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SELF-INCOMPATIBILITY IN *LOLIUM* SPECIES

1. *LOLIUM RIGIDUM* GAUD. AND *L. MULTIFLORUM* L.

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SUMMARY

Investigation of the genetic basis of self-incompatibility in F_1 families of *L. rigidum* Gaud. and *L. multiflorum* L. indicated the involvement of at least three multiallelic loci. Pollen control was found to be gametophytically determined. The results indicate that the genetic control of self-incompatibility is similar in *L. rigidum* and *L. multiflorum* although different from the majority of self-incompatible grass species.

1. INTRODUCTION

The genetic control of self-incompatibility has been investigated in several members of the Gramineae (table 1). Although the same self-incompatibility system is usually present within closely related groups or families, exceptions to this general principle have been found in the Solanaceae (Pandey, 1962; Abdalla and Hermsen, 1971) and Boraginaceae (Crowe, 1971). In the Gramineae, many species have gametophytic self-incompatibility systems controlled at two multiallelic loci (*S* and *Z*), with cooperation between alleles in the pollen and independent action between alleles in the pistil. Thus, an incompatible reaction occurs only when both alleles present in the pollen are matched in the pistil (Lundqvist, 1954, 1955, 1956, 1961*a*; Hayman, 1956). However, in contrast, the self-incompatibility systems operating in *Briza spicata* (Murray, 1979) and *Lolium* species (Hayward and Wright, 1971; Spoor, 1976; Hay, 1978) appear to be controlled by more than two loci.

This paper will deal with the results of diallel crosses carried out in three F_1 families of *L. rigidum* Gaud. and two F_1 families of *L. multiflorum*

TABLE 1

Incompatibility systems identified in grass species studied (after Østerbye, Larsen and Lundqvist, (1980))

Species	Two locus system	Other system
Secale cereale	Lundqvist (1954, 1956)	
Festuca pratensis	Lundqvist (1955, 1961*a*)	
Phalaris coerulescens	Hayman (1956)	
Hordeum bulbosum	Lundqvist (1962)	
Dactylis aschersoniana	Lundqvist 1(1965)	
Briza media	Murray (1964)	
B. spicata		Murray (1979)
Lolium perenne	Cornish, Hayward and Lawrence (1979)	Hayward and Wright (1971); Spoor (1976)
L. multiflorum		Hay (1978)

L. Results obtained in a third species, *L. perenne* L., will be published shortly.

2. Materials and methods

F_1 families were produced by controlled pollinations between self-incompatible plants from three different populations of *L. rigidum* Gaud. collected in France, and from three different commercial varieties of *L. multiflorum* L. (table 2). The progeny of 17–27 plants per family were grown in a heated glasshouse in a 16 hour day.

Table 2

Parentage of F_1 families

Species	Family	No. plants	Parents
L. rigidum	1	25	B5, O8
L. rigidum	2	25	O1, O2
L. rigidum	3	27	L3, B1
L. multiflorum	4	26	Sceemster, Combita
L. multiflorum	5	17	S22, Combita

Key:
B: Balaruc les Bains, Héroult
O: Oraison, Alpes des Haute Provence
L: Lempdes, Haute Loire

Pollinations were performed *in vitro* using the petri dish technique devised by Lundqvist (1961*b*). Whole pistils were dissected from florets immediately prior to anthesis, and the base of the ovary was placed in a recorded position on an agar plate. In this way two pistils per plant of up to 30 plants were available for pollination by one male plant. Each petri dish was therefore equivalent to a single male array of a diallel cross, and each array was replicated. Stigmas were pollinated within 8 hours of plating.

Fresh pollen was collected in bags from cut flowering heads which had just commenced anthesis, and was dusted over the pistils. The plates were incubated for 6 hours at room temperature, this being sufficient time to allow germination and pollen tube growth to the base of the stigma, in compatible crosses. The petri dishes were then stored under refrigeration (0–4°C) for a maximum of 48 hours before staining.

Whole pistils were stained on a slide in a 0·2 per cent solution of water soluble aniline blue in 0·1 M K_3PO_4 for 1–2 minutes. Excess stain was removed and the pistils were mounted in glycerol and crushed gently under a coverslip. Observations were made using a Leitz Ortholux 2 fluorescent microscope with incident light illumination.

3. Results

The results of the diallel crosses carried out in the three *L. rigidum* and two *L. multiflorum* families are presented in detail in figs. 1 to 5. Differential pollen behaviour was observed in compatible crosses (although it was not possible to divide reaction into classes such as half and three-quarters compatible), indicating gametophytic determination of pollen behaviour.

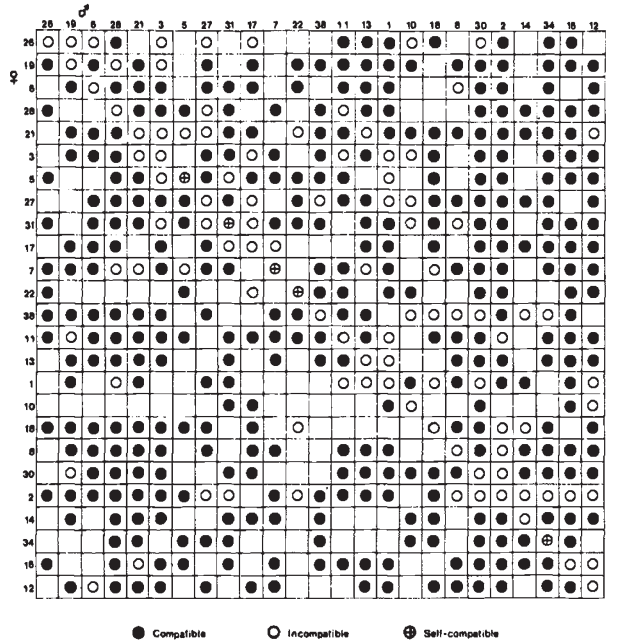

FIG. 1. Family 1: results of crosses between twenty-five plants produced by a controlled cross between two diploid *L. rigidum* plants from different populations.

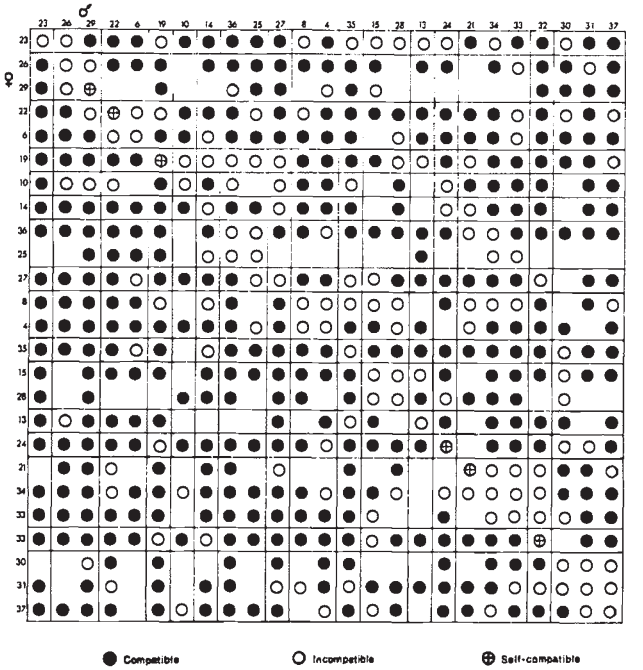

FIG. 2. Family 2: results of crosses between twenty-five plants produced by a controlled cross between two diploid *L. rigidum* plants from same population.

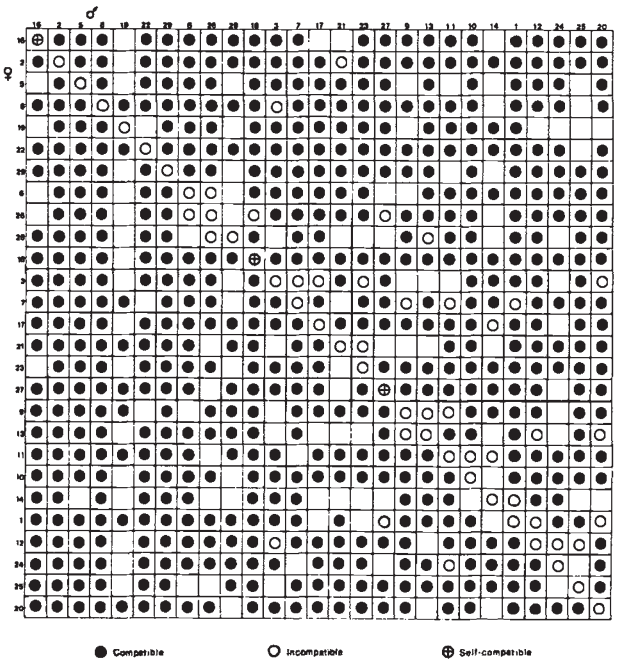

FIG. 3. Family 3: results of crosses between twenty-seven plants produced by a controlled cross between two diploid *L. rigidum* plants from different populations.

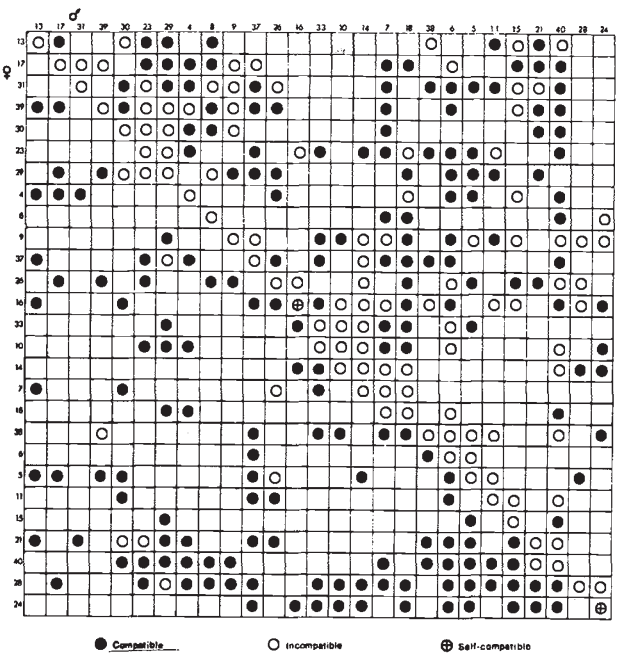

FIG. 4. Family 4: results of crosses between twenty-seven plants produced by a controlled cross between two diploid *L. multiflorum* plants from different commercial varieties.

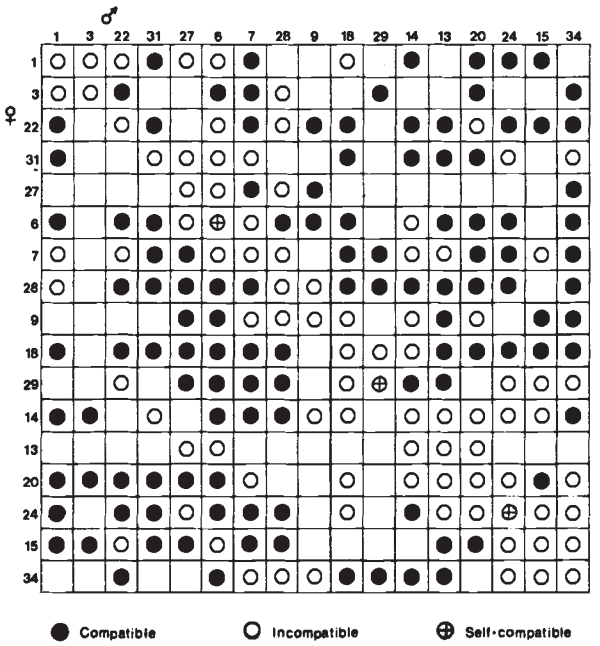

FIG. 5. Family 5: results of crosses between seventeen plants produced by a controlled cross between two diploid *L. multiflorum* plants from different commercial varieties.

Up to six plants in each family were observed to be self-compatible when excised stigmas were pollinated *in vitro*. In only one case (plant 24, family 5) were seeds produced when flowering heads of these "self-compatible" plants were bagged. Only seven seeds were produced, indicating the plant to be weakly self-compatible.

Within each F_1 family every plant appeared to have a unique mating type (figs. 1–5) and no intra-incompatible, inter-compatible groups could be constructed. Each diallel contained examples of pairs of plants which were reciprocally incompatible but had different mating types, and pairs of plants which were one way compatible (table 3).

TABLE 3

Numbers of paired reactions observed in three L. rigidum *and two* L. multiflorum *F_1 families*

Species	Family	Reaction type +↔+	−↔−	+↔−
L. rigidum	1	114	6	41
L. rigidum	2	117	9	90
L. rigidum	3	239	2	19
L. multiflorum	4	25	7	16
L. multiflorum	5	29	14	27

Key:
+↔+ reciprocally compatible
−↔− reciprocally incompatible
+↔− one way compatible

4. Discussion

In a gametophytic self-incompatible system controlled at two loci in which the parents have no common alleles, a maximum of sixteen different mating types would be expected in an F_1 family. Similarly, three and four locus systems could, at maximum, produce 64 and 256 mating types respectively. The results presented (figs. 1–5, table 3) therefore indicate the involvement of at least three loci in the genetic control of both *L. rigidum* and *L. multiflorum*. The results confirm and extend the observations made in a preliminary study of incompatibility in *L. multiflorum* (Hay, 1978).

The occurrence of one way compatible reactions between pairs of plants in each family (figs. 1–5; table 3) indicates that the parent plants used to produce the F_1's shared common alleles. The higher level of one way compatibility in family 2 (table 3) was not surprising as the parent plants came from the same source population and could be expected to have more common alleles than families produced by crossing plants from different populations.

Reciprocal incompatibility between plants with different mating types may imply that not all the alleles in the pollen have to be matched in the pistil to produce an incompatible reaction. Østerbye *et al.* (1980) proposed an incompatibility system controlled by three loci where one locus was assumed to have undergone a duplication (say $S'S''Z$), and an incompatible reaction occurred when one S (either S' or S'') and one Z allele present in the pollen was matched in the pistil.

The self-incompatibility system found to be operating in *L. rigidum* and *L. multiflorum* closely resembles that observed in *L. perenne* (Spoor, 1976), and appears to be complex with at least three *S*-loci and gametophytic determination of pollen behaviour. In addition, it is postulated that in both *L. rigidum* and *L. multiflorum* matching of some, and not all, self-incompatibility alleles in pollen and pistil could produce an incompatible reaction. Therefore, there are indications that the genetic basis of self-incompatibility may be different in *Lolium* species from most other grass species investigated to date.

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5. References

ABDALLA, M. M. F. AND HERMSEN, J. G. Th. 1971. A two-loci system of gametophytic incompatibility in *Solanum phureja* and *S. stenotomum*. *Euphytica*, *20*, 345–350.

CORNISH, M. A., HAYWARD, M. D. AND LAWRENCE, M. J. 1979. Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. *Heredity*, *43*, 95–106.

CROWE, L. K. 1971. The polygenic control of outbreeding in *Borago officinalis*. *Heredity*, *27*, 111–118.

HAY, J. M. 1978. Incompatibility in *Lolium multiflorum*. *Incompat. Newsletter*, *10*, 134–136.

HAYMAN, D. C. 1956. The genetical control of incompatibility in *Phalaris coerulescens* Desf. *Aust. J. Biol. Sci.*, *9*, 321–331.

HAYWARD, M. D. AND WRIGHT, A. J. 1971. The genetic control of incompatibility in *Lolium perenne* L. *Genetica*, *42*, 414–421.

LUNDQVIST, A. 1954. Studies on self-sterility in rye, *Secale cereale* L. *Hereditas*, *40*, 278–294.

LUNDQVIST, A. 1955. Genetics of self-incompatibility in *Festuca pratensis* Huds. *Hereditas, 41*, 518–520.

LUNDQVIST, A. 1956. Self-incompatibility in rye. I. Genetic control in the diploid. *Hereditas, 42*, 293–348.

LUNDQVIST, A. 1961*a*. Self-incompatibility in *Festuca pratensis* Huds. *Hereditas, 47*, 542–562.

LUNDQVIST, A. 1961*b*. A rapid method for the analysis of incompatibilities in grasses. *Hereditas, 47*, 705–707.

LUNDQVIST, A. 1962. Self-incompatibility in diploid *Hordeum bulbosum* L. *Hereditas, 48*, 138–152.

LUNDQVIST, A. 1965. Self-incompatibility in *Dactylis aschersoniana* Graebn. *Hereditas, 54*, 70–87.

MURRAY, B. G. 1974. Breeding systems and floral biology in the genus *Briza*. *Heredity, 33*, 285–292.

MURRAY, B. G. 1979. The genetics of self-incompatibility in *Briza spicata*. *Incompat. Newsletter, 11*, 42–45.

ØSTERBYE, U., LARSEN, K. AND LUNDQVIST, A. 1980. Comments on self-incompatibility in the Gramineae. *Incompat. Newsletter, 12*, 45–49.

PANDEY, K. K. 1962. Genetics of incompatibility behaviour in the Mexican *Solanum* species *S. pinnatisectum*. *Z. Vererb. Lehre, 93*, 378–388.

SPOOR, W. 1976. Self-incompatibility in *Lolium perenne* L. *Heredity, 37*, 417–421.