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SELF-INCOMPATIBILITY SYSTEM IN THE ETHIOPIAN POPULATIONS OF *GUIZOTIA ABYSSINICA* (L.F.) CASS. (NIGER)

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ABSTRACT: Self-incompatibility (SI) in the Ethiopian populations of *Guizotia abyssinica* (niger) was studied and detailed genetic analysis was performed. Experiments involving 1425 pollinations were carried out both by petri-dish technique and *in situ* pollination of the heads. The pollination patterns were recorded. In most cases, SI is believed to be controlled by a single S-locus (the recording of 4 self-incompatible mating groups) in the populations of *G. abyssinica* dealt with by this study. An additional S-locus, I, is proposed as part of the SI system in this crop; this is based on the recording of more than 4 self-incompatible mating groups in a considerable number of the populations studies. The type of incompatibility is found to be sporophytic. Pseudocompatibility and seeds obtained from pseudocompatible crosses were characterized. The SI system in *G. abyssinica* is characterized by the presence of two- and one-way incompatibility, reciprocal difference, and self-compatibility. Natural selection may favour the establishment of self-compatible genotypes in the populations of *Guizotia abyssinica*. The frequency of self-compatible genotypes is of a various magnitude in the Ethiopian populations of *G. abyssinica* originated from different collecting localities.

Key words/phrases: *Guizotia abyssinica*, natural selection, pollination patterns, reciprocal difference, self-incompatibility, S-locus

INTRODUCTION

Different systems of incompatibility in flowering plants were discussed in detail by Lewis (1954). The author recognized three different incompatibility systems, one of which is based on genetic control, *i.e.*, the number of alleles, the control of pollen (whether it is sporophytic or gametophytic), the interactions of S-alleles (dominance, individual), and the control of style (dominance or individual action of S-alleles). The theoretical consideration of sporophytic self-incompatibility (SI) for one gene system was worked out by Lewis (1954) and for two and three gene systems in *Eruca sativa* (Cruciferae) by Lewis (1977). Genetic control by more than one gene system has also been worked out by Lundqvist (1962) in grasses.

The basis of a biochemical (molecular) control of SI systems in flowering plants was established by Ferrari and Wallace (1976), Nasrallah and Nasrallah (1989), Nasrallah *et al.* (1972) and Mau *et al.* (1982).

The evolution of a multicellular organism was no doubt accompanied by the development of cell-cell recognition and communication (Nasrallah and Nasrallah, 1989). Cell-cell recognition plays significant roles for the continuity and integrity of the species. Sexual recognition processes have been described in ascomycets, slime molds, algae, and in gametic recognition in mammals. SI in plants is viewed as an additional mechanism evolved to prevent self-fertilization and promote outbreeding and hence genetic diversity. The failure of self-pollen to properly germinate and deliver male nucleus due to a series of complex events initiated by cell-cell recognition which itself induces signal transduction and cellular response. SI systems are helpful models for intercellular signalling in flowering plants (Dickinson, 1994). Lee *et al.* (1994) and Murfett *et al.* (1994) reported the involvement of a specific RNase to identify and reject self-pollen by a pistil.

Sporophytic SI system was recorded in some species of the family Asteraceae (Compositae): Hughes and Babcock (1950) in *Crepis foetida*, Gerstel (1950) in *Parthenium argentatum*, and Crowe (1954) in *Cosmos bipinnatus*. The known situations of S-allele interactions were illustrated by the authors and in all of the species, S-alleles exhibited different interactions both in the style and pollen.

Like some members of flowering plants such as Rubiaceae, Goodeniaceae and Campanulaceae (Robbrecht, 1988), self-pollen grains are deposited on the stigma and style of *Guizotia abyssinica*. The failure of the germination of self-pollen grains on a stigma and absence of free cross-pollination in the Ethiopian populations of *Guizotia abyssinica* were reported by Sileshi Nemomissa (1987) and Sileshi Nemomissa and Endashaw Bekele (1988). The authors suggested that this is due to the operation of a homomorphic sporophytic SI system.

Self-incompatibility is a disadvantage in the breeding system of many crops and their improvement scheme when either free intercross or inbreeding is required. Hybrid seed production of two incompatible species can only be envisaged when the SI system of each is properly understood. SI is one of the major problems which are associated with the process of screening of the national germplasm of *G. abyssinica* for improvement. Techniques to overcoming SI system in crop plants were reviewed by Hinata *et al.* (1994) and references therein. The genetic control of the SI system in *G. abyssinica* is not well-documented and extensively dealt with so far.

Therefore, the present study reports 1) the patterns of pollination which could be used as a clue to the nature of S-locus alleles, 2) the operation of reciprocal difference among different mating groups, and 3) the characteristics of seeds that resulted due to pseudo-compatibility.

MATERIAL AND METHODS

Seeds of the Ethiopian populations of *G. abyssinica* were obtained from the Biodiversity Institute, Ethiopia (formerly Plant Genetics Resources Centre, Ethiopia). The origin of the seeds is given in Table 1.

In the 1995 growing season the seeds were sown at Awassa College of Agriculture. The F_1 generation was sown at Holeta, Institute of Agricultural Research Station in the 1996 growing season.

Table 1. The Ethiopian populations of *Guizotia abyssinica* studied.

Accession	Number of crosses	Family	Origin
15067	36	*	Arsi
15008	64	45/95	Illubabor
15012	36	*	"
15055	16	*	Gojam
15180	36	*	"
15107	16	20/95	"
	64	19/95	"
15106	16	*	"

Table 1. (Contd).

Accession	Number of crosses	Family	Origin
15103	36	*	"
15108	36	*	"
15104	36	*	"
15009	49	2/95	Gondar
	36	4/95	"
	16	3/95	"
15169	36	*	"
15001	64	*	"
15094	36	*	"
	36	*	"
15093	9	48/95	"
15097	9	14/95	"
15033	64	6/95	Hararghe
	49	5/95	"
15054	36	*	"
15036	36	*	Shewa
15201	36	*	"
15202	36	*	"
15132	64	22/95	"
	64	21/95	"
15024	36	*	"
15199	36	*	"
15203	36	*	"
15151	36	*	Tigray
15085	36	*	"
15039	64	46/95	Wollega
15080	16	9/95	"
15028	36	*	"
15182	9	29/95	Wollo
15230	16	35/95	NA
15229	36	*	NA
	Total 1425		

*, Crosses made on seeds obtained after open pollination, not classified into families; NA, not applicable.

1. A diallel cross

A diallel cross pollination method was employed in two ways. All possible reciprocal crosses among the mating parents were made, *i.e.*, each parent served both as the donor (male) and recipient (female) of pollen grains to and from other mating partners during the crossing experiments. This same principle was followed during the crossing experiments undertaken in the Laboratory (Petri dish technique) and in the field (*in situ* pollination).

a) Petri dish pollination technique: Ray florets were planted on a medium composed of 2% agar, 10% sucrose and 0.001 g Boric acid (Lundqvist, 1961) and pollinated with fresh pollen grains. Self-incompatible crosses were studied by staining a pistil with a cotton blue stain 36 hrs. after pollination. Compatible pollen grains stained lighter and pollen tube were seen attached to the receptive part of the stigma. Incompatible pollen grains stained relatively darker and observed ungerminated on a stigma.

b) *In situ* pollination of capitula: 6 to 13 plants were randomly selected and bagged from each family. Intrafamilial crosses were performed by hand from 6:30 am to 10:00 am and seed set is the criteria for compatible and incompatible crosses. The seeds were checked for the presence of an embryo to discriminate compatible crosses from pseudocompatibility seeds. The latter are always characterized by well developed achene without an embryo.

A total of 1425 crosses were performed both in laboratory and in field (Table 1). Only representative crosses are included in the text.

2. Definitions and symbols

The filial generations obtained after the first growing season were assigned arabic numbers and the year when they were harvested e.g., 5/95 were considered in this study as families. Further in the text, pseudocompatibility should be understood as referring to both pseudoself- and pseudocross-compatibility.

Self-compatibility is a concealed incompatibility system with differing alleles at a heterozygote state. Both way reciprocal crosses (full compatibility) are denoted by ●; one way by ○; self-compatible by +; incompatible crosses by -. r.d. stands for reciprocal differences.

3. Interpretation

The interpretation of the cross data follows the methodology established by (Bateman, 1954; Lewis, 1954; 1977; de Nettancourt, 1977 and adopted by others elsewhere), *i.e.*, based on the patterns of pollinations obtained after crossing experiment. Dominance relationships of S-alleles increase the number of compatible crosses and co-dominance either in style or pollen drastically reduces the number of cross-compatibility.

RESULTS

These crosses were made in the families raised from parental seeds and F_1 seeds. The families were numbered and each was dealt with accordingly.

1. Family 6/95

This family was obtained from parental plants (female) 15033-1 x (male) 15033-3; only one of the parents was recorded to be self-incompatible. Eight plants were randomly selected and a diallel cross using a petri dish pollination technique was performed (Table 2a).

Table 2a. Cross data of Family 6/95 taking 7 different genotypes to show the degree of reciprocal differences in different genotypes.

Male/female	1	6	3	4	5	8	7	2	r.d.
1	-	-	-	-	-	●	-	●	0
6	-	-	-	-	-	●	-	●	0
3	-	-	-	-	-	-	-	-	0
4	-	-	-	-	-	-	-	-	0
5	○	○	○	○	-	-	●	○	5
8	●	●	○	○	-	-	●	○	3
7	○	○	-	-	●	●	-	-	2
2	●	●	-	-	-	-	○	+	1
r.d.	2	2	2	2	0	0	1	2	

Of the 56 free intercrosses only about 43% of the crosses were recorded to be compatible and the remaining 57% incompatible (Table 2a). About 58% of the compatible crosses are reciprocally incompatible. All the genotypes (mating groups) studied in this family are completely self-incompatible. An exception is plant number 2 (Table 2a) which is self-compatible. This plant is reciprocally compatible with plant number 1 and the reciprocal cross between plant numbers 2 and 7 is incompatible. If the cross with plant 2 is treated separately four mating groups could be recognized in the family 6/95 (Table 2b). Plants 3 and 4 constitute one mating group, G_1, 1 and 6 = G_4, 7 = G_3 and 5 and 8 = G_2. Both G_1 and G_4 are cross-incompatible and exhibit different degree of cross-incompatibility with G_2 and G_3.

Table 2b. Mating groups of the cross data of Table 2a. Four self-incompatible mating groups suggesting the operation of a sporophytic SI system with a single S-locus. The interactions of S-locus alleles are different in style and pollen. A co-dominant interaction of S-locus alleles highlighted.

Male/female	G_1	G_2	G_3	G_4	2
G_1	-	-	-	-	-
G_2	○	-	●	●	○
G_3	-	●	-	○	-
G_4	-	●	-	-	●
2	-	-	○	●	+

G_1 is incompatible with all mating groups as female and compatible with G_2 as male. The S-locus alleles are co-dominant in the style and one allele in pollen grains is dominant over another. G_1 is incompatible with G_2 as female but compatible when used as male parent.

Plant 2 does not fit into any of the mating groups (see discussion) and the S-locus is believed to be heterozygous and it is one-way compatible with G_3 (may be a homozygous parent) as female.

The relationships of the S-locus alleles in the family 6/95 could be perhaps conceived as different in both the style and pollen grains.

2. Family 5/95

The parental plants of this family, (female) 15033-1 x (male) 15033-1, are self-compatible.

Out of 42 possible intercrosses only 47% were found to be compatible of which about 80% are reciprocally compatible (Table 3a).

Table 3a. Cross data of Family 5/95 depicting reciprocal differences both as a female and male parent.

Male/female	1	2	3	4	5	6	7	r.d
1	-	●	-	-	-	-	●	0
2	●	+	●	●	○	○	●	4
3	-	●	-	-	-	-	●	0
4	-	●	-	+	-	●	●	0
5	-	-	-	-	-	-	-	0
6	-	-	-	●	-	+	-	0
7	●	●	●	●	○	○	+	2
r.d.	1	1	1	0	2	2	0	

The patterns of pollination are comparable to that of Family 6/95, *i.e.*, S-locus alleles perhaps behave differently both in a style and pollen grains.

Worth mentioning is also the recognition of 2 self-incompatible mating groups and 3 heterozygous self-compatible genotypes in the Family 5/95 (Table 3b). The cross data could, thus, be interpreted on the assumption of one S-locus controlling SI system in this family because this system produces a maximum of four different self-incompatible genotypes (Lewis, 1977). Similar case is also recorded in the Family 6/95. Both families (6/95 and 5/95) have the same female parent (15033-1) except that they were obtained through different cross routes.

Table 3b. Mating groups of the cross data of Table 3a. Two self-incompatible mating groups and plants 2, 4, 6, and 7 self-compatible groups exhibiting different degree of cross-compatibility to the self-incompatible mating groups suggesting the operation of a sporophytic SI system with a single S-locus. The interactions of S-locus alleles are different in style and pollen. A co-dominant interaction of S-locus alleles highlighted.

Male/female	G_1	G_2	2,7	4	6
G_1	-	-	-	-	-
G_2	-	-	●	-	-
2,7	○	●	+	●	○
4	-	-	●	+	●
6	-	-	-	●	+

3. Family 45/95

This family was obtained from (female) 15008-3 x (male) 15008-1 cross. Both parents are self-incompatible and heterozygous. Eight plants were intercrossed and the result is presented on Table 4a.

Table 4a. Cross data of Family 45/95 depicting reciprocal differences.

Male/female	1	2	8	7	4	5	6	3	r.d
1	-	-	-	-	-	-	-	-	0
2	-	-	-	-	-	-	-	-	0
8	-	-	-	-	-	-	-	-	0
7	○	○	○	-	-	-	-	○	4
4	○	○	○	-	-	●	●	○	3
5	-	-	-	-	●	-	-	●	0
6	-	-	-	-	●	-	-	○	1
3	-	-	○	-	-	●	●	-	1
r.d.	2	1	3	0	0	0	0	4	

Reciprocal incompatibility is noted and five mating groups were recognized in this family, *i.e.*, plants 1, 2 and 8 constitute one mating group, G_1, 7 is G_2, 4 is G_3 and 5 and 6 are G_4 (Table 4b). Plant number 3 was assigned as G_5. The cross data, thus, does not fit into the interpretation of the SI system on the basis of a single S-locus with 4 alleles (cf. Lewis, 1954).

Table 4b. Mating groups of the cross data of Table 4a. Five self-incompatible mating groups suggesting the operation of a sporophytic SI system with more than one S-locus. The interactions of S-locus alleles are different in style and pollen. A co-dominant interaction of S-locus alleles highlighted.

Male/female	G_1	G_2	G_3	G_4	G_5
G_1	-	-	-	-	-
G_2	○	-	-	-	○
G_3	○	-	-	●	○
G_4	-	-	●	-	●
G_5	-	-	-	●	-

All mating groups are self-incompatible. S-locus alleles in the style of G_1 are co-dominant. Both G_2 and G3 are compatible with G_1 as female and incompatible as male parents.

An additional SI factor, I, is proposed as part of the S-locus allele system of SI in Family 45/95.

The cross data for Family 42/95 [(female) 15008-3 x (male) 15008-2] is also found to be similar to that of Family 45/95 and is not depicted here. Both families have a common female parent. Note also that both parents of 42/95 are self-incompatible.

4. Family 19/95

It was obtained from a cross (female) 15107-2 x (male)15107-4 and both parents are self-incompatible.

Out of the 56 possible crosses 31 (55%) were found to be compatible (Table 5a). Of these compatible crosses, non-reciprocal difference was recorded in 16 (*c* 61%) crosses. Except for plants 1, 3, and 4, all the sampled genotypes of Family 19/95 were found to be self-compatible.

Table 5a. Cross data of Family 19/95 depicting reciprocal differences.

Male/female	1	2	3	4	5	6	7	8	r.d.
1	-	○	●	-	-	○	●	-	2
2	-	+	○	-	-	-	-	-	1
3	●	-	-	●	●	-	-	●	0
4	-	○	●	-	●	○	-	●	2
5	○	○	●	●	+	○	○	●	5
6	-	-	○	-	-	+	○	-	2
7	●	-	-	-	-	-	+	-	0
8	○	○	●	●	●	○	○	+	4
r.d.	2	4	3	0	0	4	3	0	

Three self-incompatible mating groups (G_1 = 1; G_2 = 3; G_3 = 4) and four self-compatible heterozygous genotypes (plants 2; 5; 8; 6; 7) were assigned (Table 5b). The data could be best described by a single S-locus system.

Table 5b. Mating groups of the cross data of Table 5a. Only one self-incompatible mating groups and 6 different heterozygous genotypes which exhibited self-compatibility. The interactions of S-locus alleles are different in style and pollen.

Male/female	G_1	G_2	G_3	7	6	5,8	2
G_1	-	-	-	●	○	-	-
G_2	●	-	●	-	-	●	-
G_3	-	●	-	-	○	●	○
7	●	-	-	+	-	-	-
6	-	-	-	○	+	-	-
5,8	○	●	●	○	○	+	○
2	-	○	-	-	-	-	+

5. Family 2/95

This family was obtained from a cross carried out between (female) 15009-3 x (male)15009-2 both of which are self-incompatible. Two self-incompatible mating groups (G_1 = 1,2 5; G_2 = 3, 4; cf. Table 6a and 6b) were recognized in 2/95 and two self-compatible heterozygous genotypes (plants 6 and 7). G_1 is incompatible with others as a female parent.

Table 6a. Cross data of Family 2/95 depicting reciprocal differences.

Male/female	1	2	5	6	3	4	7	r.d.
1	-	-	-	-	-	-	-	0
2	-	-	-	-	-	-	-	0
5	-	-	-	-	-	-	-	0
6	-	-	○	+	●	●	○	2
3	○	○	○	●	-	-	●	3
4	○	○	○	●	-	-	●	3
7	○	-	-	-	●	●	+	1
r.d.	3	2	3	0	0	0	1	

Table 6b. Mating groups of the cross data of Table 4a. Two self-incompatible mating groups and two heterozygous self-compatible genotypes. The interactions of S-locus alleles are different in style and pollen. A co-dominant interaction of S-locus alleles highlighted.

Male/female	G_1	G_2	6	7
G_1	-	-	-	-
G_2	○	-	●	●
6	-	●	+	○
7	-	●	-	+

Inter- and intrapopulation distribution of self-compatible genotypes

The frequency of self-compatible genotypes in populations of some selected regions was assessed. The frequency of self-compatible genotypes was 3.73% (of 241 possible crosses) for seven populations of *G. abyssinica* collected from different regions in Shewa. The interpopulation variation is remarkable, *i.e.*, the frequency of self-compatible genotypes ranges from 1.56% to 8.33%. Such a high frequency was not observed in populations from Gondar where comparable number of crosses were made. The frequency of self-compatible genotypes was 5.5% (of 200 crosses) for the populations of *G. abyssinica* from Gondar. The results obtained from crossing experiments made in populations showed that the frequency is 1.56% (of 128 crosses) for Illubabor and Hararghe and 2.21% (of 136 crosses) for Wollo. The frequency is 3.4% for populations from Wollega where only 88 crosses were made. The interpopulation variation is remarkable in populations from Wollega. The frequency in this region varies from 2.78% (of 36 crosses) to 12.5% (of 16 crosses). Such a high frequency of self-compatible genotypes was not recorded in any other populations considered in this study.

Pseudocompatibility

Pseudocompatibility is a common occurrence in *G. abyssinica*. It is characterized by well developed seeds but aborted endosperm and embryo. Pseudocompatible seeds are, thus, indistinguishable from viable seeds externally; a closer examination is required to screen them out. Furthermore, pseudocompatibility exhibited no particular association to either self- or cross-pollination.Pseudocompatibility was variously reported in *G. abyssinica* and other crop plants (Naika and Panda, 1968; Prasad, 1990; Gerstel and Riner, 1950).

DISCUSSION

Based on the present data, the following postulates were formulated:

- No self-compatibility in mating groups;
- There are two-way compatibility pairs;
- One-way compatibility pairs are common;
- Compatibility diagonal (Bateman, 1954; Lundqvist, 1990) none;
- Self-compatibility is fairly common;
- Reciprocal difference observed;
- There are more than 4 self-incompatible mating groups.

A summary of the pollination patterns leads to the following prepositions (Table 7) with regard to the interactions of S-locus alleles both in pollen and style. The data given in Tables 2–6 can be interpreted at allelic level if the conditions given in Table 7 are fulfilled.

Table 7. Summary of the interactions of S-alleles obtained from cross data (Tables 2–6) and the meaning of the signs used in Fig. 1.

Alleles	Pollen	Style
1 & 2	1 dominant over 2	Co-dominant
3	recessive to 1 & 2	dominant to 1; recessive to 2
4	recessive to 1 - 3	recessive to 2 & 3
5	co-dominant with 1	dominant to 1
I-locus (loci)	unknown	unknown
... : Individual action	→ : Dominance	= : Co-dominance

A suggested model of the SI system in *G. abyssinica* was proposed (Fig. 1). The present crossing data could be interpreted in the framework of this suggested model.

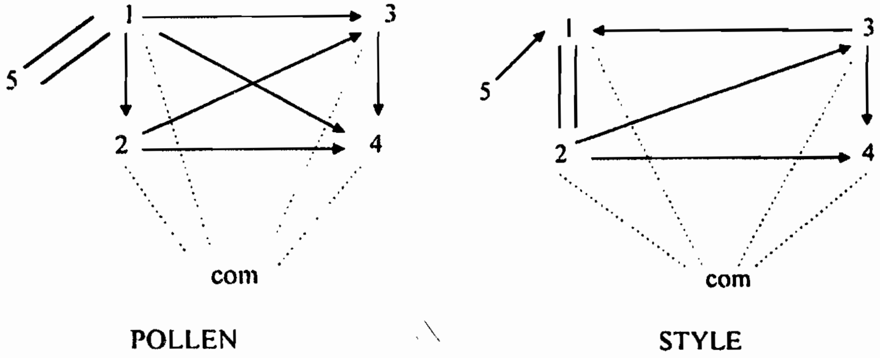

Fig. 1. Suggested self-incompatibility model for the Ethiopian *Guizotia abyssinica*.

A. Type of self-incompatibility in Guizotia abyssinica

The two types of a homomorphic SI system were set apart from each other and discussed in detail elsewhere (Lewis, 1954; Bawa and Beach, 1983; Lawrence *et al.*, 1985; Zuberi and Lewis, 1988; Speranza and Calzoni, 1988; Rioz and Shoseyov, 1995). The indication of a sporophytic SI system in *G.abyssinica* was reported elsewhere. A sporophytic system was also reported (Gerstel and Riner, 1950) in the members of family Compositae the alliance to which niger belongs. The present study further confirms the earlier report on the SI system of *G. abyssinica* to be under the direct influence of a sporophytic system. This finding bases its argument on the evidences drawn from cytology and *in situ* pollination of capitula.

1) Evidences from cytology

i) Incompatible pollen grains failed to germinate on receptive stigmas and the stigma-pollen reaction induced the production of callose.

ii) Incompatibility pollen grains may germinate (or protrude their intine content) but failed to penetrate receptive stigmas. The pollen tubes curved upward instead and grow away from the receptive stigmas and accumulated cytoplasm at the tip as to form a swelling which eventually burst.

iii) Incompatible pollen grains stained relatively darker than compatible pollen grains (Lundqvist, 1961). The latter indicates that pollen grains did not empty their content, hence, the cross is incompatible.

2) Evidence from in situ *pollination*

The recording of reciprocal differences in all pollinations without exception (Tables 2-6) manifests that SI system in *G. abyssinica* is determined sporophytically. This result was also confirmed in laboratory (petri dish pollination technique).

Generally, therefore, a sporophytic SI system is operating in *G. abyssinica*.

B. S-locus and com *alleles in the populations*

1. The interpretation of the pollination patterns of family 6/95 (Table 2a) could be made within the framework of the suggested SI model (Fig. 1) synthesized from the present data. G_1 is incompatible with all mating groups and plant 2 as a female parent suggesting that the two S-alleles are co-dominant in style. G_1 is compatible only with G_2 as male which indicates that the S-alleles of the female parents are recessive to one of the S-allele in pollen. G_1 is, thus, assigned S_1S_2 and G_2 S_2S_4. G_2 and G_3 are fully compatible pairs. G_3 is fully incompatible with G_4 and G_1 for different reasons. G_3 (female) is incompatible with G_1 may be due to that the S-alleles in the style (at least S_3) are dominant to the phenotype of the pollen grains of G_1. Note that the pollen grains of G_1 are phenotypically S_1 (Fig. 1). The incompatibility of G1 (female) to G_3 is expected because S_1 and S_2 are co-dominance in style (Lewis, 1977). G_3 (only as a female) is compatible to G_4 because G_3 and G_4 share one common S-allele (S_3). The interaction of S_3 with S_5 is, however, not yet established in this study. Alternatively, S_5 in the style may suppress the activity of S_3. G_4 was, thus, assigned S_3S_5. If G_4 is S_3S_4, the cross with G_3 (female) will not be recorded because pollen grains of G_4 are expected to be S_3 phenotypically (Fig. 1). The pollination behaviour of G_3 as a mating group could be explained within the framework of the proposed model if a homozygous state (S_3S_3) is assigned to it.

   The incompatibility of plant 2 (female) only with G_1 and G_2 could be explained if 1) the self-compatible genotype is heterozygous and 2) the self-compatibility allele, *com*, has independent interactions with S-alleles (Fig. 1). Plant 2 must thus have S_1 in style (S_1S_{com} as a genotype). It is incompatible with G_1 because S_1 in style hinders the germination of S_1 phenotype in pollen grains of G_1. Since the model envisages the dominance of S_1 in style to S_2 in pollen, plant 2 (female) is incompatible with G_2. It is to be noted that the pollen grains of G_2 are phenotypically S_2. On the other hand, plant 2 is compatible with G_3 and G_4 because G_3 produces S_3 pollen grains and G_4 produces S_3 and S_5 pollen grains. According to the model (Fig. 1), S_3 in pollen is dominant to S_1 in style and hence S_3 pollen grains germinate on the stigma of S_1S_{com} female.

Generally, the following S-locus genotypes may have been involved in 6/95 crosses: S_1S_2, S_2S_4, S_3S_3, S_3S_5, and S_1S_{com}.

2. In family 5/95, the co-dominance of S_1 and S_2 is again confirmed in G_1. G_1 and G_2 are incompatible because they may share one top dominant allele in pollen (S_1). The absence of reciprocal difference between G_2 and G_3 is due to that G_2 has an S-allele which suppresses the expression of S_1 in style, *i.e.*, S_3 is dominant to S1 in style (Fig. 1). Furthermore, G_3 has an S-allele which is dominant to S_3 in style of G_2. Therefore, the genotype of G_3 is S_2S_{com}. G_2 is fully incompatible with G_4 because they share a common allele (S_3). The genotype of plant 6 could be S_5S_{com}. The pollen grains of G_1 failed to germinate since S_5 is dominant to S_1 in pollen (Fig. 1). The S-locus genotypes involved in this particular crosses could be S_1S_2, S_1S_3, S_2S_{com}, S_3S_{com}, S_5S_{com}.

3. The co-dominance of S-alleles in pollen of G_2 (Table 4b) could be attributed to another pair of alleles. G_2 (female) is incompatible with G_1 and plant 3 because they all may have one common S-allele (perhaps top dominant, S_1) in pollen. The remaining S-allele of G_2 is again expected to be dominant to S_1 in style; this allele is S_5 according to the model (Fig. 1). The genotypes of G_2 and G_4 are S_1S_5 and S_1S_3, respectively.

   The recognition of more than 4 mating groups in family 45/95 may call for another S-locus (S-loci). This additional locus (loci) is named I (the number of alleles of this locus can not be inferred from this study). Thus, the genotype of plant 3 could be S_1I. G_3 is fully compatible with G_4 because both have different S-alleles. On the other hand, G_3 (male) is incompatible with G_2 because they may share a common allele (S_5). G_3 (male) was also incompatible with plant 3 because G_3 has an S-allele in style which is co-dominant to another S-allele in pollen (cf. Fig. 1). The genotype of G_3 could be, therefore, S_4S_5. Generally, S_1S_2, S_1S_5, S_4S_5, S_1S_3, and S_1I S-genotypes were perhaps sampled from family 45/95 in this study.

4. The S-locus genotype of G_1 could be S_1S_2 as in previous cases. Both G_1 and G_2 have one common allele, S_2 (Table 5 b). G_1 (male) was incompat-

ible to G_3 indicating that the S-allele in style is dominant to its pollen phenotype. Its incompatibility with G_4 could be explained when the S-allele in style of G_4 is co-dominant to one S-allele (S_1) in pollen of G_1. The S-locus genotype of G_4 could be, thus, S_5S_{com} and G_3 is S_3S_{com}. A homozygous state, S_2S_2, could be proposed for G_2. S_1S_2, S_2S_2, S_3S_{com}, and S_5S_{com} are believed to be the genotypes involved in the crosses of family 2/95.

5. Among the 3 incompatible mating groups (G_1 - G_3), G_1 has shown no reciprocal difference with G_2 and G_3 which may have a common S-allele. G_1 has, therefore, different S-alleles (S_2S_4) from G2 (S_1S_3) and G_3 (S_3S_3). G_2 (male) was incompatible with G_3 (female) since S_3 in style is dominant to S_1 in pollen (Fig. 1). The reciprocal cross between these genotypes was also incompatible because S_3 pollen does not normally germinate on S_3 style.

Natural selection may favour a breeding programme of *G. abyssinica* which may take self-compatible genotypes as a potential raw material for improvement. Since further crossing experiments have revealed self-incompatibility in the progeny of self-compatible parents (cf. 5/95), considerable selection procedures may be inevitable before getting entirely self-compatible quality genotypes.

The sprophytic SI system is the most complex breeding system of plants because of the presence of many crossing possibilities. Furthermore, the interactions of S-locus alleles are also equally complex. It is believed that this study has not sampled all S-locus alleles of *Guizotia abyssinica*. There may be several factors contributing to this problem. The first could be the methodology itself. The methodology is based on the formulation of postulates and preposition of an operational model. Although the model is synthesized from the crossing data, the mode of operation, *i.e.*, assigning S-alleles to the mating system and suggesting the likely interactions are made to fit into the model. Another point is the interpretation of crossing data itself. The data was interpreted by assuming that a single S-locus (with 3 and 4 alleles) produces four self-incompatible mating groups (Bateman, 1954; Crowe, 1954).

The second reason for not finding all S-alleles could be due to the information gap with regard to the adaptive value of S-alleles in different populations from different ecological settings. Why some S-alleles are sampled from a population, but not in others and why from a particular region, but not from other regions need further investigations. The present result could be, however, viewed as a preliminary undertaking meant to provoke further investigations in the same direction. The results of this study have also elucidated the magnitude of problems associated with SI system in *G. abyssinica*.

Noteworthy may be the different types of relationships exhibited by S-locus alleles both in pollen and style. S_1, S_2, S_5, and S_3 behaved differently in pollen and style. Similar patterns of the interactions of S-alleles were also reported in various crops elsewhere. The suggestion that another S-locus allele or an independent S-locus (S-loci) as part of the SI system of *G. abyssinica* originated from the documentation of more than 4 self-incompatible mating groups. This point, however, needs further verifications to uncover its modes of operation and geographical pattern.

The frequency of self-compatible genotypes of some selected regions revealed greater interpopulation variations within a given region than between regions. In fact, the regional variation is not similar. The lowest in Illubabor and Hararghe (1.56%) and highest in Gondar (5.5%) frequencies of self-compatible genotypes were recorded at regional level. It is, however, premature to state any conclusive remarks. Further studies in the future may look at and uncover 1) the relationships among the frequency of the self-compatible genotypes, 2) type and number of S-locus alleles, and 3) the additional SI factor for the Illubabor population with respect to environmental factors (forces of natural selection).

The recording of different frequencies both within a region and at interregional level may suggest that a breeder may have an enormous diversity of self-compatible genotypes to work on if the aim is to establish self-compatible genotypes. The interregional variation may also be taken into consideration for a breeding programme of *G. abyssinica*. Further studies on the same direction may reveal more and complete information on the frequency of self-compatible genotypes and even may dictate the direction of the breeding programme of this neglected crop.

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REFERENCES

1. Bateman, A.J. (1954). Self-incompatibility systems in angiosperms. II. Iberis amara. *Heredity* **8**:305–332.
2. Bawa, K.S. and Beach, J.H. (1983). Self-incompatibility systems in the Rubiaceae of a tropical lowland wet forest. *Amer. J. Bot.* **70(9)**:1281–1288.
3. Crowe, L.K. (1954). Incompatibility in *Cosmos bipinnatus*. *Heredity* **8**:1–11.
4. de Nettancourt, D. (1977). *Incompatibility in Angiosperms*. Springer - Verlag, New York.
5. Dickinson, H. (1994): Self-pollination: simply a social disease? *Nature* **367**:517--518.
6. Ferrari, T.E. and Wallace, D.H. (1976). Pollen protein synthesis and control of incompatibility in *Brassica*. *Theor. Appl. Genet.* **48**:243–249.
7. Gerstel, D.U. (1950). Self-incompatibility studies in Guayule. II. Inheritance. *Genetics* **35**:482–506.
8. Gerstel, D.U. and Riner, N.E. (1950). Self-incompatibility studies in Guayule. I. Pollen tube behaviour. *J. Heredity* 41:48–55.
9. Hinata, K., Isogai, A. and Isuzugawa, K. (1994) Manipulation of sporophytic self-incompatibility in plant breeding. - *Adv. cell. Mol. Biol. 2:102–115.*
10. Hughes, M.B. and Babcock, E.B. (1950). Self-incompatibility in *Crepis foetida* L. subsp. *rhoeadifolia* (Beib.) Schinz et Keller. *Genetics* **35**:570–588.

11. Lawrence, M.J., Marshall, D.F., Curtis, V.E. and Fearon, C.H. (1985). Gametophytic self-incompatibility re-examined: a reply. *Heredity* **54**:131–138.

12. Lee, H.-S., Huang, S. and Kao, T.H. (1994). S proteins control rejection of incompatible pollen in *Petunia inflata*. *Nature* **367**:560–563.

13. Lewis, D. (1954). Comparative incompatibility in angiosperms and fungi. *Adv. Genet*. **6**:235–280.

14. Lewis, D. (1977). Sporophytic incompatibility with 2 and 3 genes. *Proc. Royal Soc. London, B*. **196**:161–170.

15. Lundqvist, A. (1961). A rapid method for the analysis of incompatibilities in grasses. *Hereditas* **47**:705–707.

16. Lundqvist, A. (1962). The nature of two-loci incompatibility system in grasses. I. The hypothesis of a duplicative origin. *Hereditas* **48**:153–168.

17. Lundqvist, A. (1990). One-locus sporophytic S-gene system with traces of gametophytic pollen control in *Cerastium arvense* ssp. *strictum* (Carophyllaceae). *Hereditas* **113**:203–215.

18. Mau, S.L., Raff, J. and Clarke, A.E. (1982). Isolation and partial characterization of components of *Prunus avium* L. styles, including an antigenic glycoprotein associated with a self-incompatibility genotype. *Planta* **156**:505–516.

19. Murfett, J., Atherton, T.L., Mou, B., Gasser, C.S. and McClure, B.A. (1994). S-RNase expressed in trasgenic *Nicotiana* causes S-allele-specific pollen rejection. *Nature* **367**:563–566.

20. Naika, S.S. and Panda, B.S. (1968). Time of bud pollination in increasing fertility in Self-incompatible niger - *Guizotia abyssinica* (Cass.). *Indian J. Sci. & Indust.* **2** (4):177–180.

21. Nasrallah, M.E., Wallace, D.H. and Savo, R.M. (1972). Genotype, protein, phenotype relationships in self-incompatibility of *Brassica*. *Genet. Res., Camb.* **20**:151–160.

22. Nasrallah, J.B. and Nasrallah, M.E. (1989). The molecular genetics of self-incompatibility in *Brassica*. *Ann. Rev. Genet.* **23**:121–139.

23. Prasad, V. (1990). Pollen tube growth and site of incompatibility reaction in noug (*Guizotia abyssinica* Cass.). *Curr. Sci.* **59(9)**:466–468.

24. Robbrecht, E. (1988). Tropical woody Rubiaceae. *Opera Bot. Belg*. **1**:1–271.

25. Rioz, L. and Shoseyov, O. (1995). Stigmatic RNase in self-compatible peach (*Prunus persica*). *Int. J. Plant Sci.* **156** (**1**):37–41.

26. Sileshi Nemomissa (1987). The genetics of incompatibility mechanism in *Guizotia abyssinica* (L.f.) Cass. - BSc thesis, Asmara University.

27. Sileshi Nemomissa and Endashaw Bekele (1988). Self-incompatibility in noug (*Guizotia abyssinica*). Unpublished report.

28. Speranza, A. and Calzoni, G.L. (1988). *In vitro* test of self-incompatibility in *Malus domestica*. *Sex Plant Reprod.* **1**:223–227.

29. Zuberi, M.I. and Lewis, D. (1988). Gametophytic-sporophytic incompatibility in the Cruciferae - *Brassica campestris*. *Heredity* **61**:367–377.