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Review

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Pollen Stigma Interactions in *Brassica oleracea*

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Summary. Recent studies on the mechanism of self-incompatibility in *Brassica* indicate the location, nature and mode of action of the molecules involved. Characteristics of the pollen surface and the stigma surface are described in detail, together with new information pertaining to the recognition molecules located therein. A sequence of events is outlined leading from pollination, through adhesion, hydration, germination, and tube growth to acceptance and ultimate compatibility. The characteristics of rejection of incompatible grains are described for each stage of the pollen-stigma interaction. It is proposed that recognition of proteins from the coating of self-pollen by the molecules in the pellicle results in the formation of a biologically-active complex which inhibits water supply to the incompatible grain, and that all other manifestations of incompatibility are a consequence of this initial response.

Key words: *Brassica* – Pollen germination – Pollen surface – Self-incompatibility – Stigma surface

Introduction

Pollen-stigma interactions in *Brassica oleracea* are of fundamental importance as they constitute one of the best-defined processes of cell recognition found in plants. They are also of considerable interest to the plant breeder for this recognition of self pollen results in its rejection and, further, an understanding of how this system of self-incompatibility (SI) operates may make possible the modification of breeding systems by chemical treatment. For these reasons pollen-stigma interactions and the mechanism by which the SI system takes effect have been the subjects of much research and speculation over many years. (Darwin 1876; de Nettancourt 1977). They remain, however, incompletely understood.

The initial interaction between pollen and stigma occurs when the alighting grain encounters the proteinsaceous pellicle investing the stigmatic papillar cells (Mattsson et al. 1974); in a compatible cross this results in adhesion of the pollen and the subsequent processes of hydration, germination, and penetration of the stigma surface by the pollen tube. Self, or otherwise intraspecifically incompatible pollen, is inhibited soon after arrival on the stigma, and recognition has been deduced to occur within ten minutes of pollination (Kroh 1966, Ferrari and Wallace 1977). Whilst there is no doubt that the site of rejection of incompatible pollen is the stigma surface (Ockendon 1972), the manner of inhibition may vary and incompatible pollen may either fail to germinate or, when germination does occur, fail to penetrate the stigma surface. On the occasions when both germination and penetration do occur pollen tube development halts at a callose barrier synthesised by the cytoplasm of the stigmatic papillar cells (Dickinson and Lewis 1973a). Inhibition does not occur when immature stigmas in the bud are pollinated with mature self-pollen, and may be partially reduced in mature stigmas by certain environmental and chemical treatments, for high relative humidity, high CO_2 concentration, and treatments with hexane or chloroform all effect a degree of self-compatibility (Roggen 1974; Nakanishii and Hinata 1973; Carter and McNeilly 1975, 1976; Ockendon 1978).

Genetic control of the SI system of the Cruciferae is sporophytic (Bateman 1955) in that pollen compatibility is determined by the genotype of the sporophyte, and such SI systems have certain correlated characteristics. These characteristics include trinucleate pollen, 'dry' papillate stigmas and superficial rejection of self-pollen (Brewbaker 1957; Heslop-Harrison 1975). These features and the nature of inhibition described above must surely give some indication as to the mechanism of the SI system, but a far more precise characterisation of the pollen-stigma interactions and the molecules involved is needed. Results

from recent work with *Brassica* may now be profitably drawn together with pre-existing information to produce a more complete understanding of this particular plant cell-recognition system. Information on the stigma and pollen are probably more conveniently considered first in isolation, followed by what is known of their participation in the pollen-stigma interaction.

The Stigma

The Proteins of the Stigmatic Papillae

Components of the stigmatic papillae that may be involved in interactions between pollen and stigma have been identified by a number of methods. Using immunological techniques Nasrallah and Wallace (1967) and Sedgely (1974) have detected the presence of incompatibility (S) allele-specific antigens in the stigma, while Nasrallah et al. (1970) describe similar polypeptides, identified by electrophoresis. Using electrofocussing, Nishio and Hinata (1977) were able to demonstrate the presence of S-specific glycoproteins in homogenates of *Brassica* stigmas. However, little of this work using stigmatic extracts has pointed to the way in which these polypeptides might be involved in any process of recognition. On the other hand, experiments using mature pollen and immature flowers have revealed the SI system not to be expressed in 'bud' stigmas (el Murabaa 1957) and it has been proposed that a new component is added to the stigmatic pellicle enabling the recognition and rejection of self-pollen (Shivanna et al. 1978). For this reason, the detection by electrofocussing of a single glycoprotein whose appearance coincides with the development of the SI response (Roberts et al. 1979a) may well be significant.

Unfortunately, further evidence pointing to the direct involvement of these stigmatic glycoproteins in the SI response has not been forthcoming. They have, however, been shown to be exclusive to the stigma, to bind the lectin Concanavalin A, and to be S-gene specific (Nishio and Hinata 1978), properties which might reasonably be expected from molecules involved in recognition events.

The Stigma Surface

The scanning electron microscope (SEM) reveals the surface of the papillae to be chracterised by small swellings some 6 μm in radius. These features of the surface are identical in stigmas of buds and mature flowers, and neither their frequency nor their distribution varies with ageing. The behaviour of these swellings in stains and dehydration media has indicated them to be composed, at least in part, of lipid material (Dickinson and Lewis 1973a).

Papillae from both buds and mature flowers bear a thin proteinaceous pellicle (Mattsson et al. 1974) which, owing to the fact it contains an esterase, may be detected with both light and electron microscopes. Stigmatic pellicles are also possessed by species of the Graminae, and the lectin concanavalin A has been demonstrated to bind to the pellicle of *Phalaris* sp. (Heslop-Harrison 1976) indicating that, in this plant, the pellicle contains glycoproteins. In *Brassica*, the cuticle underlying the pellicle is thin and apparently composed of aggregates of cutinised material rather than a continuous layer. Heslop-Harrison and Shivanna (1977), in their survey of stigmatic types. classify *Brassica* as 'dry', although it is clear that this stigma surface is particularly well-adapted to the release of water, on demand (Mattsson et al. 1974).

The Pollen

The Proteins of the Pollen

Brassica pollen is a typical trinucleate grain and, in addition to these nuclei, contains mitochondria, lipid globules, microbodies, endosplasmic reticulum, dictyosomes, ribosomes, mitochondria and a vast population of vesicles many apparently concerned with wall synthesis. The cavities of the exine contain a tapetally-derived lipo-protein complex known as the pollen coat or tryphine. Isoelectric focussing of pollen homogenates reveals some thirty bands (Nishio and Hinata 1978) but neither S-gene specific differences, nor S-gene specific antigens have been reported (Nasrallah and Wallace 1967). Certainly an impressive array of enzymes will readily diffuse from the pollen of many species (Knox et al. 1970) but, as yet, little is known of their function. In *Brassica*, it has been proposed that failure to identify proteins involved in the SI system may stem from the masking effects of the lipids of the pollen coating (Roggen 1975). New evidence suggesting that the pollen coat itself contains a spectrum of proteins that are not released during aqueous diffusion has been

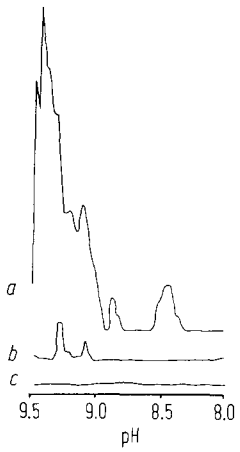

Fig. 1. 'Chromoscan' trace of electrofocussing plate loaded with extracts from *Brassica* pollen, see Roberts et al. (1979a) for details. a) Pollen extracts obtained by mixing rapidly for 2 min in extracting medium containing 0.5% Triton X-100 and 12% sucrose in 0.1M Tris-HCl buffer pH 7.2. 10mg of pollen were extracted per 0.1ml of medium and the extract was filtrated using Millipore filters (45 micron mesh). b) As in a) but pollen given prior treatment with chloroform. c) As in a) using extracting medium containing no Triton X-100

obtained in experiments using the neutral detergent Triton X-100 (Fig.1). Using this method, proteins not normally observed in electrofocussing gels at all are released from the pollen within 2 minutes. Examination of these grains with the SEM reveals that the pollen coating is absent, supporting the hypothesis that these proteins, which focus between pH8 and pH9.5, are indeed those of the 'sporophytically-coded' pollen coat (Dickinson and Lewis 1973b) and hence those most likely to be involved in the SI response. The pollen coating may also be removed using covalent solvents (Roberts et al. 1979b) and extracts made from this coat-less pollen do not contain these proteins. It is also interesting that these proteins appear not to be released into solutions containing ionic detergents such as sodium dodecyl sulphate.

The Hydration and Germination of Pollen in vitro

An understanding of the behaviour of *Brassica* pollen in vitro is of considerable value when interpreting the interactions occurring in vivo. Hydration in vitro may be followed by supplying dry pollen grains with controlled quantities of water from atmospheres of known relative humidity (R.H.) (Stead et al. 1979). Whilst little change in volume is detected there is a considerable increase in fresh weight together with a change of shape; after exposure to atmospheres in excess of 75% RH for 18h pollen changes from elipsoid to spherical, and from a water content of 16.3% to 39.3%. Similar changes are observed in vivo.

Germination of *Brassica* pollen is not easily achieved in vitro for trinucleate pollens are notoriously difficult to culture. Although initial tube growth may occur in a humid environment more complex media are required for sustained growth, such as that containing polyethylene glycol described by Ferrari and Wallace (1975). Such media must contain substances that act in a manner similar to the factors present at the stigma surface. A medium has been developed recently to reproduce as closely as possible the environment at the stigma surface (Dawes et al. in preparation). It appears that the crucial factors are the osmotic potential and the physical environment offered the pollen; the former must resemble that of the papillar cells (i.e. equivalent to 17% sucrose), the latter must have a similar surface tension to the pellicle (i.e. equivalent to 4% agar) and may control the rate of water uptake. Physical properties of the media are also likely to affect the behaviour of the tryphine and this is significant when considering the differences in tryphine mobility occurring after cross and self pollination.

The Coating of the Pollen Grains

The pollen coat or tryphine invests the grain and is the first component of the pollen to come into contact with the stigma. It is derived from the tapetum and is thus of sporophytic origin (Dickinson and Lewis 1973b). It has been suggested that the S-gene coding is carried in the tryphine (Heslop-Harrison 1975) and indeed tryphine extracts have been shown to elicit the callose rejection when applied to self stigmas (Dickinson and Lewis 1973b). It is therefore reasonable to conclude that the S-gene determinants are carried in the tryphine but they have failed to be identified in any of the chemical analyses so far carried out, possibly because of the nature of the tryphine proteins discussed above.

The components of the tryphine visible in the SEM are completely extracted by chloroform, and lipids, proteins and carbohydrates may be detected in this extract (Roggen 1974). The lipids may be resolved into seven fatty acid components by GLC (Roberts et al. 1979b). More recently, C-13 NMR analyses of the chloroform-extractable coat have given a more complete indication of its chemical nature, but shown little to indicate any role that it may play in the SI response, save to indicate that the covalent moiety of the coats of both compatible and incompatible pollen grains alters chemically on pollination.

The Pollen-Stigma Interaction

Events Prior to Pollen Germination

The rapidity with which recognition apparently occurs lends even greater significance to the events prior to germination than previously believed. The major pre-germination events are the capture and adhesion of the pollen, the interaction of pollen-coat and pellicle, and the hydration of the grain.

Differences in adhesion between self- and cross-pollen may occur within a few minutes of pollination as, at 1-15 min. after pollination, self-pollen is easier to remove than cross-pollen (Roggen 1975). Quantitative studies of the adhesion of pollen grains to the stigma reveal that self-pollen is initially less firmly bound than cross-pollen (Stead et al. 1979). However, after 2h on the stigma self pollen becomes adhered to an extent comparable with cross pollen, whereas no increase in adhesion of cross pollen over this period is detected. It is also interesting to note that the pollen of self-compatible genotypes adheres to a self-stigma in a manner comparable with an ordinary self-pollination.

The components of the stigma responsible for binding the pollen have been shown to be the proteins of the pellicle (Stead et al. 1980). Digestion of the stigma surface with protease adversely affects pollen grain adhesion, but stigmas are capable of recovering their adhesive properties if there is an interval of over 1.5h between digestion and pollination, and experiments with cycloheximide indicate that the surface proteins can have a rapid turnover rate

comparable with some animal cell-surface proteins (Vernay et al. 1978). Isoelectric focussing of stigma homogenates after protease treatment reveals that the concentration of at least three different protein bands is reduced, including one previously shown to appear concomitant with the development of the SI system (Roberts et al. 1979). Enzymic removal of the pellicle also prevents the entry in the Caryophyllaceae indicating that the importance of the pellicle extends to all facets of grain development (Heslop-Harrison and Heslop-Harrison 1975).

Most recently, the physical character of the pellicle has been studied, and, while the stigmatic surface can be in no way regarded as 'wet', it has the constitution of a viscous fluid. Using techniques devised to measure the adhesion of pollen, it has been possible to determine that the force required to detach a pollen grain is of the order of 1×10^{-7} Newtons. A new picture of the pellicle thus emerges, not of a dry 'crusty' layer but of a viscous, fluid mosaic in which components are continually under renewal, and in which constituents once removed, may swiftly be replaced.

Since the component of the pollen likely to be involved in adhesion is its coating, it is interesting to note that the mobility of this coating, as evidenced by the fluorescent probe 1-ANS, is greater after self-pollination than after cross-pollination (Stead et al. 1979). The chemical basis of this difference is not known, but an experiment measuring the force required to detach pollen grains indicates some change within 15 minutes. It may be that a gelatinisation occurs via cross-linking within the coat, and perhaps between the coat and stigma surface. Certainly potential components of a 'glue' are present in the pellicle and pollen coating as noted by Clarke et al. 1979. No fundamental changes occur in the pollen coat lipids after cross and self-pollination (Roberts et al. 1979b), and the only evidence of chemical changes in the coat following pollination comes from the N.M.R. spectrometer.

In the light of these data, therefore, the pollen grain coating emerges as a substance much resembling the stigmatic pellicle; a viscous, lipid-rich layer, containing a mosaic of proteins. Naturally these proteins are not under constant renewal. The pollen coating also appears to be curiously hydroscopic, in that it will flow off the grain in a humid environment. The delicate relationship between pollen and water has been noted from germination experiments *in vitro*, but the hydration of pollen on the stigma has yet to be followed satisfactorily.

Some indication that hydration is affected by self-recognition is obtained from observations of shape changes occurring on the stigma (Fig. 2). In a cross the long-axis to short-axis ratio may change from 2.0 (ellipsoidal) to 1.3 (spherical) within 3h of pollination, and remain spherical thereafter. Few such changes in the shape of self pollen are detected.

Since it seems reasonable to propose that the change in shape is a consequence of hydration, these data must provide evidence that incompatible pollen fails to hydrate in a manner similar to compatible grains.

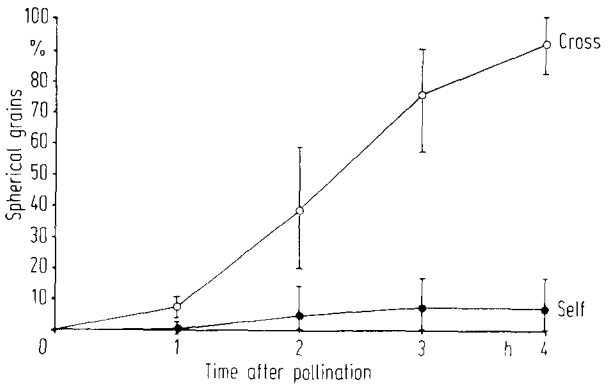

Fig. 2. Change in shape of pollen following compatible and incompatible pollination. Stigmas were pollinated with approximately 30 grains and observed using a high power stereoscopic microscope. At hourly intervals each stigma was scored for the number of spherical grains and this was expressed as a percentage of the total number of grains (the means of 8 replicates are presented, bars representing maximum and minimum results)

The Mechanism by Which Compatibility is Established

From the foregoing, we may now propose the following sequence of pollen-stigma interactions leading to compatible pollen development:

(a) The pollen adheres rapidly, the coat does not flow and probably undergoes a gelatinisation. This process occurs 10-15 minutes after pollination.

(b) The grain hydrates during the first hour after pollination, a process possibly facilitated by the change in the coat.

(c) Germination occurs and perfect short tubes attach directly to nearby papillae.

(d) The cutinase from the pollen tube effects penetration of the stigma surface by the tube which then grows down the papillae between its cuticle and the subjacent pectocellulosic wall (Kroh 1964).

The Characteristics of Rejection

Pollen development following incompatible matings is inhibited by constraints which act at all stages of pollen-stigma interaction. Adhesion is a slow process and the coat is mobile. Grains appear to experience difficulties in obtaining sufficient water for complete hydration. Although in many cases the grains germinate, and some do develop quite long malformed tubes which encircle the papillae, these tubes rarely penetrate the cuticle and, if they do

succeed, often stimulate the deposition of callose in the stigma (Dickinson and Lewis 1973a).

Conspectus

The SI system in *Brassica* is thus a well-documented phenomenon of biological recognition; considerable information exists for each of the processes leading from the moment of pollination to acceptance and ultimate cross-fertilisation, or to rejection and the prevention of self-fertilisation. What then of the precise mechanism involved?

All data are compatible with the hypothesis that the informational molecules are carried in the coating of the pollen and the pellicle of the stigma. Experiments with pollen-coat extracts and chemical alteration of the stigma lead directly to this conclusion. There is strong circumstantial evidence that the reacting molecules of the pellicle are glycoproteins which bind the pollen; data from other biological recognition systems indicate the importance of glycoproteins in mediating cognitive adhesions. (Bolwell et al. 1979).

We can thus be fairly confident of the location and characteristics of the molecules involved. How then do the determinants of the stigma interact with the molecules of the pollen coating to effect acceptance or rejection? It has been deduced that the recognition step is completed within 10-15 minutes of the grain alighting, and this would indicate that the initial recognition occurs during the process of pollen grain adhesion. Thus it is not unreasonable to propose that the differences of adhesion and coat mobility detected between cross and self pollinations are the first events to follow the recognition process. These may result directly or indirectly, for example, in a self pollination molecules of tryphine and pellicle may fail to adhere, causing coat flow and failure to establish further contact, or, alternatively, interaction between tryphine and self pellicle may result in a biologically active complex which disrupts subsequent development. However, self compatible genotypes exhibit poor adhesion and coat flow of self pollen suggesting that adhesion is not critical for development (Stead et al. 1979). Thus, we currently favour the latter mechanism as the acquisition of a new glycoprotein by maturing stigmas and the data of el Murabaa (1957) and Shivanna et al. (1978) suggests that the SI system is superimposed on a preexisting 'compatibility' system involving the pellicle proteins present in the bud. This is also evidenced by the work of Heslop-Harrison and Heslop-Harrison (1975) as enzymic removal of the pellicle was found to prevent tube entry in the Caryophyllaceae.

Whatever the biochemical interactions, the pollen grain coating certainly becomes more rigid only minutes after a compatible cross, and the grain begins to take up water. The possibility thus exists that the coat changes occurring subsequent to pollination serve to meter the ingress of water into the grain. Since water must clearly come from the stigma, changes in the pellicle may also take place and it has already been noted (Mattsson et al. 1974) that the pellicle is well adapted to regulate water supply; it appears fissured when the stigma is turgid, but may close to prevent loss of water during periods of stress.

Clearly the supply of water from the stigma to the grain is the single most important requirement for germination and should self pollen differ solely in its ability to accumulate water, the variation in development and effect of environmental treatments are anticipated. If the SI system is based on a factor so simple as water relations, it is not easy to explain how the few incompatible pollen tubes that do form are rejected by the stigma. However, stigma papillae will react similarly to mechanical damage and the ingress of foreign protein. It is possible that the water-stressed incompatible tube fails to synthesise sufficient callose in a manner which isolates efficiently the tube content (in higher plants sporophyte and gametophyte are always separated by callose) from the sporophytic papillar cytoplasm, and the papilla thus rejects this 'foreign' protein as it would any other. An alternative possibility is that the lack of control of carbohydrate biosynthesis so evident in incompatible tubes results in a too-massive deposition of callose during early pollen tube growth. If this takes place at the tip in addition to the walls, of the tube the cell will immediately become rigid, with its tip inextensible. In these circumstances a rapid turgor rise would occur in the cell, perhaps causing rupture of the tube wall at its weakest point, the tip, releasing gametophytic cytoplasm onto, or into the sporphytic papillae. Equally, the reaction of the stigma may simply be to an interaction between elements of the pollen coating and the pools of pellicle proteins known to be held in the stigmatic cytoplasm (Stead et al. 1980), an interaction similar to that which has already taken place on the surface of the papilla.

We may conclude that our understanding of the pollen-stigma interactions underlying the SI system in *Brassica* has improved considerably in recent years. Knowledge of the molecular architecture of the pollen surface and the changes occurring in the coat would enable the construction of the first detailed model of a higher plant recognition system.

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Literature

Bateman, A.J. (1955): Self-incompatibility in Angiosperms. III. Cruciferae. Heredity 9, 52-68

Bolwell, G.P.; Callow, J.A.; Callow, M.E.; Evans, L.V. (1979): Fertilisation in brown algae. II. Evidence for lectin-sensitive complementary receptors involved in gamete recognition in *Fucus serratus*. J. Cell Sci. 36, 19-30

Brewbaker, J.L. (1957): Pollen cytology and incompatibility systems in plants. J. Hered. 48, 271-277

Carter, A.L.; McNeilly, T. (1975): Effects of increased humidity on pollen growth and seed set following self-pollination in Brussels sprout (*Brassica oleracea* var. gemmifera). Euphytica 24, 805-813

Carter, A.L.; McNeilly, T. (1976): Increased atmospheric humidity post-pollination: a possible aid to the production of inbred line seed from mature flowers in the Brussles sprout (*Brassica oleracea* var. 'gemmifera'). Euphytica 25, 531-536

Clarke, A.E.; Gleeson, P.; Harrison, S.; Knox, R.B. (1979): Pollen-stigma interactions: Identification and Characterisation of surface components with recognition potential. Proc. Natl. Acad. Sci. (Wash.) 76, 3358-3362

Darwin, C. (1876): Effects of Cross and Self Fertilisation in the Vegetable Kingdom. 2nd Ed. 1878. New York: D. Appleton

Dickinson, H.G.; Lewis, D. (1973a): Cytochemical and ultrastructural differences between intraspecific compatible and incompatible pollinations in Raphanus. Proc. Roy. Soc. (Lond.) B. 183, 21-38

Dickinson, H.G.; Lewis, D. (1973b): The formation of the tryphine coating pollen grains of *Raphanus*, and its properties relating to the self-incompatibility system. Proc. Roy. Soc. (Lond.) B. 184, 149-165

Ferrari, T.E.; Wallace, D.H. (1975): Germination of Brassica pollen and expression of incompatibility in vitro. Euphytica 24, 757-765

Ferrari, T.E.; Wallace, D.H. (1977): A model for self-recognition and regulation of the incompatibility response of pollen. Theor. Appl. Genet. 50, 211-225

Heslop-Harrison, J. (1975): Incompatibility and the pollen stigma interaction. Ann. Rev. Plant Physiol. 26, 403-425

Heslop-Harrison, J.; Heslop-Harrison, Y. (1975): Enzymic removal of the proteinaceous pellicle of the stigma papilla prevents pollen tube entry in the Caryophyllaceae. Ann. Bot. 39, 163-165

Heslop-Harrison, Y. (1976): Localisation of concanavalin A binding sites on the stigma surface of a grass species. Micron 7, 33-36

Heslop-Harrison, Y.; Shivanna, K.R. (1977): The receptive surface of the angiosperm stigma. Ann. Bot. 41, 1233-58

Knox, F.R.; Heslop-Harrison, J.; Reed (1970): Localisation of antigens associated with the pollen grain wall by immunofluorescence. Nature 225, 1066-1068

Kroh, M. (1964): An electron microscopic study of the behaviour of Cruciferae pollen after pollination. In: Pollen Physiology and Fertilisation Linskens, H.F., pp. 221-224. Ed.: Amsterdam: N. Holland Publ. G.

Kroh, M. (1966): Reaction of pollen after transfer from one stigma to another. Der Züchter 36, 185-189.

Mattsson, O.; Knox, R.B.; Heslop-Harrison, J.; Heslop-Harrison, Y. (1974): Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature 213, 703-704

Murabaa, A.I.M. el. (1957): Factors affecting seed set in Brussels sprouts, radish and cyclamen. Meded. Landbouwhogesch. Wageningen 57, 1-33

Nakanishii, T.; Hinara, K. (1973): An effective time for CO_2 gas treatment in overcoming self-incompatibility in *Brassica*. Plant Cell Physiol. 14, 873-879

Nasrallah, M.E.; Wallace, D.H. (1967): Immunogenetics of self-incompatibility in *Brassica oleracea* L. Heredity 22, 519-527

Nasrallah, M.E.; Barber, J.T.; Wallace, D.H. (1970): Self-incompatibility proteins in plants: detection, genetics and possible mode of action. Heredity 25, 23-27

Nettancourt, D. de (1977): Incompatibility in Angiosperms. Monographs on Theoretical and Applied Genetics 3. Berlin, Heidelberg, New York: Springer-Verlag.

Nishio, T.; Hinata, K. (1977): Analysis of S-specific proteins in stigma of *Brassica oleracea* L. by isoelectric focussing. Heredity 38, 391-396

Nishio, T.; Hinata, K. (1978): Stigma proteins in self-incompatible *Brassica campestris* L. and self-compatible relatives, with special reference to S-allele specificity. Jap. J. Genet. 53, 197-205

Ockendon, D.J. (1972): Pollen tube growth and site of incompatibility in *Brassica*. New Phytol. 71, 519-522

Ockendon, D.J. (1978): Effect of hexane and humidity on self-incompatibility in *Brassica oleracea*. Theor. Appl. Genet. 52, 113-119

Roberts, I.N.; Stead, A.D.; Ockendon, D.J.; Dickinson, H.G. (1979a): A glycoprotein associated with the acquisition of the self-incompatibility system by maturing stigmas of *Brassica oleracea*. Planta 146, 179-183

Roberts, I.N.; Stead, A.D.; Dickinson, H.G. (1979b): No fundamental changes in lipids of the pollen grain coating of *Brassica oleracea* following either self- or cross-pollinations. Incompatibility Newsletter 11, 77-83

Roggen, H.P. (1974): Pollen washing influences in compatibility in *Brassica oleracea* Varieties. In: Fertilisation in Higher Plants Ed.: Linskens, H.F., pp. 273-278. Amsterdam North Holland Publ. Co.

Roggen, H.P. (1975): Stigma application of a extract from rape pollen (*Brassica rapus* L.) effects self-incompatibility in Brussels sprout (*Brassica oleracea* var. 'gemmifera'). Incompatibility Newsletter 6, 80-84

Sedgely, M. (1974): Assessment of serological techiniques for S-allele Identification in *Brassica oleracea*. Euphytic 23, 543-551

Shivanna, K.R.; Heslop-Harrison, Y.; Heslop-Harrison, J. (1978): The pollen stigma interaction: bud pollination in the Cruciferae. Acta Bot. Neerl. 27, 107-119

Stead, A.D.; Roberts, I.N.; Dickinson, H.G. (1979): Pollen-pistil interaction in *Brassica oleracea*: events prior to pollen germination. Planta 146, 211-216

Stead, A.D.; Roberts, I.N.; Dickinson, H.G. (1980): Pollen-stigma interaction in *Brassica oleracea*: the role of stigmatic proteins in pollen grain adhesion. J. Cell Sci. 42, 417-423

Vernay, M.; Cornic, M.; Aubrey, M. and Bourrillon, R. (1978): Relationship between regeneration of cell surface glycoproteins in trypsin-treated chick embryo fibroblasts and cell adhesion to the substratum. Experientia 34, 736-737

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