Journal of Applied Horticulture, 15(2): 128-132, 2013

Pollen storage and use for enhancing fruit production in kiwifruit (*Actinidia deliciosa* A. Chev.)

Sharmistha Naik*, Poonam[1] and Vishal Rana

Department of Fruit Science, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230, India.
*[1]Central Potato Research Institute, Shimla 171 001, India.*E-mail: naiksharmistha@gmail.com*

Abstract

Kiwifruit is a dioecious plant and there is a strong correlation between fruit size and the number of fertilized ovules. Insufficient pollination due to asynchrony between staminate and pistillate blossoms and unfavourable environmental conditions results in reduced fruit size and unequal fruit shape. In the present investigation an attempt was made to develop a simple and reliable method for storage of kiwifruit pollen and their utilization in hand pollination in following year. Pollen were stored at different temperatures [room temperature (25 ± 2 ^{0}C), 4, 0 and -20 ^{0}C] for a period of one year. Pollen viability was determined at monthly intervals using 2,3,5-triphenyltetrazolium chloride (TTC) staining and percent *in vitro* germination using 14 % sucrose,1.7 mM calcium nitrate and 3mM Boric acid. Initial TTC stainability (78.83 %), *in vitro* germination (65.55%) and absolute viability (51.72 %) of fresh pollen went on reducing with storage periods. -20 ^{0}C was the best temperature at which maximum viability of kiwifruit pollen can be retained up to one year. Pollination using pollen stored for one year showed that pollen stored at -20 ^{0}C were able to set 100 % fruits, pollen stored at 0 ^{0}C could set 36 % fruits, while there was no fruit setting with pollen stored at room temperature and 4 ^{0}C. These findings have practical implications for kiwifruit production in India. Artificial pollination with stored pollen can circumvent several uncertainties of natural pollination and guarantee adequate pollination in kiwifruit.

Key words: Kiwifruit, pollen storage, pollen viability, *in vitro* pollen germination, absolute pollen viability, TTC staining.

Introduction

Kiwifruit is dioecious plant, with pistillate and staminate flowers occurring on separate plants (Ferguson, 1990). Insufficient pollination due to frequent asynchrony between staminate and pistillate blossoms and unfavourable environmental conditions results in reduced fruit size and unequal shape which is a major problem in kiwifruit production. Growers resort to hand pollination using pollen collected from their own orchard or from other sources for production of large size fruits. In kiwifruit, pollen acquisition is costly, often prohibiting the use of supplementary pollination technique. In artificial pollination, the gap between pollen harvesting and application ranges from few hours to several months. In such cases, pollen need to be stored under desirable conditions to retain their viability. Optimum pollen storage conditions vary from one species to another (Shivanna and Johri, 1985). It has been reported that pollen stored at -10 to -20 ^{0}C can retain viability for years in many plant species (Hanna and Towill, 1995). Bomben *et al.* (1999) and Abreu and Oliveira (2004) reported successful storage of kiwifruit pollen for varying periods (54 to 160 weeks) at varying temperatures. In the present investigation, we report a simple and reliable method for storage of kiwifruit pollen for a period of one year and their use in hand pollination to produce normal kiwifruits.

Material and methods

Experiments were performed on kiwifruit cultivar Allison in 26 year old experimental orchard in Nauni, District Solan (Himachal Pradesh) during 2010-2011. The latitude and longitude of Nauni is 30° 50' North and 77° 11' 30" East, respectively. The location is 1260 m above mean sea level and receives annual precipitation of 1000-1300 mm, with most rainfall occurring from June-September. Male flower buds were collected before anthesis in early morning (before 0800 hrs) and kept under incandescent table lamp for 12-18 hrs. The pollen were separated by fine brush and collected after sieving through clean and dry 0.12 mm mesh to separate anther filaments. Collected pollen were stored in glass vials at different temperatures [room temperature (25 ± 2 ^{0}C), 4, 0 and -20 ^{0}C] for a period one year.

Pollen viability using TTC stain: Percentage of stainable pollen was measured after staining with TTC (2, 3, 5-triphenyltetrazolium chloride). The stain was prepared in 0.05 M phosphate buffer, pH 5.8. TTC stain (1 %) was prepared by dissolving 100 mg of 2,3,5-triphenyltetrazolium chloride in 10 mL of above 0.05M phosphate buffer. The stain was filter sterilized using Millipore syringe filter and refrigerated in dark bottle**.** Pollen viability was expressed as % TTC stainability. Pollen viability was studied in the beginning and at 11 intervals of one month each on the pollen stored at different temperature.

***In vitro* pollen germination:** Pollen viability was also studied by germinating pollen in liquid medium containing 14 % sucrose, 3 mM boric acid and 1.7 mM calcium nitrate. The pH of the medium was adjusted to 5.6 before autoclaving**.** Pollen germination was carried out in Cellstar tissue culture plates having 6 wells. About 5-10 mg pollen were mixed in 5 mL pollen germination medium in wells of culture plate and the plates were incubated in dark for 6 hours at 37 ^{0}C on incubator shaker running at 25 revolutions per minute (rpm). Germinated (with pollen tube) and non-germinated (without pollen tube) pollen were counted under microscope at 200 X magnification.

Absolute pollen viability: Absolute pollen viability or the effective germination capacity was calculated using following formula given by Visser *et al.* (1977).

$$\text{Absolute pollen viability} = \frac{\text{TTC stained pollen (\%) x Germinated pollen (\%)}}{100}$$

***In vivo* viability of stored pollen:** Pollen stored at different temperatures for a period of 1 year were used to pollinate Allison female flowers in 2011. Twenty nine to 41 flowers were hand pollinated in each treatment. The percent fruit set was recorded 15 days after pollination.

Arc sine transformation and statistical analyses were carried out using MSTAT-C software.

Results

TTC staining, *in vitro* germination and absolute viability of pollen stored at different temperatures: Results obtained in the present study indicated that percentage of TTC stained pollen went on reducing with storage period. This reduction was more pronounced in pollen stored at relatively higher temperatures. From the initial 78.83 % TTC staining in fresh pollen, the percentage staining was reduced to 5.47, 18.71, 37.13 and 46.95 % in pollen stored at room temperature, 0, 4 and –20 ^{0}C, respectively, in a period of 11 months (Table 1). There were significant differences in % TTC staining over storage periods, storage temperatures and their interactions (Fig. 1 and 2).

Similar reduction was observed *in vitro* germination of stored pollen (Fig. 1 and 2). The reduction in pollen viability was more rapid in pollen stored at higher temperatures. From the initial 65.55% *in vitro* germination in fresh pollen, the percentage germination was reduced to 0, 8.99, 24.15 and 35.37 % in pollen stored at room temperature, 0, 4 and –20 ^{0}C, respectively, in a period of 11 months (Table 2). There were significant differences in % *in vitro* germination over storage periods, storage temperatures and their interactions.

Per cent absolute pollen viability, which is a measure of effective germination capacity, is lower than % TTC staining and % *in vitro* germination at all temperature regimes and intervals starting from fresh pollen. From the initial 51.72 % absolute viability in fresh pollen, the viabilities were reduced to 0 %, 1.68 %, 8.97 % and 16.60 % in pollen stored at room temperature, 0 ^{0}C, 4 ^{0}C and –20 ^{0}C, respectively, in a period of 11 months (Table 3). There were significant differences in % absolute viability over storage periods, storage temperatures and their interactions.

Correlation coefficients between TTC staining (%), *in vitro* (%) germination and absolute viability (%) were significantly positive with highest r-value between % *in vitro* germination and % absolute viability (0.976) and lowest between TTC staining (%) and absolute viability (%) (r=0.930) (Table 4).

Fruit setting using one year old pollen stored at different temperature regimes: Pollen stored for a period of one year at different temperatures [room temperature (25±2 ^{0}C), 4 ^{0}C, 0 ^{0}C and -20 ^{0}C] were used in hand pollination. There was no fruit set in pollen stored at room temperature and at 4 ^{0}C. Fruit set to the tune of 36 % was recorded in pollen stored at 0 ^{0}C. In case of pollen stored at – 20 ^{0}C, the fruit set was 100% (Table 5).

Discussion

The initial TTC stainability (78.83 %), *in vitro* germination (65.55 %) and absolute viability (51.72 %) of fresh pollen went on reducing with storage periods. The reduction in viability parameters were drastic in storage at higher temperatures, moderate at relatively lower temperatures and least at -20 ^{0}C (Table 1-3). Our results are supported by most of studies conducted on storage of pollen at different temperatures.

Storage of pollen at temperature above 0 ^{0}C usually does not allow pollen viability for more than few months (Van der Walt and Littlejohn, 1996). It has been shown that pollen stored at -10 to -20 ^{0}C can retain viability for years in many plant species (Hanna and Towill, 1995). Bomben *et al.* (1999) and Abreu and Oliveira (2004) reported successful storage of kiwifruit pollen for varying periods (54 to 160 weeks) at varying temperatures (20, -20, -80, -196 ^{0}C). Abreu and Oliveira (2004) observed that

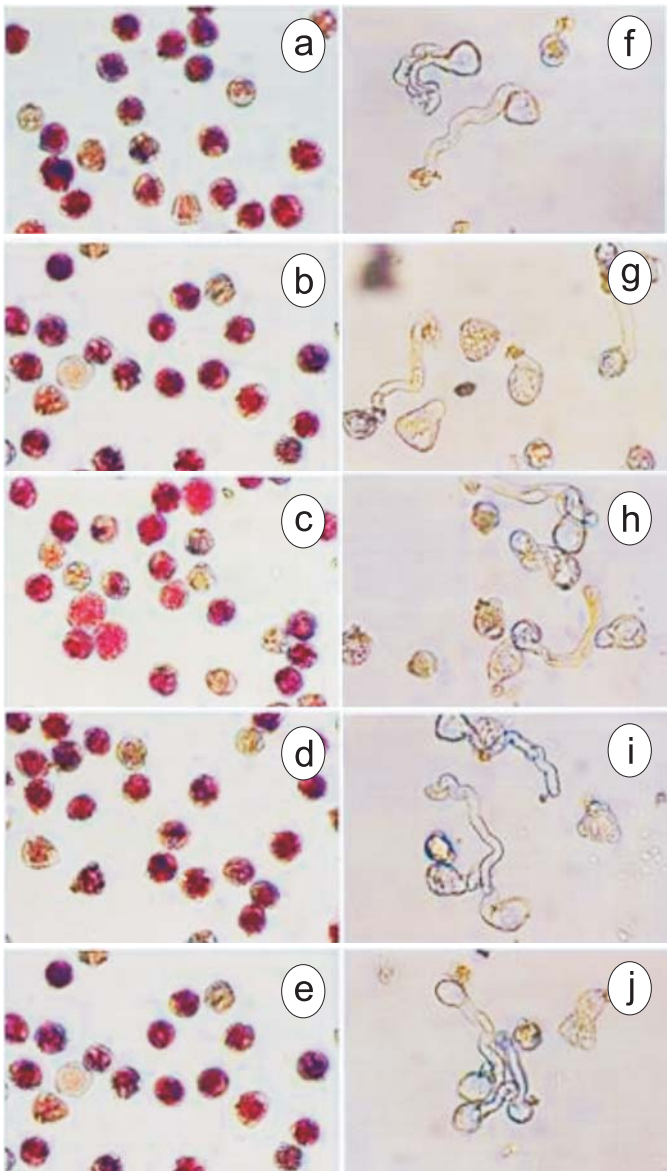

Fig. 1. Pollen staining and *in vitro* germination in fresh and stored pollen for 1 month at different temperatures. a, b, c, d, e depict pollen staining of fresh pollen and stored pollen at room temperature, 0, 4 and -20 ^{0}C, respectively. Fig f, g, h, i, j depict pollen germination in fresh pollen and pollen strored at room temperature, 0, 4 and -20 ^{0}C, respectively.

-20 ^{0}C was the best temperature at which pollen of *A. deliciosa* retained high viability and germination. We also observed that amongst the used temperature regimes, -20 ^{0}C is the best storage temperature.

In plants, vital stains have been used extensively in many crops as indicators of the viability and it has been observed that the pollen stainability reduces with storage time and in many cases staining do not correlate well with *in vitro* pollen germination (Heslop-Harrison *et al.,* 1984). We also recorded that pollen stainability by TTC stain progressively reduces with storage period, however, TTC staining and *in vitro* germination are positively correlated (r = 0.97) in kiwifruit.

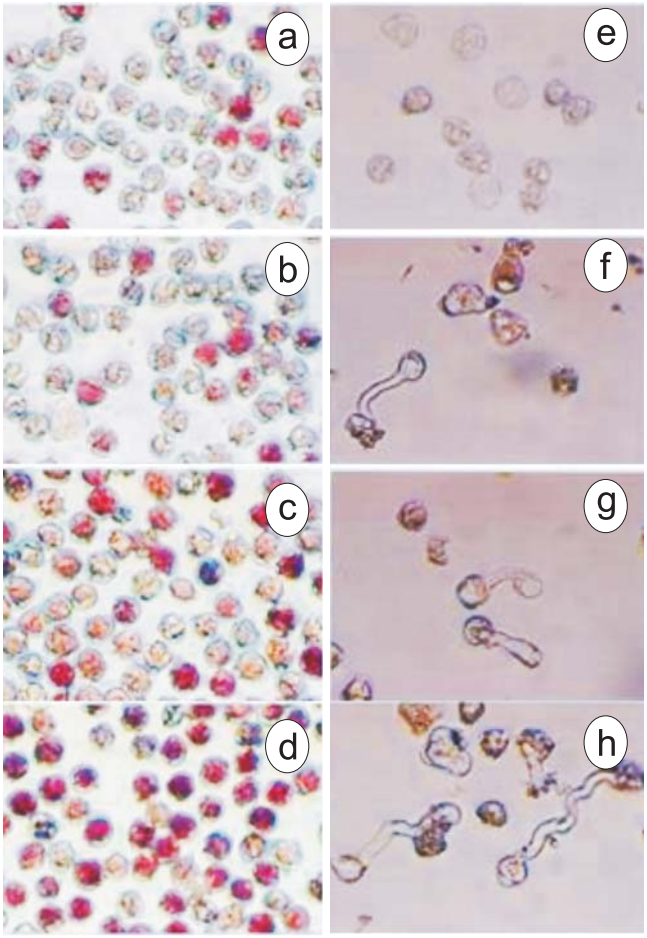

Fig. 2. Pollen staining and *in vitro* germination in stored pollen for 11 months at different temperatures. a, b, c, d depict pollen staining of pollen stored at room temperature, 0, 4 and -20 ^{0}C, respectively. Fig e, f, g, h depict pollen germination in pollen strored at room temperature, 0, 4 and -20 ^{0}C, respectively.

In vitro germination on artificial medium is the most commonly used test to determine pollen viability. Several studies on determining viability of kiwifruit pollen using *in vitro* germination method have been reported. Miaja and Me (1992) and Holcroft and Allan (1994) used *in vitro* germination tests to study viability of pollen stored for varying periods under different conditions. Miaja and Me (1992) observed that (i) kiwifruit pollen can be stored up to 3 months at 4, -18 and -196 ^{0}C without much effect on viability, (ii) *in vitro* pollen germination declined after 3 months and after one year there was 20 and 28 % loss of viability in pollen of Matua and Tomuri cultivars, respectively stored at -18 or -196 ^{0}C, (iii) pollen of both these pollinizers stored at 4 ^{0}C did not germinate after one year, and (iv) pollen viability evaluated with fluorochromatic reaction (staining) was always 1-2 % higher than germinability. Similar results were obtained in our studies, pollen stored at -20 ^{0}C exhibited highest *in vitro* germination and % TTC staining was always higher than % *in vitro* germination at all storage periods under different temperature regimes.

Per cent absolute pollen viability, which is a measure of effective germination capacity, was lower than *in vitro* germination at all storage periods and storage temperatures. Similar to TTC staining and *in vitro* germination, the highest and lowest absolute pollen viabilities were recorded at -20 ^{0}C and room temperature, respectively at all storage periods. Absolute pollen viability was also positively correlated with TTC statining (r = 0.93) and *in vitro* germination (r = 0.97).

Our results show that -20 ^{0}C was the best temperature at which maximum viability of kiwifruit pollen can be retained up to one year and all the three methods used for viability determination in our study are equally reliable because all of them were strongly and positively correlated with each other (Table 4). The most

Table 1. Effect of different storage temperatures on the percent pollen stainability of kiwifruit cv Allison

Storage period	Temperature				
	Room temperature	4 ^{0}C	0 ^{0}C	-20 ^{0}C	Mean
1 Month	42.99* (46.47)**	49.71 (58.14)	52.05 (62.13)	56.86 (70.05)	50.40 (59.20)
2 Months	39.17 (39.86)	48.76 (56.50)	51.78 (61.68)	55.72 (68.20)	48.85 (56.56)
3 Months	38.85 (39.32)	46.55 (52.67)	50.77 (59.95)	54.37 (66.01)	47.63 (54.48)
4 Months	36.69 (35.67)	45.71 (51.19)	50.33 (59.21)	53.17 (64.02)	46.48 (52.52)
5 Months	34.31 (31.75)	44.43 (48.97)	49.40 (57.61)	52.29 (62.54)	45.11 (50.22)
6 Months	20.13 (11.91)	43.78 (47.83)	47.00 (53.45)	52.13 (62.26)	40.76 (43.86)
7 Months	19.11 (10.70)	37.81 (37.55)	46.62 (52.79)	50.65 (59.75)	38.55 (40.20)
8 Months	17.16 (8.70)	36.87 (35.97)	45.67 (51.14)	49.07 (57.04)	37.19 (38.21)
9 Months	15.55 (7.18)	33.90 (31.09)	44.42 (48.94)	47.82 (54.87)	35.42 (35.52)
10 Months	14.49 (6.26)	30.63 (25.95)	43.45 (47.27)	46.25 (52.14)	33.71 (32.90)
11 Months	13.50 (5.47)	25.64 (18.71)	37.55 (37.13)	43.27 (46.95)	29.99 (27.06)
Mean	26.54 (22.12)	40.35 (42.24)	47.19 (53.75)	51.05 (60.35)	
LSD(*P*=0.05) Temperature: 0.55					
LSD(*P*=0.05) Month: 0.91					
LSD(*P*=0.05) Interaction (Month x Temperature): 1.83					

* Arc sine transformed values; ** Original values

Table 2. Effect of different storage temperatures on the percent *in vitro* pollen germination of kiwifruit cv Allison

Storage period	Temperature				
	Room temperature	4 ^{0}C	0 ^{0}C	-20 ^{0}C	Mean
1 Month	37.96* (37.80)**	44.68 (49.40)	48.00 (55.18)	50.80 (60.00)	45.36 (50.59)
2 Months	33.29 (30.11)	42.52 (45.64)	44.23 (48.61)	49.68 (58.06)	42.42 (45.60)
3 Months	24.61 (17.33)	36.96 (36.13)	44.40 (48.29)	48.49 (56.02)	38.53 (39.44)
4 Months	23.14 (15.43)	35.88 (34.33)	43.44 (47.24)	47.93 (55.06)	37.60 (38.01)
5 Months	17.42 (8.96)	34.94 (32.77)	42.53 (45.40)	47.22 (53.82)	35.49 (35.24)
6 Months	7.23 (1.58)	33.64 (30.67)	40.59 (42.30)	46.37 (52.34)	31.96 (31.72)
7 Months	4.95 (1.12)	25.19 (18.10)	37.32 (36.73)	45.01 (49.97)	28.12 (26.48)
8 Months	0.00 (0.00)	24.39 (17.06)	35.41 (33.56)	43.95 (48.12)	25.94 (24.69)
9 Months	0.00 (0.00)	20.89 (12.18)	33.36 (30.22)	41.01 (43.02)	23.81 (21.49)
10 Months	0.00 (0.00)	18.10 (9.65)	30.08 (25.09)	38.42 (38.58)	21.65 (18.33)
11 Months	0.00 (0.00)	17.46 (8.99)	29.44 (24.15)	36.51 (35.37)	20.85 (17.13)
Mean	13.51 (10.21)	30.42 (26.86)	38.93 (39.71)	45.03 (50.03)	
LSD (P=0.05) Temperature: 0.57					
LSD (P=0.05) months: 0.95					
LSD (P=0.05) Interaction (Month x Temperature): 1.90					

* Arc sine transformed values; ** Original values

Table 3. Effect of different storage temperatures on the percent absolute pollen viability of kiwifruit cv Allison

Storage period	Temperature				
	Room temperature	4 ^{0}C	0 ^{0}C	-20 ^{0}C	Mean
1 Month	24.79* (17.59)**	32.41 (28.70)	35.86 (34.29)	40.43 (42.03)	33.37 (30.65)
2 Months	20.28 (12.01)	30.53 (25.78)	33.20 (29.96)	39.03 (39.63)	30.76 (26.85)
3 Months	15.13 (6.80)	25.87 (19.03)	32.57 (28.97)	37.47 (36.98)	27.76 (22.95)
4 Months	13.57 (5.51)	24.76 (17.52)	31.94 (27.96)	36.42 (35.22)	26.67 (21.55)
5 Months	9.70 (2.84)	23.61 (16.03)	30.78 (26.17)	35.48 (33.67)	24.89 (19.68)
6 Months	2.48 (0.19)	22.53 (14.68)	28.40 (22.60)	34.82 (32.58)	22.06 (17.51)
7 Months	1.64 (0.12)	15.12 (6.80)	26.14 (19.40)	33.14 (29.86)	19.01 (14.04)
8 Months	0.00 (0.00)	14.34 (6.15)	24.48 (17.18)	31.60 (27.44)	17.61 (12.69)
9 Months	0.00 (0.00)	11.45 (3.94)	22.62 (14.78)	29.07 (23.58)	15.78 (10.58)
10 Months	0.00 (0.00)	9.12 (2.51)	20.14 (11.85)	26.66 (20.11)	13.97 (8.62)
11 Months	0.00 (0.00)	7.46 (1.68)	17.42 (8.97)	24.06 (16.60)	12.23 (6.81)
Mean	7.96 (4.10)	19.74 (12.98)	27.59 (22.01)	33.47 (30.70)	
LSD (P=0.05) Temperature: 0.39					
LSD (P=0.05) months: 0.64					
LSD (P=0.05) Interaction (Month x Temperature): 1.28					

* Arc sine transformed values; ** Original values

Table 4. Correlation coefficients among pollen viabilities determined using different methods

Parameter	TTC staining (%)	*In vitro* germination (%)
In vitro germination	0.97	1.00
Absolute viability	0.93	0.97

Table 5. Effect of the stored pollen at different temperatures for one year on the fruit set of Kiwifruit cv Allison

Pollen stored for one year at	Number of flowers pollinated	Number of fruits set	Fruit set (%)
Room temperature (25±2 ^{0}C)	29	0	0.0
4 ^{0}C	30	0	0.0
0 ^{0}C	33	12	36.4
-20 ^{0}C	41	41	100.0

accurate test of pollen viability is the ability of pollen to effect fertilization and set seeds. It was observed that pollen stored at -20 ^{0}C were able to set 100 % fruits, pollen stored at 0 ^{0}C could set 36% fruits, while there was no fruit setting with pollen stored at room temperature and 4 ^{0}C (Table 5). These findings are in accordance with Shivana and Johri (1985). They reported that in many plant species *in vitro* germination is positively correlated with ability to set fruits and seeds.

The findings of present investigation have practical implications for kiwifruit production in India. Artificial pollination with stored pollen can circumvent several uncertainties of natural pollination and guarantee adequate pollination in kiwifruit. In conclusion, the results indicate that -20 ^{0}C was the best temperature at which maximum viability of kiwifruit pollen can be retained up to one year and this pollen can be further used for artificial pollination.

Acknowledgement

This work was supported by Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. Facilities provided by the Central Potato Research Institute, India are thankfully acknowledged.

References

Abreu, I. and M. Oliveira, 2004. Fruit production in kiwifruit (*Actinidia deliciosa*) using preserved pollen. *Austral. J. Agri. Res.,* 55: 565-569.

Bomben, C., C. Malossini, G. Cipriani and R. Testolin, 1999. Long term storage of kiwifruit pollen. *Acta Horiculturae,* 498: 105-110.

Ferguson, A.R., 1990. The Genus *Actinidia*. In: *Kiwifruit: Science and Management*. Warrington, I.J. and G.C. Weston (Eds.). The New Zealand Society for Horticultural Science, Auckland, N. Z.

Hanna, W.W. and L.E. Towill, 1995. Long term pollen storage. *Plant Breeding Reviews,* 13: 179-207.

Heslop-Harrison, J., Y. Heslop-Harrison and K.R. Shivanna, 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical Applied Genetics,* 67: 367-375.

Holocroft, D.M. and P. Allan, 1994. Storage of kiwifruit pollen. *J. Southern African Society Horticulture Science,* 4:21-23.

Miaja, M.L. and G. Me, 1992. Viability and germinability of fresh and stored pollen of *Actinidia deliciosa*. *Acta Horticulturae,* 297: 191-196.

Shivanna, K.R. and B.M. Johri, 1985. *The Angiosperm Pollen Structure and Function*. Wiley Eastern Ltd. Publisher, New Delhi.

Van der Walt, I.D. and G.M. Littlejohn, 1996. Storage and viability testing of *Protea* pollen. *J. Amer. Society Hort. Sci.,* 121: 804-809.

Visser, T., D.P. De Vries, G.W.H. Wells and J.A.H. Scheurink, 1977. Hybrid tea rose pollen. I. Germination and storage. *Euphytica,* 26: 721-728.

Received: March, 2013; Revised: March, 2013; Accepted: April, 2013