Papillar integrity as an indicator of stigmatic receptivity in kiwifruit (*Actinidia deliciosa*)

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Abstract

Stigmatic receptivity is a major factor limiting fruit set in kiwifruit (*Actinidia deliciosa*). The aim of this work was to know what determines the cessation of stigmatic receptivity in this species. Stigmatic receptivity has been analysed in kiwifruit through the capacity of the stigma to sustain pollen germination and has been related to stigmatic development and degeneration. The stigma of kiwifruit flowers has a papillate surface which from anthesis is covered with an abundant exudate. Papillae are unicellular and contain a number of phenolic deposits. During the lifetime of the flower these papillae gradually lose turgidity, while stigmatic secretion increases until 5 d after anthesis when papillae start rupturing and the papillar content is liberated into the germination medium. Stigmatic receptivity is high at anthesis and lasts for the next 4 d. However, it drastically decreases 5 d after anthesis and it is nil 2 d later. The pattern of stigmatic receptivity closely fits that of papillar integrity, indicating that stigmatic receptivity relies on this integrity. Since papillar integrity can easily be evaluated in stigmatic arms, this criterion can be used as a quick method to estimate stigmatic receptivity.

Key words: *Actinidia deliciosa*, kiwifruit, stigma, receptivity, pollen.

Introduction

Kiwifruit (*Actinidia deliciosa* (Chev.) Liang and Ferguson) is a species that is highly dependent on pollination, since fruit size is positively related to the number of seeds per fruit (Pyke and Alspach, 1986). Thus, to ensure that a good crop forms, the time that a flower is receptive to

pollen is critical. However, in kiwifruit, this time is particularly short, being a limiting factor for fruit set (Galimberti *et al.*, 1987; González *et al.*, 1994a). The reason this period is short is because the stigma is receptive for only a short period (González *et al.*, 1994a), and is not due to a slow growth of the pollen tube or to a short ovule longevity as in other species (Williams, 1965). Even when the stigma is hand-pollinated, it is unable to sustain pollen germination and tube growth. This situation is not exclusive to kiwifruit. It is the main limiting factor for fruit set in other species like sour cherry (Furukawa and Bukovac, 1989) and apricot (Egea and Burgos, 1992). While stigmatic receptivity has a clear significance in an agricultural context is also has wider implications since it can be involved in pollen competition (Murdy and Carter, 1987; Hormaza and Herrero, 1994).

Much work has been done on stigmatic anatomy and structure and a comprehensive review on stigma types has been published by Heslop-Harrison and Shivanna (1977). Since then stigma anatomy has been studied in a number of species (Cresti *et al.*, 1986; Baird *et al.*, 1988; Ciampolini *et al.*, 1990; Clifford and Owens, 1990), as well as an assessment of the role of the stigma in pollen retention (Slater and Calder, 1990), in insect attraction (Kenrick and Knox, 1981) and in the self-incompatibility responses (Nasrallah and Nasrallah, 1993). Likewise, the onset of stigmatic receptivity has been associated with the production of stigma secretion and different parameters have been explored as indicators of this receptivity. Thus, esterase activity in some instances appears to be associated with stigmatic receptivity (Mattson *et al.*, 1974; Bernhardt *et al.*, 1980), and in others peroxidases has been evaluated (Galen and Plowright, 1987), although a wide use of these methods is still not clear (Shivanna and Sastri, 1981). However, it is striking that information is lacking about what makes a stigma lose its receptivity.

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In the work reported here, stigma development and degeneration is followed in kiwifruit and the observed changes are related to stigmatic receptivity, in an attempt to clarify what determines the cessation of stigmatic receptivity in this species.

Materials and methods

Plant material

This work was carried out in the north of Spain on kiwifruit mature (8-years-old) female vines of cultivar 'Hayward' grafted on 'Bruno' seedlings. Pollinations were performed with pollen from a male (male C) selected as a good pollinator in these conditions (González *et al.*, 1994b). To obtain pollen, male flowers were collected just before anthesis; anthers were removed and left to dry on paper at room temperature for 24 h. Pollen was separated from the dehiscent anthers and other impurities with the help of a fine mesh. In all the experiments, the female flowers were bagged prior to anthesis to prevent uncontrolled pollination, and were pollinated on the day of anthesis. A batch of flowers was similarly treated, but left unpollinated to follow stigma development in them.

Histochemical preparations

At anthesis, 2 d before anthesis and for the next 15 d following anthesis, flowers were fixed sequentially. Five flowers per day and per treatment were fixed in 2.5% (v/v) glutaraldehyde in 30 mM phosphate buffer (Sabatini *et al.*, 1963) at pH 6.8, dehydrated in an ethanol series and embedded in Historesin (Reichert-Jung). Sections, 2 µm thick, were stained with PAS (0.5% periodic acid-Schiff reagent) for carbohydrates (Feder and O'Brien, 1968), with a double staining PAS–0.02% toluidine blue for general histological examination (modified from Feder and O'Brien, 1968), with 0.07% calcofluor white for cellulose (Hughes and McCully, 1975) and with 0.01% auramine in 0.05 M phosphate buffer (pH 7.38) for cuticle (Heslop-Harrison, 1977). Phenolic compounds were visualized with a combined staining of 1% safranin in 50% ethanol for 30 s followed by 1% methylene blue for 2 min (Gutmann, 1993) and with 0.02% toluidine blue for 30 s at 60 °C following a modification of Feder and O'Brien (1968). With this stain, metachromasia is produced and while RNA is stained purple and DNA blue, phenolic compounds are evidenced by a green coloration (Feder and O'Brien, 1968), indicating that they are tannins and/or polyphenols (Ramalingan and Ravindranath, 1970).

Stigmatic receptivity

To determine stigmatic receptivity, flowers isolated 1 d before anthesis were pollinated at different intervals, so that the flowers were 0, 1, 2, 3, 4, 5, 6, or 7-d-old at the time of pollination. One day after pollination, 10 flowers per day were fixed in formaldehyde:acetic acid:70% ethanol (1:1:18, FAA) (Johansen, 1940). The capability to sustain pollen germination was evaluated by sampling three stigmatic arms in each of these 10 flowers, and observing pollen germination. Pollen and pollen tubes were stained in squash preparations with 0.1% aniline blue in 0.1 N K₃PO₄ (Linskens and Esser, 1957), after washing the fixative off with water and softening the tissue by boiling in 5% Na₂SO₄ (Jefferies and Belcher, 1974). Data were analysed using Duncan's multiple range test.

Stigma degeneration

Papillar integrity was recorded in stigmatic arms after staining the papillar wall with 0.07% (w/v) calcofluor white (Hughes and McCully, 1975). This was performed on a sample of three stigmatic arms per flower and in the same population of flowers as used to evaluate the stigmatic receptivity. Data were also analysed using Duncan's multiple range test.

Results

The pistil of kiwifruit is composed of a number, generally over 30, of stigmatic arms. Each stigmatic arm has an inner transmitting tissue and ends in a papillate stigma (Plate 1A) made up of unicellular papillae (Plate 1B) covered by a cuticle (Plate 1C). The stigma is of a wet type. At anthesis, papillae contained starch (Plate 1D) and a number of inclusions that did not stain with PAS, but stained red with safranin–methylene blue or green with toluidine blue, indicating the presence of phenolic compounds (Plates 1E, F).

Sequential histological study of the papillae showed that the stigma experienced a number of changes as the flower aged. Starch vanished from the cells as secretion increased and papillae gradually lost turgidity during the 4 d following anthesis (Plate 2A) until their abrupt rupture, when the papillar contents were liberated into the germination medium. From these contents, the most conspicuous staining was the green coloration from the remnants of the phenolic inclusions (Plate 2B). This pattern and timing was followed in a similar way in both pollinated and unpollinated stigmas.

Stigmas were highly receptive at anthesis and pollen germinated profusely on them (Plate 2C). Stigmatic receptivity remained high for the next 4 d following anthesis. Subsequently, stigmatic receptivity decreased. While this process is not simultaneous for all the stigmatic arms of a flower, it does occur abruptly in each stigmatic arm and they appear to pass from an ability to sustain pollen germination to a situation where the pollen germination was nil. During the 4 d following anthesis, on average, 80% of the stigmatic arms were able to sustain pollen germination. However, receptivity significantly decreased ($P \leq 0.05$) 1 d later, when only 39% of the stigmatic arms supported pollen germination and 7 d after anthesis it was nil (Fig. 1; Plate 2D).

To evaluate if stigmatic receptivity was inversely related to stigmatic degeneration, this parameter was assessed through papillar integrity in a population of flowers of different ages from anthesis to 7 d later. It could easily be evaluated in stigmatic arms, stained with calcofluor white and observed with epifluorescence (Plate 1A). While all the stigmatic arms of a flower do not degenerate at the same time, degeneration appears to occur abruptly inside each stigmatic arm and all their papillae appear to rupture simultaneously. Papillar integrity, measured as

Plate 1. (A) Stigma at anthesis stained with calcofluor white, papillae covering all the stigmatic surface. Bar=310 μm . (B) Unicellular papillae (asterisks) from a stigma at anthesis. Calcofluor white-stained 2 μm Histoiresin section. Bar=120 μm . (C) Unicellular papilla from a stigma 2 d before anthesis and showing its cuticle. Auramine-stained 2 μm Histoiresin section. Bar=10 μm . (D) Stigma at anthesis. Papillae contain starch (arrows) and other unstained inclusions. PAS-stained 2 μm Histoiresin section. Bar=50 μm . (E) Stigmatic papilla at anthesis with phenolic inclusions (arrows). Safranin-methylene blue double-stained 2 μm Histoiresin section. Bar=20 μm . (F) Stigmatic papilla at anthesis with phenolic inclusions (arrows). Toluidine blue-stained 2 μm Histoiresin section. Bar=20 μm .

Plate 2. (A) Integral stigmatic papilla 4 d after anthesis. While papillae are less turgid they keep their phenolic contents (arrows). Toluidine blue-stained 2 μ m Histoiresin section. Bar=20 μ m. (B) Phenolic contents (arrows) liberated into the germination medium after papillar autolysis 7 d after anthesis. Toluidine blue-stained 2 μ m Histoiresin section. Bar=20 μ m. (C) Stigma pollinated at anthesis showing germinated pollen grains (asterisks) and callose plugs (arrows) of pollen tubes. Aniline blue-stained squash preparation. Bar=25 μ m. (D) Ungerminated pollen on a 7-d-old stigma. Aniline blue-stained squash preparation. Bar=25 μ m.

the percentage of stigmatic arms with integral papillae, when examined sequentially, was maintained for the 4 d following anthesis in 80% of the stigmatic arms, then it sharply decreased to 50% 1 d later (significant at $P \leq 0.05$), before declining to zero 7 d after anthesis. This process followed a pattern that closely fitted the one of stigmatic receptivity measured as the capability to sustain pollen germination (Fig. 1). Thus, both parameters were highly

correlated by regression analysis ($R^2 = 0.95$, significant at $P \leq 0.01$).

Discussion

Papillar cells of kiwifruit follow a developmental and degeneration process very similar to the one described for other species. The production of secretion by papillar

Fig. 1. Percentage of receptive stigmatic arms (□), evaluated by the capability to sustain pollen germination, and of stigmatic arms with papillar integrity (●) in flowers pollinated 0, 1, 2, 3, 4, 5, 6, and 7 d after anthesis. Correlation coefficient $R^2=0.95$, significant at $P\leq 0.01$.

cells has been recorded in species with wet stigmas (Heslop-Harrison, 1977; Sedgley, 1979; Sedgley and Scholefield, 1980). Likewise, autolysis of these papillae has also been described in different systems (Herrero and Dickinson, 1979; Sedgley, 1979; Mackenzie *et al.*, 1990). This is not surprising since stigmatic papillae appear to behave as secretory cells (Gasser and Robinson-Beers, 1993) and autolysis is a common phenomenon in this kind of cell (Fahn, 1988). The process of stigma development and degeneration appears to be developmentally regulated for it also occurs in unpollinated flowers, although pollination accelerates secretion in most species which exhibit a wet stigma (Sedgley and Blesing, 1982) and induces conspicuous changes in species with dry stigmas (Elleman *et al.*, 1992).

The involvement of the stigmatic papillar cells in stigmatic receptivity has been established in *Brassica* with ablation experiments (Kandasamy *et al.*, 1993). Due to the incapacity of the stigma to support pollen germination, the ablation of papillar cell function resulted in female sterility. In kiwifruit, the cessation of stigmatic receptivity as the flower ages could be interpreted as a decline in exudate quantity or quality as a result of the declining metabolic activity of senescent papillae. However, the fact that both cessation of stigmatic receptivity and papillar rupture occur abruptly and concomitantly indicates that these two processes are interrelated. Thus, although a number of changes, indicative of ageing, occur in the papillar cells during the 4 d following anthesis, they do not appear to affect pollen germination. Stigmas are equally able to sustain pollen germination although papillae lose turgidity, starch vanishes from these cells and secretion increases. However, this capability is impaired at the very moment of papillar autolysis. This might be associated with the disfunctioning of these cells and also to the liberation of some toxic cellular contents into the germination medium. The most con-

spicuous substances liberated into the medium seem to be phenolic compounds as shown by the green coloration after toluidine blue staining (Feder and O'Brien, 1968; Ramalingam and Ravindranath, 1970). While they are present in the stigma, inside the papillae, right from anthesis, they remain apparently unaltered until papillar rupture when they are massively liberated into the medium.

The presence of phenolic compounds in the stigma has been previously reported in a number of different species (Kristen *et al.*, 1979; Villar *et al.*, 1987; Shivanna *et al.*, 1989) and appears to be one of the main components of stigmatic exudate where they bind to sugars (Martin, 1969). While a clear function has yet to be allocated to them, their effects on pollen germination and pollen tube growth have been discussed recently (Mascarenhas, 1993). Thus, the addition of certain kind of flavonoids into the medium has been reported to promote pollen germination and tube growth in kale and tobacco (Sedgley, 1975; Ylstra *et al.*, 1992), while an inhibitory effect has been reported in sour cherry pollen (Szember and Wociór, 1991). Likewise, pollen germination and/or tube growth are inhibited in chalcone synthase deficient transgenic plants producing flavonoid-defective pollen (Coe *et al.*, 1981; Taylor and Jorgensen, 1992). Further work is necessary to elucidate if the intracellular phenolic content reported in kiwifruit might play a role in pollen germination.

On the other hand, presynthesized phenols in the cells appear to be involved in defence mechanisms (Nicholson and Hammerschmidt, 1992). It is possible that the observed phenols in the kiwifruit stigma also function in a defence mechanism. The stigma is a very vulnerable tissue, with a rich medium where pathogens could well develop. The liberation of phenolic contents into the stigmatic medium, once the flower is old, could be a means of poisoning this medium and thus protecting against pathogen infection through this necrotic tissue. Whatever their primary role, these phenols could also indirectly impair pollen germination and, thus, limit flower receptivity.

Results presented here are indirect evidence of papillar integrity being responsible for stigma receptivity. While this association may be a pure coincidence in kiwifruit, it may prove valuable to evaluate to what extent it can be regarded as a more general phenomenon. Autolysis of papillar cells has been described in most species where the structure of stigma development has been considered. While differences in the time when this autolysis occurs have been recorded (Sedgley, 1982), the extent to which this time is limited to the duration of stigmatic receptivity is unknown. However, the fact that papillar integrity and stigmatic receptivity have such a close fit, suggests that these parameters, being easy to evaluate, are useful both in an agricultural and an ecological context.

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