International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 8 (August-2015) pp. 188-195

www.ijcrar.com

Floral morphology, pollen viability and pollinizer efficacy of kiwifruit

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KEYWORDS

Kiwifruit,
Floral morphology,
Pollen viability,
Pollinizer efficacy

A B S T R A C T

Experiment was conducted during 2012-2013 at the experimental orchard of Department of Fruit Science, Dr Y S Parmar University of Horticulture and Forestry Nauni, Solan (H P). Four pistillate and two staminate cultivars of kiwifruit were studied for floral morphology. The flowers of staminate and pistillate cultivars exhibited different morphology with respect to flower size and the presence / absence of reproductive parts. The pistillate cultivars also exhibited different flower morphology with respect to the position of the style on the ovary (either erect or recumbent). The pollen grains of both the staminate cultivars were ovate when dry and assumed a circular shape on imbibition of moisture. Maximum pollen viability of both the cultivars was observed in Acetocarmine solution, followed by Tetrazolium Chloride and lowest in Erythrosine B. The highest pollen germination (54.31 %) in Allison was recorded in 10 % sucrose solution followed by 15 % (47.12%) and lowest in distilled water (6.73 %). Both the pollinizers proved to be effective in inducing high fruit set but the cultivar Allison (♂) was considered better because of synchronization with other pistillate varieties.

Introduction

The chinese gooseberry popularly known as kiwifruit is a native of South West China and grown commercially in New Zealand, China, Australia, France, Spain, Italy and many other countries. It belongs to the genus *Actinidia* and family Actinidiaceae. The genus contains more than 50 species distributed in the temperate and subtropical regions of the Asian continent. All member of the genus *Actinidia* are functionally dioecious, with pistillate and staminate flowers occurring on the separate plants (Ferguson, 1990). Both male and female vines produce large numbers of visually conspicuous flowers with large white to cream petals and numerous stamens with yellow anthers. Flowers are borne in small dichasia in lower leaf axils of flowering shoots of current season. These flowering shoots arise from axillary buds on lateral

canes that had developed the previous growing season. Pistillate flowers are typically larger than staminate flowers but male vines usually carry more flowers. Pollen grains are small, lack pollenkitt (Goodwin, 1987) and are shed abundantly in clumps from both pistillate and staminate flowers. Pollen produced by pistillate flowers is usually nonviable lacking cytoplasm and nuclei. Flowers are scented, particularly staminate flowers, but produce no nectar (Schmid, 1978). Mouat (1958) studied that the principle cultivars like Allison, Hayward and Bruno exhibit different type of flower morphology with differences in petal outline. He observed differences in the position of styles (erect or recumbent). These differences can be used as an aid in cultivar identification. Pollen with cytoplasmic contents and empty pollen can be easily distinguished by staining with acetocarmine and pollen viability can be assessed using fluoresce in diacetate. Pollen viability can also be assessed by germination in shake culture at 22^0 C for 3.5 hours in a solution containing 10% (w/v) sucrose and 0.01% (w/v) boric acid (Hopping and Jerram 1980). Kiwifruit pollens show very good pollen viability in fact close to 85% and the time course of pollen tube growth (increased over time of tube mass) was linear over a 6 hour incubation period (Speranza *et al.* 2001).

Material and Methods

Data on different parameters of the flower were recorded with respect to flower size, colour, number of anthers, number of styles, number of sepals, number of petals, their size and stamens length. Fresh pollen grains were studied for their shape in dry stage under the microscope after placing them on a clean dry slide. Pollen grains were also examined by the acetocarmine solution as follows.

Pollen was placed on the slide and 2–3 drops of acetocarmine solutions were added. After covering with cover slip excess of solution was drawn off and left as such for 10 minutes and then this slide was examined for studying the pollen morphology. Pollen viability was tested by using staining test and pollen germination. Staining solutions used for testing pollen viability were acetocarmine (1%), tetrazolium test (1%) and erythrosine B test (0.1%). Pollen viability was observed in one per cent acetocarmine solution. One to two drops of acetocarmine solution was placed on the slide and then the pollen grains were dusted, covered with a cover slip carefully to avoid the formation of air bubble and examined under the microscope. Deeply stained and normal looking pollen grains were considered to be viable, whereas shrivelled, lightly stained or colourless pollen grains were counted as non-viable. In tetrazolium test the stained pollens i.e. red stained are considered viable while unstained are non viable. In Erythrosine B solution the unstained pollen grains were considered viable in this method and stained pollen grains were considered as non-viable.

For pollen germination the freshly dehisced pollen grains were used for *in vitro* germination test. The different concentrations of sucrose *i.e.* 0, 5, 10, 15 and 20 per cent were prepared by dissolving 5, 10, 15 and 20 grams of sucrose in 100 ml of distilled water, respectively. Different sucrose solutions were placed in cavities of different microscopic slides and pollen grains were dusted over them. After placing cover slips on their edges were smeared by Vaseline and the slides were inverted instantly, leading to the formation of a hanging drop over the cover slips. These cavity slides were placed in Petri dishes containing moist filter paper to ensure uniform and high relative humidity. Pollen

tube growth was assessed for each cultivar under microscope after 24 hours. The pollen grains having pollen tube at least two times longer than pollen size were considered to be germinated and percentage of germinated pollen grains under three microscopic fields was worked out, which were treated as one replication.

Two pollinizers namely Allison and Tomuri were used to study the pollinizers efficacy. Pistillate varieties used were Allison, Hayward, Bruno and Monty. Bagging was done in Allison, Hayward, Bruno and Monty before anthesis. The pollens were collected from Allison male and Tomuri and then crossed with the pistillate varieties to study the pollinizers efficiency of different pollinizers and data up to fruit set was recorded.

Results and Discussion

Data pertaining to floral morphology of different cultivars is presented in table 1. It is evident from the table that flowers of all the cultivars were creamish white large and attractive with highest diameter of flowers in Allison variety (6.1 x 6.1cm) and lowest in Allison (♂) (4.1 x 4.1cm). The sepals were brown and densely tomentose and varied from 4 - 7 in different cultivars. In Hayward the number of sepals were constant i.e. six. Maximum size of sepals was recorded in Allison (1.2 x 0.9cm) and minimum in Bruno (0.9 x 0.6cm). Petals were creamish white in colour and their numbers varied from 5-9 in different cultivars. Tomuri recorded the highest number of petals (5-9) and lowest number was found in Monty (4-6). Maximum petal size was recorded in Allison (2.9 x 2.0 cm) and minimum in Allison (♂) (2.1 x 1.6 cm). Number of stamens were highest in Bruno (123 to 153) and lowest were recorded in Tomuri (96 to 119). The stamen length ranged from (1.0-1.3 cm) being maximum in Tomuri (1.3 cm) and minimum in Bruno and Monty (1.0 cm). Number of styles varied from 24 - 40 in different cultivars being maximum in Hayward (35 to 40) and minimum in Bruno (24 to 31). The ovary was superior, multicarpellate and densely tomentose in all cultivars except in Allison (♂) and Tomuri. These cultivars exhibit different type of flower morphology with differences in petal outline and the position of the styles (either erect or recumbent) (Plate 1).

The results of the present findings on the floral morphology of staminate and pistillate flowers are in accordance with the findings of Ferguson (1984), who reported that male and female flowers of kiwifruit differ in pistil morphology. He reported that pistil is formed in the male flowers but carpel locules within the ovary remain compressed and there is no development of ovules. The styles are short without the stigmatic papillae. In contrast, female flowers contain two rows of ovules within each carpel locules. Stamens of male and female flowers are morphologically very similar but male flowers produce viable binucleate pollen whereas female flowers produce only empty pollen grains (Rizet, 1945; Schmid, 1978; White, 1990). An interesting observation made in the present study was the position of styles on the ovary of pistillate flowers. The cultivars, Allison (♀) and Bruno had styles which were parallel to the flower petals (recumbent) or close to the stamens whereas Hayward and Monty had styles which grew upward or were partially erect. The high percentage of fruit set in Allison and Bruno under natural pollination may be due to the fact that honey bees while working the flowers have most contact with stigmas close to the stamens. The erect position of stigmas in Hayward may permit the bees to easily work the flower without touching them resulting in poor or low fruit set compared to other varieties.

Table.1 Floral morphology of different kiwifruit cultivars

Characters	Allison (♀)	Hayward (♀)	Monty (♀)	Bruno (♀)	Allison (♂)	Tomuri (♂)
Flower Diameter (cm)	6.1 X 6.1	5.5 X 5.3	5.0 X 5.0	5.0 X 5.1	4.1 X 4.1	5.0 X 4.9
Flower colour	White	White	White	White	White	White
Number of Sepals	5 - 6	6	4 - 6	5 – 7	5 – 7	6 - 7
Sepal size (l x b) cm	**1.2 x 0.9**	1.04 X 0.8	1.06 X 0.8	**0.9 X 0.6**	0.9 X 0.7	1.0 X 0.7
Number of Petals	5 to 6	6	6	5 to 6	5 to 6	**5 to 9**
Petal size (l x b) cm	**2.9 x 2.0**	2.6 X 2	2.5 X 2.1	2.3 X 1.6	2.1 X 1.6	2.3 X 1.8
Number of Stamens	118 - 135	113 – 138	108 - 138	**123 – 153**	104 - 117	96 - 119
Stamen length(cm)	1.1	1.1	1.0	1.0	1.2	1.3
Number of styles	28 - 36	**35 – 40**	27 - 30	24 – 31	-	-

Table.2 Pollen viability of Allison (♂) and Tomuri cultivars with different staining solutions

Cultivars	Acetocarmine (1%)	Tetrazolium chloride (1%)	Erythrosine B (0.1 %)	Mean
Allison	96.67 (9.83)*	93.67 (9.68)	85.33 (9.24)	91.89 (9.58)
Tomuri	94.00 (9.7)	92.33 (9.61)	84.00 (9.17)	90.11 (9.49)
Mean	95.33 (9.76)	93.00 (9.64)	84.67 (9.20)	

C D $_{0.05}$

Cultivars (C)	0.06
Staining solution (S)	0.07
C x S	0.1

*Figures in parentheses are square root transformed values

Table.3 Germination of pollen grains in different concentration of sucrose solution

Cultivars	Distilled water	5%	10%	15%	20%	Mean
Allison	6.73 (15.03)*	34.12 (35.73)	54.31 (47.45)	47.12 (43.33)	34.66 (36.05)	35.39 (35.52)
Tomuri	6.77 (15.07)	34.07 (35.69)	52.76 (46.56)	46.97 (43.25)	33.82 (35.22)	34.88 (35.22)
Mean	6.75 (15.05)	34.09 (35.71)	53.53 (47.01)	47.05 (43.29)	34.24 (35.8)	

C D $_{0.05}$
Cultivar (C) 0.08
Solutions (S) 0.13
C x S 0.19
*Figures in parentheses are arc sine transformed values

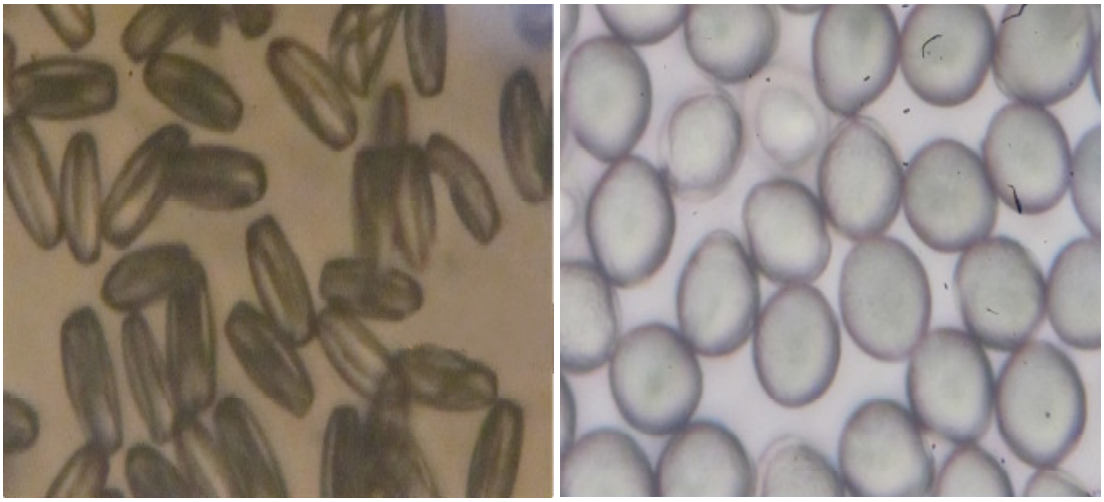
Table.4 Effect of pollinizers on fruit set of different kiwifruit cultivars

Cross combinations	Number of flowers pollinated	Fruit Set (%)
Allison x Allison (♂)	45	100.00 (10)*
Allison x Tomuri	45	98.67 (9.93)
Monty x Allison (♂)	45	100.00 (10)
Monty x Tomuri	45	98.67 (9.93)
Bruno x Allison (♂)	45	97.33 (9.87)
Bruno x Tomuri	45	98.67 (9.93)
Hayward x Allison (♂)	45	98.67 (9.93)
Hayward x Tomuri	45	98.33 (9.92)
CD $_{0.05}$		0.08

*Figures in parentheses are square root transformed values

Plate.1 Flower morphology showing recumbent (Bruno & Allison) and erect styles (Hayward & Monty)

Plate.2 Pollen morphology of kiwifruit



(DRY) (HYDRATED)

The shape of the pollen was pyramidal to ovate when dry and became more spherical when hydrated or with the addition of Acetocarmine solution (Plate - 2). Similar observations were also made by Hopping and Jerram (1979).

Pollen viability was assessed in different staining solutions and data is presented in table 2. The maximum viability was recorded in the cultivar Allison with Acetocarmine solution (96.67 %) followed by Tetrazolium chloride (93.66 %) and lowest (85.33 %) with Erythrosine B. A similar trend of pollen viability was observed in the cultivar Tomuri with different staining solutions. Although different staining methods of testing viability are in use but they all are a crude measure of pollen viability. The pollen viability was highest with Acetocarmine followed by Tetrazolium chloride and lowest with Erythrosine B. This is because in Acetocarmine solution even the dead pollen grains are also counted as viable as they also get stained because of the presence

of protein (Sharma, 2001). Hopping (1981) reported that in general, viability of pollen from early season clones exceeds 80 % while that of pollen from mid to late season clones range from 65–75%.

The viability of pollen was also assessed by in vitro germination in different concentration of sucrose solutions and the data is presented in table 3. The highest germination was recorded in 10 % followed by 15 % and thereafter there was a deleterious effect on the germination probably due to plasmolysis. Several studies on determining viabilities of kiwifruit pollen using in vitro germination methods have been reported (Holocroft and Allan, 1991; Gonzalez *et al.* 1994 and Korkutal *et al.* 2004). Maximum pollen germination (52.76 %) of Tomuri cultivar was also recorded in 10 % sucrose solution by Borghezen *et al.* (2011).

In order to study the efficacy of two pollinizers namely Allison and Tomuri, they were crossed with four pistillate cultivars and the data on fruit set (recorded 25 days after pollination) is presented in table 4. From the perusal of data it is evident that the fruit set was very high and ranged from 97.33 to 100 % in different cross combinations.

Highest fruit set (100 %) was recorded when Allison was used as a pollinizer for Allison (♀) and Monty. However Tomuri also recorded a high fruit set of more than 98 % under different cross combinations and was considered equally at par with Allison. The results of the present finding are in accordance with the observation of Maria *et al.* (1994) who evaluated different pollinizers for the cultivar Hayward. They concluded that pollen source had no significant effect on fruit set, fruit weight and fruit diameter.

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