Acta Bot. Neerl. 31(5/6), November 1982, p. 429–439.

THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 4. AN INTERPRETATION OF THE SELF-INCOMPATIBILITY RESPONSE

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Key words: Gametophytic self-incompatibility, Gramineae, pollen-stigma interaction, pollen-tube inhibition, cell recognition

SUMMARY

Features of the self-incompatibility system in the grasses are reviewed, and related to characteristics of the stigma and pollen tube growth. From a consideration of the existing data, a general hypothesis is advanced for the self-incompatibility reaction, based upon the following propositions: (A) the pistil-side incompatibility factors are proteins (probably glycoproteins) with lectin-like properties present in the stigma surface secretions and in the transmitting tracts; (B) their binding specificities are such that they are complementary to sugar sequences or arrays displayed by wall carbohydrate in the growth zone of incompatible pollen tubes, but not complementary to those presented by the compatible tubes; (C) binding at the tip of an incompatible tube leads to a disruption of apical growth by preventing the dissociation of the polysaccharide content of the wall precursor bodies and interfering with the extension of polysaccharide microfibrils in the sub-apical zone. The implications of the hypothesis are briefly discussed, and also its relevance to other gametophytic self-incompatibility systems.

1. INTRODUCTION

The first three papers of this series have been concerned with the structural and cytochemical characteristics of the grass stigma, with its reaction to pollination and the penetration of compatibility response itself as expressed in the pollen grain and pollen tube (J. & Y. HESLOP-HARRISON 1980, 1981; SHIVANNA, Y. & J. HESLOP-HARRISON 1982, referred to hereafter as papers 1, 2, & 3). The body of information now available, although still far from complete, already provides a basis for interpreting some of the principal features of the self-incompatibility response. In the present paper we briefly review some of the more significant facts, and offer the outlines of an hypothesis, based in part on suggestions made earlier concerning the nature of pollen-tube inhibition in the self-incompatibility reaction (J.HESLOP-HARRISON 1978a, 1978b, 1982; Paper 3).

2. CHARACTERISTICS OF THE SELF-INCOMPATIBILITY SYSTEM

2.1. Genetical features

The genetics of self-incompatibility in the Gramineae have been discussed in detail in the reviews of LUNDQUIST (1975) and DE NETTANCOURT (1977). The system is of the gametophytic type, governed, according to the general view, by two polyallelic loci, designated as *S* and *Z*. Eight species of seven genera are listed by CORNISH, HAYWARD & LAWRENCE (1979) and ØSTERBYE, LARSEN & LUNDQUIST (1980) as having this type of control, although the latter authors point out that the data are at present perhaps still inadequate to permit generalisation throughout the family.

The essential characteristic of a polyallelic, single-locus gametophytic system is that pollen-tube growth is governed by the *S*-allele of the haploid genotype of the individual pollen grain on the male side, and by the alleles at the incompatibility loci acting independently in the diploid tissue of the pistil on the female side. A two-locus system such as that of the grasses involves a further requirement, namely that there should be some form of co-operative interaction between the *S* and *Z* loci. The working of such a system is well described by the wording of LUNDQUIST (1975) – "Each specific pair of an *S* and a *Z* allele is the determinant of one unique specificity, there being formed as many such specificities as there are *S*–*Z* pairs of alleles, and identity between pollen and pistil in one such specificity is sufficient to lead to incompatibility." A comprehensive description of the function of the system must therefore account for allelic independence in diploid cells and *S*–*Z* interaction in both haploid and diploid cells. The genetical evidence demands, further, that any interpretation of the response must also allow for the fact that there is no interaction in respect to *S*, *Z*-gene products between contiguous spores and gametophytes in the anther, on the stigma, or during growth through the transmitting tracts.

2.2. Physiological features

We have already given reasons for excluding the possibility that the discrimination between compatible and incompatible tubes in the self-incompatibility response in the grasses results solely from the promotion of germination and growth in compatible tubes by some unknown constituent or property of the stigma and transmitting tracts (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978). Germination of pollen is readily obtained *in vitro* on media of known composition, and the pollen-tube growth rates subsequently achieved approach those attained *in situ*. Moreover, the tubes sometimes reach lengths matching those observed in the pistil. We may conclude, then, that the discrimination results from the arrest or retardation of the incompatible pollen tubes.

Four seemingly essential characteristics of the reaction are now well established:

A. The *S*, *Z*-allele combination does not significantly affect the initial hydration and germination of the pollen. This has been shown for *Secale cereale* and

Gaudinia fragilis using controlled single-grain pollinations (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978; J. HESLOP-HARRISON 1978a; Paper 3). Irrespective of the compatibility of the applied pollen, the tube tip emerges at about the same time after the first contact of the grain with the receptive surface of the stigma, and the tubes grow initially at similar rates.

B. In an incompatible combination, although hydration and germination are not affected simply by contact between the exine and the stigma, the growth of the tube ceases when the tip touches the stigma surface, or very soon thereafter. HAYMAN (1956) found evidence of very early inhibition in *Phalaris caerulescens*, and this is generally seen in grass species with a "strong" form of self-incompatibility. However, weaker forms are known in which incompatible tubes are not arrested until after the tip has passed through the cuticle of the contiguous stigma papilla and the tube has begun growth in the intercellular spaces of the secondary stigma branches (Paper 3, J. HESLOP-HARRISON 1982).

C. The arrest or retardation of growth in an incompatible tube is associated with the formation of nodules, seemingly composed of microfibrillar pectins, in the wall at the extreme apex (Paper 3). This is the first observable structural change associated with the self-incompatibility response, and it is seen both where the inhibition is on the surface of the stigma and before pollen-tube penetration, and where the arrest is delayed until after the penetration of the tip into the intercellular spaces of the transmitting tract. From an initial thickening of the wall in the extreme apex, the pectic accumulations increase until the tip is occluded. The deposition of callose, a conspicuous marker for inhibited tubes (DE NETTANCOURT 1977), follows after the initial change in the pattern of wall growth at the tube tip.

D. As judged from cyclosis, starch and lipid degradation and the continued deposition of callose, the general metabolism of the male gametophyte is not blocked following upon the arrest of tube growth in an incompatible pollination. This has been established both for species with early inhibition such as *Gaudinia fragilis* and *Secale cereale*, and for those with delayed inhibition, such as *Alopecurus pratensis* (Paper 3, and unpublished).

These findings, taken with the genetical evidence, raise three key questions, the answers to which could obviously provide a valuable guide to the physiological basis of the incompatibility reaction. They are as follows:

A. Since the pollen response follows immediately upon contact of the tube tip with the stigma or shortly after penetration, the primary interaction must be with factors present on the surface, or immediately accessible in the intercellular spaces of the adjacent transmitting tract. *What factors, then, are synthesised in the stigma as it becomes receptive and are transferred both into the intercellular spaces of the tract and onto the surface?*

B. Recognition and response are both localised in the pollen tube wall in the growth zone, and the incompatiblity factors on the pollen side are not diffusible, even between tubes in contact. *What constituents are synthesised post-meiotically but before the onset of pollen dormancy, and are presented for interaction with*

the stigma immediately following upon germination while remaining anchored in the wall?

C. The inhibition of an incompatible tube involves a rapid modification of the growth of the wall in the tip region, but this is not associated with a general blockage of tube metabolism. *How does the tube wall normally extend in the tip region, and what is the nature of the abnormality induced by the incompatibility response?*

In the following sections we review the evidence bearing upon these questions.

3. STIGMA FEATURES AND POLLEN-TUBE GROWTH

3.1. The secretory system of the stigma

The principal structural features of the grass stigma have been described in paper 1. The papillar cells, which are the sites of pollen capture (Paper 2), show characteristics suggesting specialisation for both internal and external secretion. The intercellular cavities of the transmitting tissue of the secondary stigma branches receive secretion products from the adjacent cells; these include acidic pectic polysaccharides, and also a protein constituent. The receptive surface of the stigma papillae bears two extracuticular layers, an outer sheath of protein some 15–20 nm in thickness corresponding to the proteinaceous pellicle known from other stigmas of the "dry" type (including those of species with sporophytic self-incompatibility systems; MATTSSON *et al.* 1974; ROBERTS *et al.* 1979), and an inner mucilaginous pectic layer. The developmental sequence suggests that the outer coatings of the stigma represent samples of the secretions of the papillar cells and the cells of the transmitting tract, passed through the discontinuities of the cuticle of the receptive zone of the papillae during the maturation of the stigma. Dictyosomes are relatively inactive during the accumulation of the intercellular and surface secretions, but the secretion mechanism may nevertheless be granulocrine, involving paramural bodies (Paper 1). Following upon pollination the remaining membranes of the receptive cells undergo dissolution, and this may lead to further transfer of proteins into the transmitting tract through a form of holocrine secretion (Paper 2). Obviously, however, the products released after pollination are unlikely to be involved in the early interactions of the self-incompatibility system.

The surface proteins of the stigma are highly heterogeneous (J. HESLOP-HARRISON 1978b; 1982). Some 30 proteins have been separated from the surface eluate of *Pennisetum glaucum* stigmas, the principal one being a glycoprotein of molecular weight *c.* 170,000 daltons. A corresponding glycoprotein, also dominating the spectrum, has been detected among the stigma proteins of *Secale cereale.*

The stigma secretions are produced at the right time, and presented in the right sites, for interaction with the pollen tube. Especially may it be noted that the external secretion layer is the first contact point for the tube tip, and that it is in this site that inhibition is imposed in an incompatible combination in plants with the "strong" type of self-rejection reaction.

3.2. The mode of pollen-tube growth

In the normally-growing grass pollen tube there is a transition in the structure of the wall over the distal 10–15 μm from the thin apical sheath of short, randomly disposed microfibrils, through the zone of longer and partly oriented microfibrils without an inner callosic layer, to the bi-layered wall of the older tube with its outer zone of oriented polyaccharide microfibrils and inner sheath of callose (J. HESLOP-HARRISON 1979). This organisation of the apical region is comparable with that known in the pollen tubes of other families, and various aspects of the structure were described in the pioneering work of O'KELLEY & CARR (1954), ROSEN *et al.* (1964) and SASSEN (1964). DASHEK & ROSEN (1966) concluded from cytochemical evidence that the principal component of the wall at the extreme apex of the pollen tube of *Lilium longiflorum* is pectic in nature, and the wall in the apical region of the tube has been shown to be the richest in pectins in other species (MATCHETT & NANCE 1962; ROGGEN & STANLEY 1971). The principal constituent of the wall at the growing tip of the grass pollen tube is also pectic (Paper 3 and unpublished). The micro-fibrillar component of the older wall has not as yet been characterised in the grasses, although analogy with the work of HERTH *et al.* (1974) suggests that it is probably not exclusively cellulosic. These authors showed that the microfibrillar polysaccharide of the tube wall of *Lilium* is heterogeneous, with both β-1,3- and β-1,4-linkages.

As in other tip-growing plant cells, the extension of the pollen tube is associated with the apposition of polysaccharide-containing vesicles or particles. The evidence of electron microscopy indicates that these are likely to be a source of wall material, and they are added both to the tip where extension is occurring and to the sub-apical region where the wall is thickening. VAN DER WOUDE *et al.* (1971) found that these bodies from the pollen tube of *Lilium* were rich in galacturonic acid, and concluded that they serve as the principal means of transfer of wall precursors. They suggested further that they are likely to be involved in metabolic conversions among the wall carbohydrates and perhaps in the synthesis of cellulose. HELSPER *et al.* (1977) showed that the vesicle fraction from pollen tubes of *Petunia* has β-glucan synthetase activity, and is capable of synthesising alkali-insoluble glucan with both β-1,3- and β-1,4-linkage *in vitro*.

It has generally been accepted that the vesicles originate through the activity of the Golgi system, and in several of the investigated species (including *Lilium longiflorum*, VAN DER WOUDE *et al.* 1971) a zone of active dictyosomes has been identified in the extending tube, lying towards the tip, but proximal to the main site of wall growth.

The situation in the grasses is rather different. The ungerminated pollen grain itself contains a large reserve of the precursor bodies ("P-particles", J. HESLOP-HARRISON 1979; J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1982), and there is little or no dictyosome activity during the extension of the pollen tube after germination. The P-particles are transferred to the tip by active cyclosis within the tube, where they are evidently incorporated into the growing wall much as in the *Lilium* pollen tube.

The grasses therefore depart from the general pattern exemplified by *Lilium*, and this is certainly associated with the very rapid development of the male gametophyte following upon hydration, seen in the speed of germination and the high subsequent rate of tube growth, which is as much as ten times greater than that achieved by the *Lilium* tube. This behaviour is correlated with various other characteristics of the pollen, including its tricellular structure, short life and high respiratory rate (HOEKSTRA & BRUINSMA 1975, 1978). The developmental basis of the difference lies in a shift in the timing of the principal events. In *Lilium*, the generative cell division occurs after germination while the tube is growing, and during the same period the wall precursor vesicles are actively produced by the dictyosome population. In the grasses, in contrast, the division of the generative cell occurs before the pollen enters its period of temporary dormancy in the anther, and the synthesis of the P-particles, associated with dictyosome activity in the latter stages of pollen development, is also completed in the anther before dispersal.

A partial characterisation of the P-particles stored in the grass pollen grain has indicated that they are likely to be similar in composition to those produced during the growth of the lily pollen tube, with a considerable pectic component (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1982). They are originally membrane-bounded, but the membranes are not retained intact, and the particles frequently fuse in the grain and during tube growth. In the present context it may be significant that the polysaccharide is consistently associated with protein which is perhaps not exclusively derived from the residual membranes.

3.3. Pollen-tube inhibition

The two circumstances already mentioned, namely, (1) that the initial incompatibility response is strictly localised to the extreme apex of the pollen tube where extension growth is normally centred, and (2) that there is no evidence of any general metabolic inhibition in the male gametophyte associated with the arrest of growth, point to the likelihood that the cessation of tube extension in an incompatible combination is brought about by a specific and local disruption of the mechanism of wall growth.

The fine-structural manifestations provide a clue as to the nature of the disruption (Paper 3, and unpublished). The first indication of the breakdown of the normal zonation is an excessive thickening of the pectic sheath at the extreme tip. This is followed by a further build-up of microfibrillar pectins resulting from the continued apposition of the precursor particles without dissociation of the content, as evidenced by the persistence of flattened profiles of the particles in the occluded tip zone. When extension growth is checked, the zonation of the wall near the tip is lost, and the inner callose sheath advances to envelop the protoplast completely.

4. THE HYPOTHESIS

On the basis of the foregoing reasonably well-established facts we may now consider an hypothesis for the working of the incompatibility reaction in the grasses. The outlines can be conveyed in the following statements:

A. The pistil-side incompatibility factors are proteins (most probably glycoproteins), with lectin-like properties, present in the surface secretions and in the intercellular spaces of the transmitting tract.

B. Their binding properties are such that they are complementary to sugar sequences or arrays displayed by wall carbohydrate in the growth zone of the incompatible pollen tubes, but not complementary to those presented by the compatible tubes.

C. Binding at the tip of an incompatible tube leads to a disruption of apical growth by preventing the normal dissociation of the content of the precursor bodies – probably through cross-linking – as they are transferred into the wall, and subsequently interferes with the re-orientation and extension of polysaccharide microfibrils in the immediate sub-apical zone by blocking the access of the appropriate transferases.

These propositions may be supplemented to provide a scheme for information flow in the grass self-incompatibility systems by the following more speculative riders:

D. On the pistil side, the incompatibility genes are transcribed and translated in the secretory cells of the stigma and transmitting tract. The result of the co-operative action of each combination of the *S, Z* alleles is one class of lectin molecule, with its sugar-binding specificity determined jointly. The synthesis is probably trans-membranal, and the products are moved out of the cell by a granulocrine system. They accumulate in the intercellular spaces of the transmitting tract, and also pass onto the stigma surface.

E. On the pollen side, the incompatibility genes are transcribed in the spore and developing pollen grain before the release from the anther. Co-operative action of the *S, Z* alleles is again involved, the ultimate expression being in the structure of a carbohydrate moiety that is ultimately incorporated into the wall in the growth zone of the pollen tube. A possible – although at this stage quite hypothetical – sequence might begin with the trans-membrane synthesis of the elements of a specific glycosyl-transferase battery in the endoplasmic reticulum, followed by transfer to the Golgi system, then the translation of the specificity into sugar sequences or arrays in the polysaccharide synthesised in the dictyosome vesicles and the storage of the product as the P-particle population of the vegetative cell of the grain, and finally the passage of the P-particle polysaccharide into the wall at the extending apex where the specific arrays are exposed for challenge by the complementary stigma-side factors.

6. GENERAL DISCUSSION

The conception of a recognition reaction based upon carbohydrate-protein interaction is implicit in the schemes offered for many animal and microorganism systems (see, for example, various reviews in CURTIS 1978). Interactions that appear to be of this nature have been investigated in various lower plant groups, including slime moulds (BARONDES & ROSEN 1976) and algae (*Chlamydomonas*, WIESE & WIESE 1978), and systems of the same type have been postulated for host-parasite and host-symbiont interactions in higher plants (e.g., ALBERSHEIM & ANDERSON-PROUTY 1975; BOHLOOL & SCHMIDT 1974).

The idea incorporated in the riders (D) and (E) to the present hypothesis that the recognition systems may be based upon glycosyl transferase polymorphism and on interactions with some similarities to that between enzyme and substrate owes its origin to the observations and proposals of ROTH et al. (1971), ROTH (1973), MCLEAN & BOSMANN (1975) and PARISH (1977), and is at present without any form of evidence from angiosperm self-incompatibility systems. The suggestive scheme offered by PARISH (1977) is concerned with the recognition factors produced by invertebrates. These are large molecules with specificity for carbohydrate structures, possessing polyvalent properties which give them agglutinating capacities; they are secreted into the haemolymph where they persist in soluble form. PARISH proposes that the recognition factors are composed of a cluster of glycosyl transferases of the same nature as those concerned in the synthesis of the animal's own carbohydrate side chains. His model for the action of the recognition factor assumes that each transferase acts as a recognition sub-unit, the permutation of sugar specificities and spatial arrangements providing for a wide specificity range in the whole grouping. Although there are conceptual difficulties in translating such schemes as this to plant self-incompatibility systems, they obviously provide a fruitful basis for speculation. It might not be difficult, for example, to devise a model for self-recognition which would account for the fact that it is allelic identity that leads to rejection, based upon the proposition that the transferase system responsible for assembling an array of sugars might act in another mode in the other partner as a recognition factor for the same array. However, in the absence of any pertinent evidence, we refrain from further speculation along these lines.

Two features of the basic hypothesis contained in paragraphs (A), (B) and (C) may be noted.

Firstly, it is based upon the supposition that the recognition molecules on the pistil side are diffusible, and indeed present in an essentially fluid phase, while the complementary factors on the pollen side are anchored in the wall, probably forming part of the structural polysaccharide itself. This would account for the pervasive nature of the pistil-side control over the whole of any captured pollen population, while explaining how it is that the behaviour of an individual pollen tube is rigorously determined by its own incompatibility genotype, even when it is growing through a liquid medium in close physical

contact with other tubes with different incompatibility genotypes. That the pollen-side factors are non-diffusible would also account for the fact that no differences are found among the antigens of leachates from grass pollens of different *S, Z*-genotypes (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1979).

Secondly, the proposition that the pistil-side recognition factors are diffusible secretion products of the cells of the stigma and transmitting tract indicates how the strength of the incompatibility reaction might vary while its specificity is rigidly preserved. The explanation lies in the likelihood that the secretory activity is itself variable, both in response to environmental factors influencing the behaviour of the stigma cells, and in relation to the genetic background. A polygenic control of the rate of secretion would provide a basis for modulating the intensity of the incompatibility response without affecting the segregation of the *S, Z* system that determines the specificity of the control (J. HESLOP-HARRISON 1982). In the extreme instance the segregation might be masked altogether because effective levels of the secretion are never reached in the stigma, in which case the plant would be self-compatible.

Although it is not our intention in this paper to discuss in detail the possible implications of the present proposals for other families with gametophytic systems, two aspects may be noted. The early inhibition of incompatible pollen that is so much a characteristic of the grass system is accounted for here by the proposition that the pollen-side incompatibility factors are synthesised premeiotically and are held in the P-particle population of the mature grain, to be displayed as soon as the tube emerges. Obviously enough, this cannot happen when the wall precursors are not synthesised until after germination and during the growth of the tube. In these cases, the inhibition will be delayed until the tube is growing through the transmitting tracts (fig. 3 in J. HESLOP.HARRISON 1978a). This is evidently the situation in most families with gametophytic systems, including Liliaceae, Solanaceae, Rosaceae and Leguminosae, all with relatively slow pollen tube growth rates and stylar inhibition of incompatible tubes.

Then, in respect to the pistil-side control, if the mechanism in other families with gametophytic systems is generally similar to that proposed here for the grasses, the differences in the sites of action can be readily enough accounted for by the different distributions of the cells secreting the incompatibility factors. The stigmas of Liliaceae, Solanaceae, Rosaceae and Leguminosae are not at all discriminating; but in all these instances control is imposed in the transmitting tracts of the style. For example, it was shown by EAST (1934) that whereas compatible tubes pass through the transmitting tissue of *Nicotiana* at a uniform rate, incompatible tubes grow more slowly or are arrested in what SEARS (1937) referred to as an "interference zone". Those that survive the passage through this zone resume normal growth and may eventually reach the ovary. Obviously this effect would be accounted for were the secretion of the incompatibility factors to be restricted to the cells of the interference zone, and were the concentration to vary across the transmitting tract in this zone in such a manner that the disruption of apical growth was slight enough in some tubes merely to bring

about a retardation from which recovery could be made rather than a complete arrest.

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