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

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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

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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) (Issarakraisil 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.

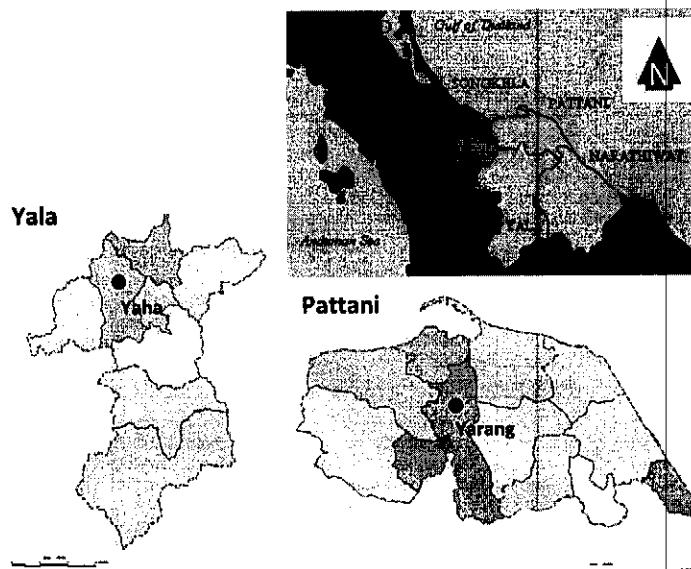


Fig. 1. Location of Pattani and Yala provinces, southern Thailand

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 0.1MK₃PO₄. 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).

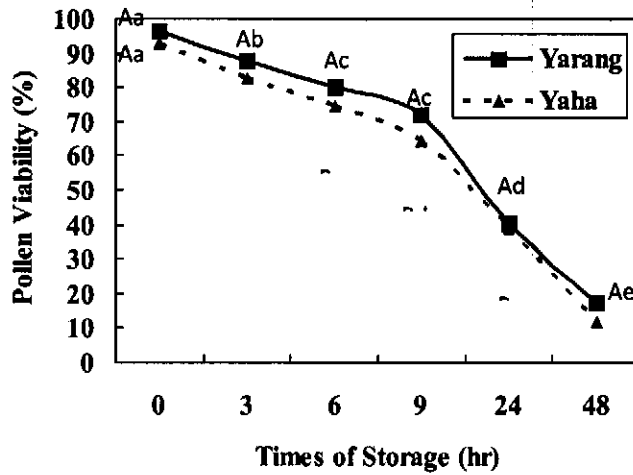


Fig. 2. Percentage of pollen viability at different times storage) 0, 3, 6, 9, 24 and 48 hr (at Yarang and Yaha districts.

*Means with the different first letters)same times storage (and second letters)same district (are significantly different ($P \leq 0.01$)

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).

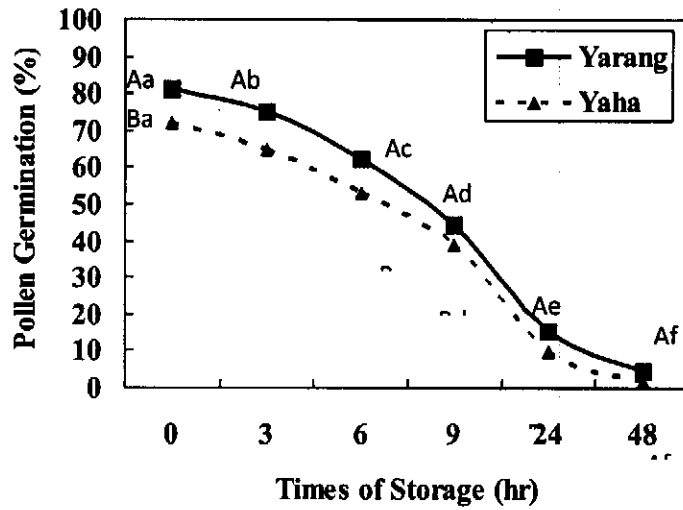


Fig. 3. Percentage of pollen germination at different times storage) 0, 3, 6, 9, 24 and 48 hr (at Yarang and Yaha districts.

*Means with the different first letters)same times storage (and second letters)same district(are significantly different ($P \leq 0.01$)

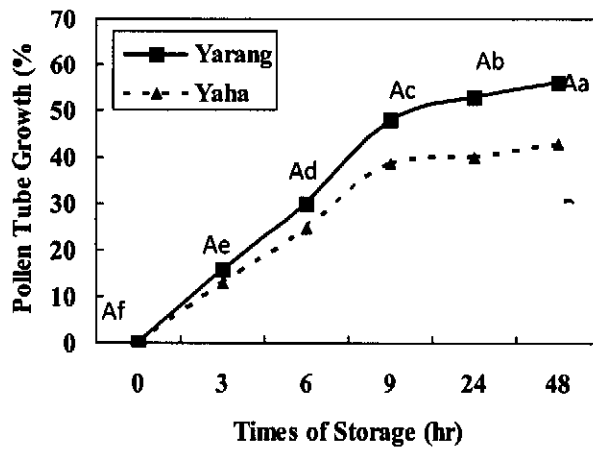


Fig. 4. Percentage of pollen tube growth at different times storage)0, 3, 6, 9, 24 and 48 hr (at Yarang and Yaha districts.

*Means with the different first letters)same times storage (and second letters)same district(are significantly different ($P \leq 0.01$)

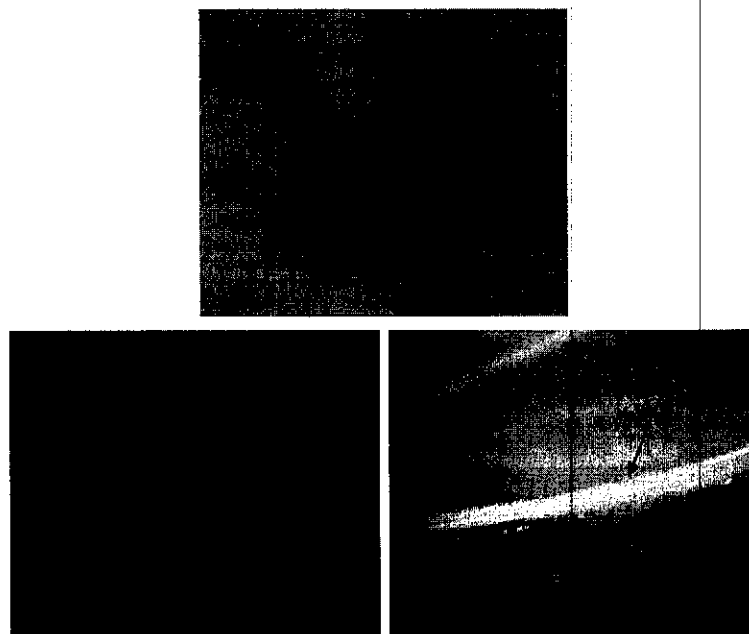


Fig. 5. Pollen viability (A), pollen germination (B) and pollen tube growth when it penetrated Through style (C), under microscopic.

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.

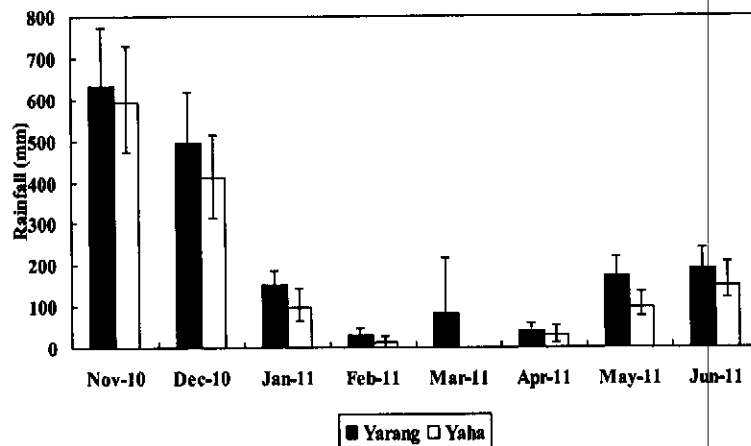


Fig. 6. The monthly rainfall at Yarang and Yaha districts.

*The vertical bars represents the standard deviation of the mean (P = 0.01)

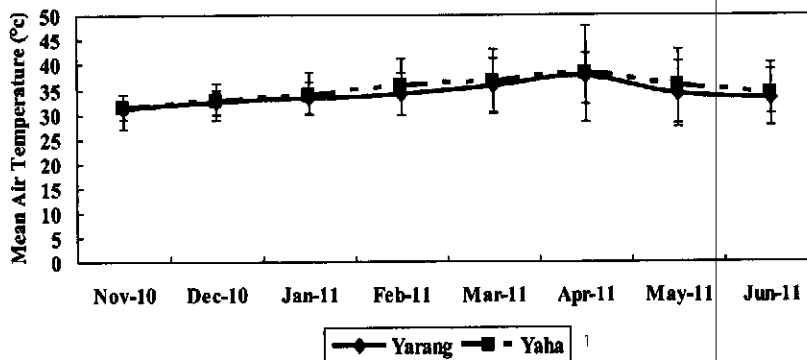


Fig. 7. The mean air temperature at Yarang and Yaha districts.

*The vertical bars represents the standard deviation of the mean (P = 0.01)

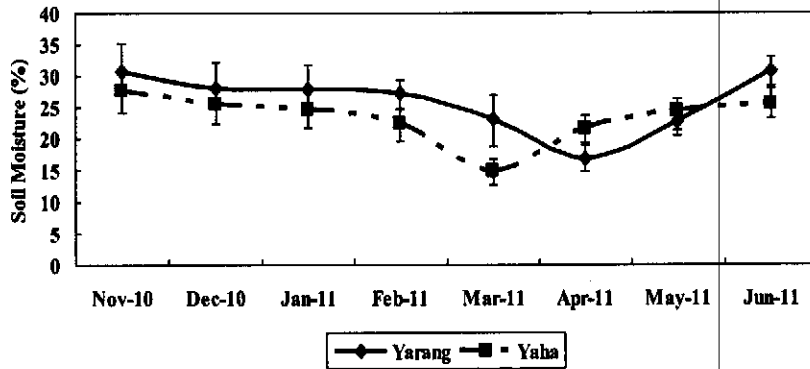


Fig. 8. The soil moisture at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

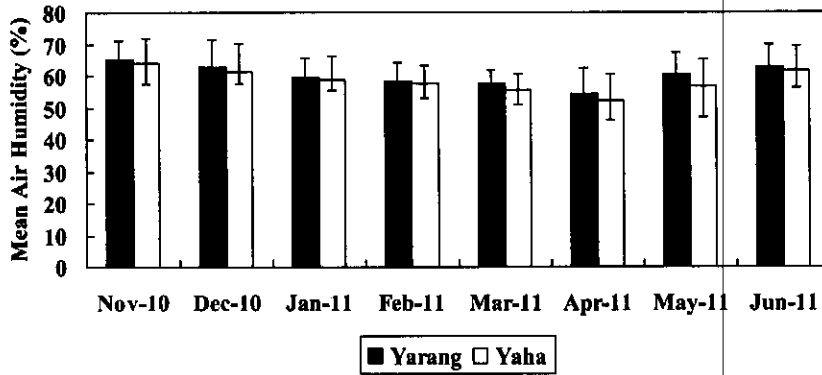


Fig. 9. The mean air humidity at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

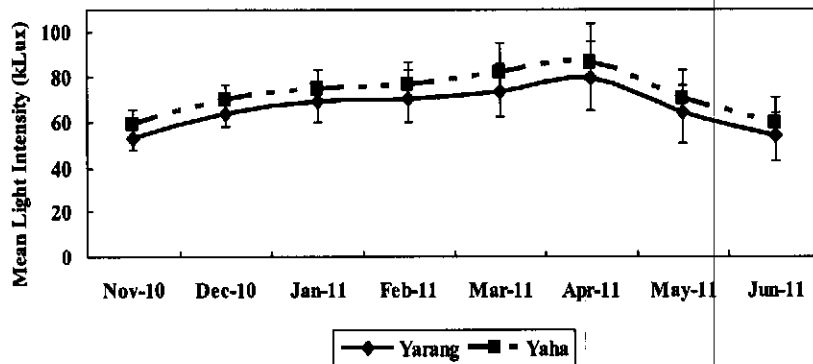


Fig. 10. The mean light intensity at Yarang and Yaha districts.
*The vertical bars represents the standard deviation of the mean (P = 0.01)

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