Journal of Agricultural Technology 2012 Vol. 8(7): 2297-2307
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Pollen viability, pollen germination and pollen tube growth of Shogun (*Citrus reticulata* Blanco) under climate variability in southern Thailand

Isma-ae Chelong and Sayan Sdoodee*

Department of Plant Science, Faculty of Natural Resources, Prince of Songkhla University, Hat Yai, Songkhla 90112, Thailand.

Isma-ae Chelong and Sayan Sdoodee (2012) Pollen viability, pollen germination and pollen tube growth of Shogun (*Citrus reticulata* Blanco) under climate variability in southern Thailand. Journal of Agricultural Technology 8(7):2297-2307.

The Shogun (*Citrus reticulata* Blanco) is commonly grown in southern Thailand and made one of important commercial fruit crops of its region. Recently, climate variability has affected the pollen characteristics of the shogun. Pollen viability, pollen germination and pollen tube growth were conducted. Investigation was observed in 2 areas: 1) Yarang district, Pattani Province and 2) Yaha district, Yala Province, during November 2010 to June 2011. Rainfall, air temperature, air humidity, soil moisture and light intensity were recorded. The experiment was designed as completely randomized design (CRD). Result showed that high viability of the fresh dehisced pollen in Yarang and Yaha were 96.47 and 93.01%, respectively, but the viability at Yaha was slightly lower than that of Yarang region. The pollen germination *in vitro* (15% sucrose solution) within 3 hr at Yarang and Yaha were 81.24 and 71.98%, respectively. The germination was lowest within 48 hr storage. The pollen tube growth in to ovary within 2 and 3 hr were observed at Yarang and Yaha, respectively. The climate variability during flowering period at Yaha was higher than that at Yarang. Their suggested that tended to influence pollen viability, pollen germination and pollen tube growth of shogun. Therefore, the effect of climate on fruit setting is needed to investigate further.

Key words: Pollen viability, Pollen tube, *Citrus reticulata* Blanco, Climate, Fruit set

Introduction

The Shogun (*Citrus reticulata* Blanco) is member of tangerine citrus. It is believed that its origin was in Southeast Asia, then it was distributed widely e.g., China, India, and southern Thailand. In current, it is on important economic plant in Pattani and Yala provinces, southern Thailand (Spiegel-Roy and Goldschmidt, 1996; Mukhopadhyay, 2004; Chelong, 2010; Dorji and Yapwattanaphun, 2011). It has specific characteristics with soft waternary juice

* Corresponding author: Sayan Sdoodee; e-mail: sayan.s@psu.ac.th

and sweet taste (Lim, 2009). Normally, shohun is fruit-set by cross-pollination (Faegri and Pijl, 1979; Chelong, 2006, 2012; Chelong and Wunnachit, 2007). Pollen viability, pollen germination and pollen tube growth are important factors on productivity of citrus (Ahmed *et al*., 2007; Sedgley and Griffin, 1989; Godini *et al.*, 1992). The climatic particularizes are factors on pollen characteristics, particularly with temperature affecting pollen grain germination and longevity (Pio *et al*., 2004; Buitink *et al*., 2000; Shivanna *et al*., 1991; Young *et al*., 2004; Aronne, 1999). The night temperatures below 10°C resulted in mango cv. 'Kensington’ pollen grains with a low viability (<50%) but temperature between 15 and 33°C during the phase from meiosis to the pre-vacuolate microspore was optimum for pollen development (70-85% pollen viability) (Issarakraisilal and Considine, 1994). The growth temperatures of grain-sorghum (*Sorghum bicolor* (L.) Moench) at 36/26 8°C significantly decreased pollen production, pollen viability, seed-set, seed yield and harvest index compared to 32/22 8°C (Prasad *et al*., 2006). The effects of drying pollen of the oil palm (*Elaeis guineensis*) and storage under various conditions, oven-drying at 37°C for 2–8 hr followed by storage in a deep freezer proved to be the best method of storing the pollen. Pollen treated in this way and stored for 12 months was capable of producing fruits which were equal in weight and appearance to those produced by fresh pollen (Ekaratne and Senathirajah, 1983). The cultured pollens of Rosaceae family were incubated in dark condition at 25°C for 24 hr and then, pollen germination percentage and pollen tube length were evaluated using light-microscope. Results showed significant differences among cultivars and finally favorable cultivars/genotypes of each genus with high pollen germination percentage and tube growth (Sharafi, 2011). Perveen and Khan (2008) studied pollen germination of *M. pumila* L., beyond 48 weeks in the refrigerator (+4°C), freezer (-20°C, -30°C) and freeze drier (-60°C) in different concentration of sucrose and boric acid solution; results showed that pollens stored at low temperature had higher germination percentage compared to pollens stored at +4°C; and in fresh pollen also, freezer dried pollen (-60°C) showed the highest germination percentage. The neck orange (*Citrus reticulata* Blanco) in south of Thailand, is blooming in summer and had a high initial pollen viability of 90.1% that decreased slightly to 62.1% after 48 hr storage. The pollen penetrated the ovary within 3 hr with the highest peak at 48 hr. The receptivity of the pistil to pollen penetration was the highest within 3 hr of the flower opening (Chelong and Wunnachit, 2007) The objective of this work was to determine pollen viability, pollen germination and pollen tube growth of shogun under climate variability in different regions in southern Thailand.

Materials and methods

The pollen viability, pollen germination and pollen tube growth of shogun (*Citrus reticulata* Blanco) were conducted in the farmer orchards at the Yarang district, Pattani Province and Yaha district, Yala Province (Fig. 1) during November 2010 to June 2011. The 8 years old of shogun trees (4×6 m spacing) was used for as a study. The experiments was designed completely randomized design, therefore, 20 trees were used in each location.

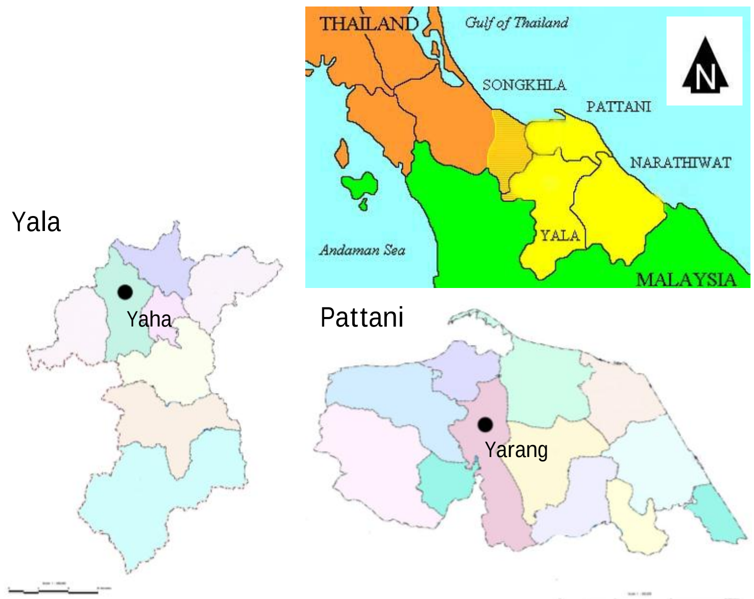

Fig. 1. Location of Pattani and Yala provinces, southern Thailand

Pollen viability

The flowers (1 hr before anthesis) were collected and placed in the Petri dishes. Then, anthers were sampled and squashed on glass slides. Pollens were stained with acetocarmine, then, immediately covered with cover slips. At 0, 3, 6, 9, 24 and 48 hr after staining, the pollen viability was estimated by counting the numbers of stained pollen grains on 5 points per slide 5 replications were used by Stereo microscope and compound microscope. The percentage of pollen viability was calculated by

$$\%\text{Pollen viability} = \frac{\text{No. of stained pollen} \times 100}{\text{Total of pollen}}$$

Pollen germination

The pollen were incubated on germination culture medium including 15% (w/v) sucrose, 50 ppm boric acid, 100 ppm calcium nitrate (CaNO3) and solidified with 1% (w/v) agar. Pollens were spreaded uniformly on the germination medium in 6 cm diameter Petri dishes. At 0, 3, 6, 9, 24 and 48 hr (15 samples were used) after incubation, germinated pollens were counted when the pollen tube length exceeded twice the pollen diameter. 5 randomed microscopic fields were counted in 5 Petri dishes.

Pollen tube growth

Pollen tube growth was carried out by hand cross-pollination. The flowers were emasculated in the morning before anthesis. Then, flowers were covered with paper bags. When anthesis, flowers were pollinated and re-covered with paper bags again. At 0, 3, 6, 9, 24 and 48 hr (15 samples were used each time) after pollinated and immediately fixed in Carnoy's. The pistils were softened for more than 1 hour in 0.1N NaOH at 60°C and then stained with 0.1% (w/v) decolorized aniline blue in 0.1MK_3PO_4. They were laid on a glass slide, and then gently squashed with a coverslip. Pollen tubes were observed using a fluorescence microscope. Pollen grains were counted on the stigma and pollen tubes were counted in style.

Climatic factor

Data of rainfall, air temperature, air humidity, soil moisture and light intensity were recorded by a data logger at the both sites of shogun orchards.

Experimental design and data analysis

The experiment was carried out as a completely randomized design (CRD). Data were analyzed using SAS software and comparison of means was carried out with Duncan's multiple range tests.

Results and discussions

Pollen viability

At Yarang, the fresh dehisced pollen after storage at 0, 3, 6, 9, 24 and 48 hr were 96.47, 87.54, 80.21, 71.87, 40.25 and 17.13%. Which at Yaha, were 93.01, 82.87, 74.25, 64.36, 38.58 and 11.57%, respectively. The pollen viability at Yaha was slightly significant ($P < 0.001$) lower than that at Yarang region (Fig. 2, 5A).

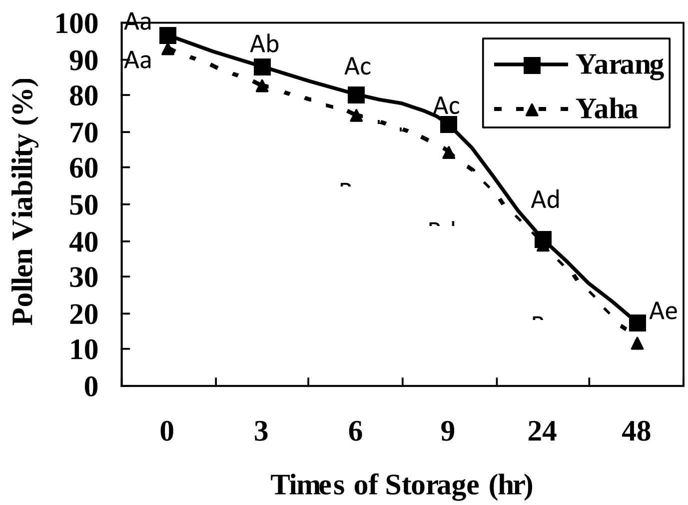

Fig. 2. Percentage of pollen viability at different times storage)0, 3, 6, 9, 24 and 48 hr at(Yarang and Yaha districts.
*Means with the different first letters)same times storage and(second letters)same district (are significantly different (*P Ò*)0 . 0 1

Pollen germination

The pollen germination after storage at 0, 3, 6, 9, 24 and 48 hr in Yarang were 81.24, 74.86, 62.41, 44.63, 15.54, 4.34% and Yaha were 71.98, 64.71, 53.14, 38.64, 9.47 and 1.68%, respectively (Fig. 3, 5B).

Pollen tube growth

The pollen tube growth and reached in to the ovary at 0, 3, 6, 9, 24 and 48 hr in Yarang were 0.00, 15.71, 30.16, 47.85, 52.87, 56.13% and Yaha were 0.00, 13.03, 24.80, 38.65, 40.17 and 42.83%, respectively (Fig. 4, 5C).

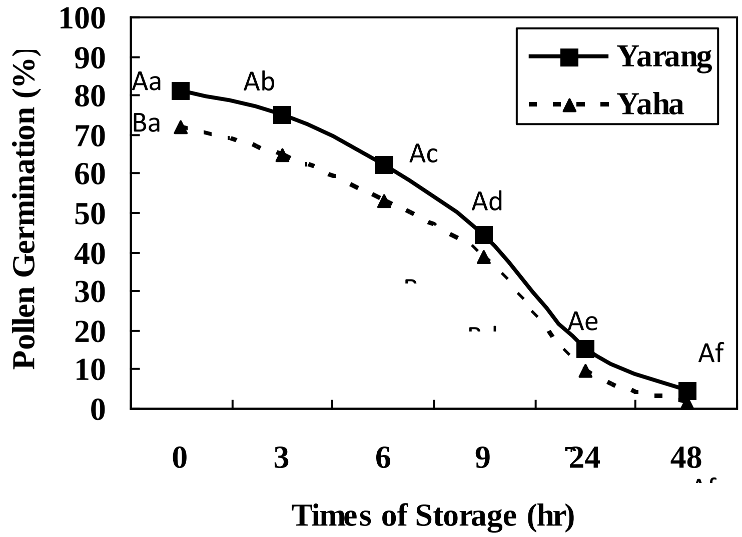

Fig. 3. Percentage of pollen germination at different times storage) 0, 3, 6, 9, 24 and 48 hr at(Yarang and Yaha districts.
*Means with the different first letters)same times storage and(second letters)same district (are significantly different (*P* Ò)*0 . 0 1*

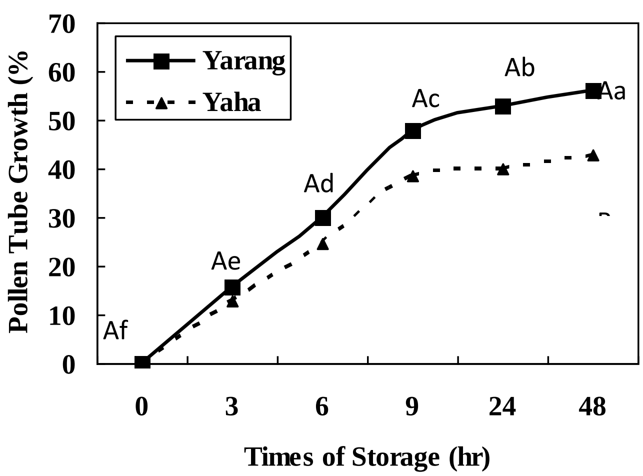

Fig. 4. Percentage of pollen tube growth at different times storage)0, 3, 6, 9, 24 and 48 hr at(Yarang and Yaha districts.
*Means with the different first letters)same times storage and(second letters)same district (are significantly different (*P* Ò)*0 . 0 1*

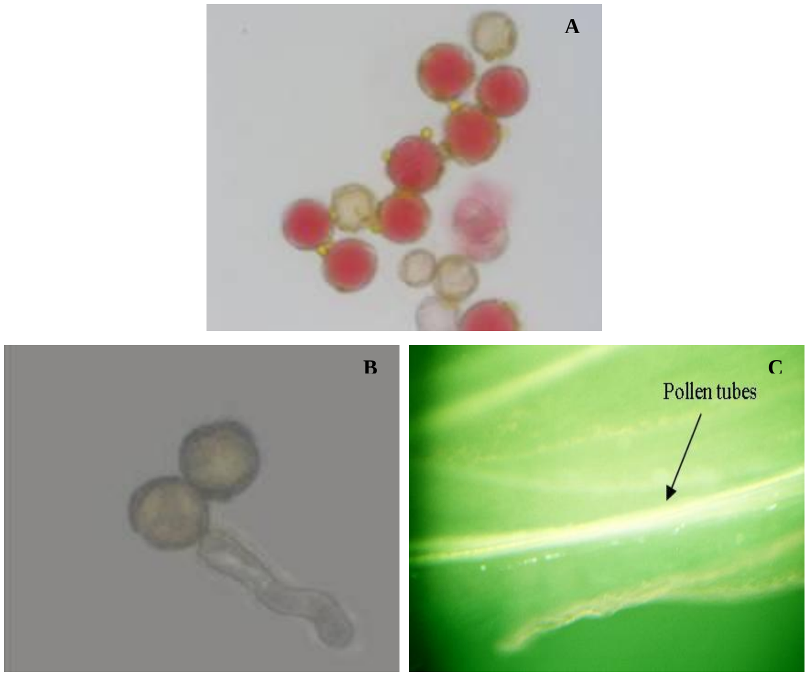

Fig. 5. Pollen viability (A), pollen germination (B) and pollen tube growth when it penetrated Through style (C), under microscopic.

Climatic factors

Monthly rainfall, soil moisture and air humidity in Yarang and Yaha trended to fluctuate with decreasing between January-April 2011 (Fig. 6, 8 and 9). In southern Thailand, flowering of shogun occurred in summer. During the study period that was high temperature and light intensity but low rainfall (Fig. 6, 7 and 10), relative humidity and soil humidity, leading to stress induction. The climates are important factors for pollen viability, pollen germination and pollen tube growth (Fig. 2, 3 and 4). Currently, climate has influenced on pollen development and pollen abortion. This led to the number of pollen germination and pollen tubes being reduced after examination. Chakrabarti *et al.*, (2010) reported that increased temperature due to global warming may reduce pollen germination and induce spikelet sterility in rice crops. Anthesis is the most sensitive stage in rice and exposure to high temperature during this period may cause reduction in floral reproduction. Increased temperature caused different effects on different rice varieties. The germinated pollen grains reduced 13 times when the temperature slightly increased over the optimal (Pressman *et al.,* 2002). Johkan *et al*., (2010) reported that crop germination and growth rely on optimal temperatures during the period of greatest growth rate (Datta *et al.,* 2011). Therefore, non-optimal temperatures reduced the growth rate or stop growth altogether. The limiting temperatures for growth are minimum and maximum temperatures, and these vary among crops, crop varieties (Prasad *et al.,* 2006; Shivanna *et al.*, 1991) and among different growth stages in the same crop. In particular, temperature strongly affects crops during their reproductive period, from pollen formation to fertilization. Low or high temperatures during this period can prevent crop fertilization and cause seed abortion (Thompson, 1975; Aerts *et al.*, 2004).

However, this effect depends upon the species, location and the strength of the climate stress. Deleterious effects of climate condition on pollen viability, pollen germination and pollen tube growth could pose a further threat to shogun production.

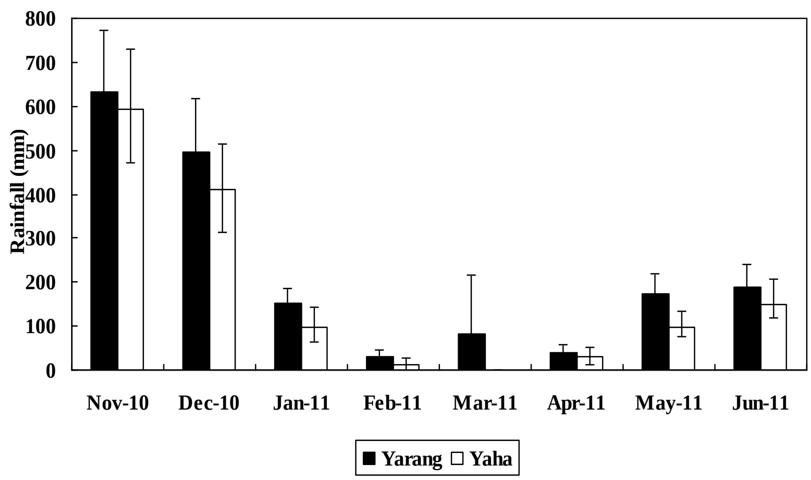

Fig. 6. The monthly rainfall at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

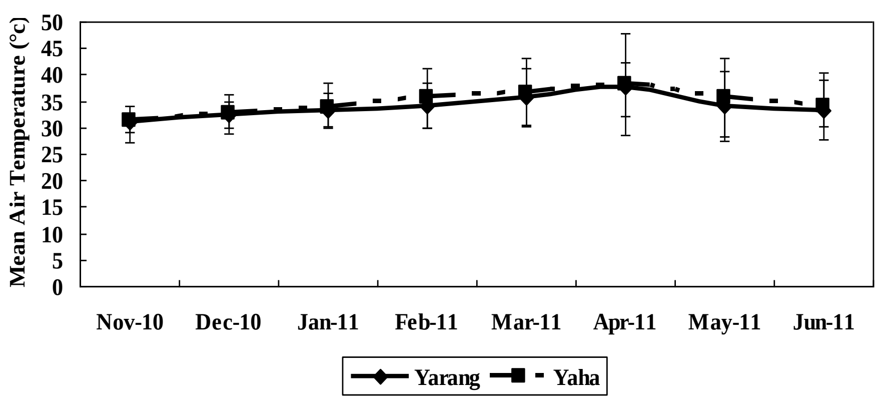

Fig. 7. The mean air temperature at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

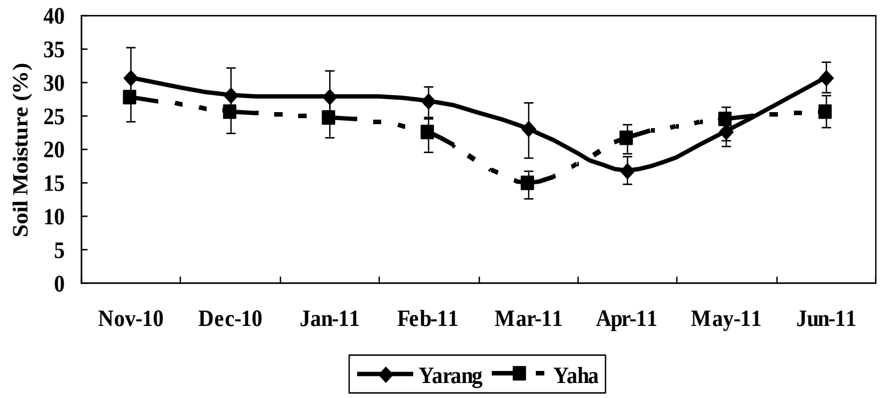

Fig. 8. The soil moisture at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

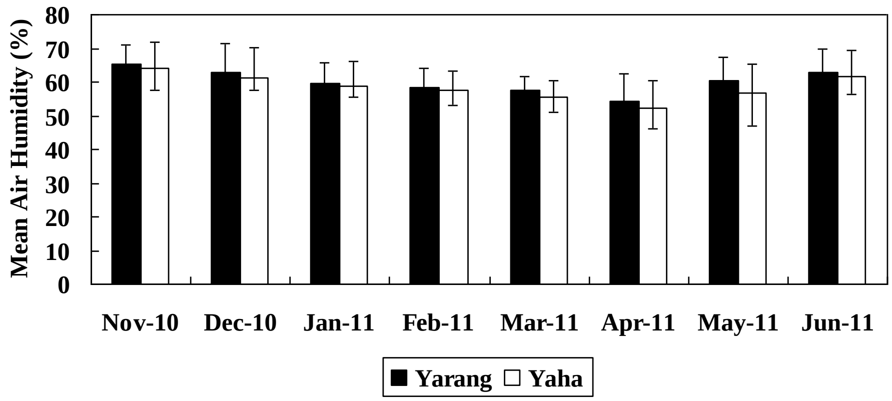

Fig. 9. The mean air humidity at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

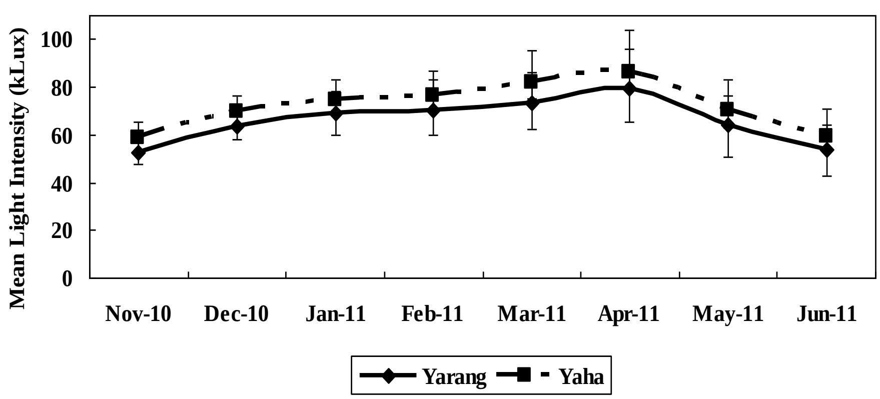

Fig. 10. The mean light intensity at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

Acknowledgements

This work was by financial supported strategic scholarships fellowships frontier research networks 2011 (specific for southern region and Thailand Research Fund - TRF. Department of Plant Science, Faculty of Natural Resources, Prince of Songkhla University, HatYai, Songkhla Province also provided the facility.

References

Aerts, R., Cornelissen, J.H.C., Dorrepaal, E., Van Logtestijn, R.S.P. and Callaghan, T.V. (2004). Effects of experimentally imposed climate scenarios on flowering phenology and flower production of subarctic bog species. Global Change Biology 1 :01 5 –9196 .0 9

Ahmed, W., Ziaf, K.M., Nawaz, M.A., Saleem, B.A. and Ayyub, C.M. (2007). Studies on combining ability of Citrus hybrids with indigenous commercial cultivars. Pak. J. Bot. 39:47-55.

Aronne, G. (1999). Effects of relative humidity and temperature stress on pollen viability of *Cistus incanus* and *Myrtus communis*. Grana 38:364-367.

Buitink, J., Leprince, O., Hemminga, M.A. and Hoekstra, F.A. (2000). The effect of moisture and temperature on the ageing kinetics of pollen: interpretation based on cytoplasmic mobility. Plant, Cell and Environment 23:967-974.

Chakrabarti, B., Aggarwal, P.K., Singh, S.D., Nagarajan, S. and Pathak, H. (2010). Impact of high temperature on pollen germination and spikelet sterility in rice: comparison between basmati and non-basmati varieties. Crop and Pasture Science 61:363–368.

Chelong, I. (2006). Floral biology and pollination of neck orange (*Citrus reticulata* Blanco). M.Sc. Thesis. Prince of Songkla University.

Chelong, I. (2010). Survey of Shogun Propagation and Production. Yala : Yala press.

Chelong, I. (2007). Palynology of cultivated plant species at Yala Rajabhat University, Thailand. KKU Sci. J. 39:433-446.

Chelong, I. and Wunnachit, W. (2007). Floral biology and pollination of neck orange (*Citrus reticulata* Blanco). Thai Agricultural Research Journal 25:58-73.

Datta, J.K., Mondal, T., Banerjee, A. and Mondal, N.K. (2011). Assessment of drought tolerance of selected wheat cultivars under laboratory condition Journal of Agricultural Technology 7:383-393 .

Dorji, K. and Yapwattanaphun, C. (2011). Assessment of morphological diversity for local mandarin (*Citrus reticulata* Blanco.) accessions in Bhutan Journal of Agricultural Technology 7:485-495 .

Ekaratne, S.N.R. and Senathirajah, S. (1983). Viability and storage of pollen of the Oil Palm, *Elaeis guineensis* Jacq. Ann Bot. 51:661-668.

Faegri, J. and Pijl, L.V.D. (1979). The Principles of Pollination Ecology. London : Pergamon Press.

Godini, A., Palma, L. and Palasciano, M. (1992). Role of self pollination and reciprocal stigma / anthers position on fruit set of eight self compatible almonds. Horticultural Science 27:887-889.

Issarakraisilal, M. and Considine, J.A. (1994). Effects of temperature on pollen viability in Mango cv. 'Kensington'. Annals of Botany 73:231-240.

Johkan, M., Oda, M., Maruo, T. and Shinohara, Y. (2010). Crop Production and Global Warming. Graduate school of Horticulture, Chiba University And Graduate school of Life Science and Environment, Osaka Prefecture University, Japan.

Lim, M. (2009). Orange production. Songkhla Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hatyai, Songkhla.
Mukhopadhyay, S. (2004). Citrus: Production, Postharvest, Disease and Pest Management. New Hampshire : Science publishings.
Perveen, A. and Khan, S.A. (2008). Maintenance of pollen germination capacityof *Malus Pumila* L., (Rosaceae). Pak. J. Bot. 40:963-966.
Pio, L.A.S., Ramos, J.D., Pasqual, M., Santos, F.A. and Junqueira, K.P. (2004). Receptiveness of the stigma and *in vitro* germination of orange pollen, submitted to different temperature. Ciênc. Agrotec. 28:1087-1091.
Prasad, P.V.V., Boote, K.J. and Allen Jr., L.H. (2006). Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum (*Sorghum bicolor* (L.) Moench) are more severe at elevated carbon dioxide due to higher tissue temperatures .Agricultural and Forest Meteorology 139:237–251.
Prasad, P.V.V., Boote, K.J., Allen Jr., L. H., Sheehy, J. E. and Thomas, J. M.G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crop Research 95:398-411.
Pressman, E., Peet, M.M. and Pharr, D.M. (2002). The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in developing anthers. Annals of Botany 90:631-636.
Sedgley, M. and Griffin, A.R. (1989). Sexual Reproduction of Tree Crops. London : Academic Press.
Sharafi, Y. (2011). Study of pollen germination in pome fruit tree of Rosaceae family *in vitro.* African Journal of Plant Science 5:483-488.
Shivanna, K.R., Linskens, H. F. and Cresti, M. (1991). Pollen viability and pollen vigor. Theoretical and Applied Genetics 81:38-42.
Shivanna, K.R., Linskens, H.F. and Cresti, M. (1991). Responses of tobacco pollen to high humidity and heat stress: viability and germinability *in vitro* and *in vivo*. Sexual Plant Reproduction 4:104 -109.
Spiegel-Roy, P. and Goldschmidt, E.E. (1996). Biology of Citrus. Cambridge: Cambridge University Press.
Thompson, L.M. (1975). Weather variability, climatic change, and grain production. Science 188:535-541.
Young, L.W., Wilen, R.W. and Bonham-Smith, P.C. (2004). High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion and disrupts seed production. Journal of Experimental Botany 55:485-495.

(Received 24 September 2012; accepted 30 November 2012)