubes alone or in combination of these abnormalities might have resulted into incompatibility. In compatible crosses pollen tubes had thin-walled tips and regularly spaced callose plugs (4, 18).

Tatebe (17) and Khan *et al.* (6) reported contradictory results about the compatibility between *S. melongena* and *S. integrifolium*. However, the present investigation conforms the results of Ludilov (8) and Nishio *et al.* (12). Our results in relation to seed set in interspecific crosses between eggplant and *S. gilo*, *S. indicum*, *S. mammosum*, *S. torvum* and *S. xanthocarpum* are in partial to complete agreement with the results of Rajasekaran (15, 16), Nasrallah and Hopp (11) and McCammon and Honma (9). Rajasekaran (15) and Nishio *et al.*

(12) reported that *S. indicum*, as the seed parent only, was compatible to eggplant. But in our investigations eggplant 'Senryo 2 gou' as the seed parent also produced viable seeds with this species (Fig. 1 and Table 3). Fruit and seed setting ability of this cultivar indicated rather wide range of crossability. The conflicting results of interspecific compatibility might be due to genotypic difference of the material within and between species used by different authors. As the pollen parent both of the eggplant cultivars showed higher crossability than as the seed parent indicating some sort of cytoplasmic regulation on the success of interspecific crosses.

To overcome the barriers pertaining to pre-fertilization incompatibility intraovarian pollination or *in vitro* fertilization may bring success. In some cases post-fertilization incompatibility are resulted into seed abortion as it was observed in the combinations of *S. melongena*, *S. indicum* and *S. torvum*. To overcome such incompatibility, excised pre-mature embryo, ovule or ovary culture *in vitro* may be appropriate. For the most difficult but important combinations, somatic hybridization through protoplast fusion is the last tool to get the hybrid.

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ナス (*Solanum melongena* L.) と近縁野生種との交雑和合性について

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摘 要

ナスと近縁野生種とを交配し、花粉管の伸長行動と種子稔性について調べた。

S. gilo, *S. insanum*, *S. integrifolium* とナスとは高い交雑和合性が認められた。 *S. indicum* は‘千両2号’とは正逆交雑で多くのしいなをえたが、‘Uttara’とは種子親の場合に50%の稔実種子をえた。 *S. surattense* と *S. xanthocarpum* は‘千両2号’が花粉親の場合に少量の発芽力のある種子をえた。 *S. mammosum* の花粉管は‘千両2号’の花柱内を正常に生長したが、この

組合せでは雑種種子はえられず、単為結果にとどまった。なお‘千両2号’は8つの近縁種の花粉で単為結果を生じたが、‘Uttara’はそれらを花粉親にした交配では全く着果しなかった。

交雑不和合の組合せでは、花粉の不発芽または発芽不良、花粉管の生長遅延・子房到達前の生長停止・微弱的な螢光発色、カロースプラグの不規則な沈積・分布・大きさ、花粉管先端の肥厚や分岐などが、単独あるいは複合して観察された。