Kasetsart J. (Nat. Sci.) 30 : 373-377 (1996)

Effects of Prehydration, Sucrose Concentration and Developmental Stage of Anther on Pollen Germination of Chili cv. Kheenuu (*Capsicum frutescens* L. cv. Kheenuu)

Niran Juntawong and Kanita Deepralad [1]

ABSTRACT

Pollen germination of Chili cv. Kheenuu (*Capsicum frutescens* L. cv. Kheenuu) cultured in liquid medium comprising 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2$, 200 ppm $MgSO_4.7H_2O$ and 100 ppm KNO_3 was studied. The result revealed that prehydration of pollen in a humid chamber for 30 min. significantly increased the germination percentage from 7.6 to 48.0. The medium with 15% sucrose (w/v) added had the best result for pollen germination. Moreover, pollen harvested from indehiscent anther germinated better than those from dehiscent anther. Fluorescence micrograph of pollen stained with 0.2% aniline blue fluorochrome showed the callosic layer of pollen tube.

Key words : pollen culture, *Capsicum frutescens*, prehydration, callose, osmoticum

INTRODUCTION

The essential function of pollen grain and tube emerging is to delivery two male gametes into the embryo sac of angiosperm. The haploid male gametophyte that produces and conveys the gamete is structurally simple, but the attainment of its functional objective demands considerable physiological sophistication, expressed in a complex adaptations associated with dispersal, interaction with stigma and style, nutrient and growth. Heslop-Harrison (1987) reported that pollen of some species showed low germinability in culture media because during the final period of maturation in anther pollen grains undergo rapid dehydration. In *Carex ovalis*, maximum germination of pollen is obtained only when it is conditioned for 30 min. in an atmosphere of 95% relative humidity (Shivanna and Heslop-Harrison,1981). Another important factor is carbohydrate or sugar. Role of carbohydrate or sugar on the germination of pollen is a much debated question. So far as known, sugars may regulate the osmotic pressure as well as supply nutrition to the growing tube (Vasil, 1987) and its concentration for pollen germination varies among species. In Chili cv. Cheefaa (*Capsicum annuum* L cv. Cheefaa.), 25% sucrose significantly increased germination of pollen (Juntawong, 1994). Developmental stage of anther has an effect on germination of pollen. Furthermore, Juntawong (1994) found that pollen from indehiscent anther germinated better than those from dehiscent anther in dark condition and concluded that its germination was restricted by light. Nevertheless, preliminary study still showed a very low germination

[1] Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

percentage of pollen. Aim of this investigation is to study effect of prehydration, sucrose concentration and developmental stage on pollen germination of Chili cv. Kheenuu (*Capsicum frutescens* L. cv. Kheenuu).

MATERIALS AND METHODS

1. **Culture medium and experimental design** : Liquid medium was prepared by following the method of Brewbaker and Kwack (1963). It consisted of 100 ppm H3BO3, 300 ppm $Ca(NO_3)_2$, 200 ppm $MgSO_4.7H_2O$ and 100 ppm KNO_3. The pH of the medium was adjusted between 6.6-6.8. The hanging-drop technique was utilized for culturing the pollen grains in the liquid medium. Then, the pollen was allowed to grow in darkness for 3 hours. The experiments were run in Completely Randomized Design (CRD) with 5 replications. For each germination test, random counts of 500-1,000 pollen grains from 10 different fields on the slide were carried out under light microscope. Air temperature and relative humidity were 30°-32° Celsius and 40%, respectively.

2. **Effect of prehydration** : Collected pollen from dehiscent anther was divided into two groups. For the first group, the pollen was placed on a petridish and was prehydrated in a closed chamber of 95% relative humidity. For the second group, the pollen was placed on a petridish in an opened-air chamber (control). Both groups were kept at room temperature for 30 min. After that, the pollen was mixed with a few drop of culture medium with 15% sucrose (w/v). Germination of pollen from both groups was observed and counted under light microscope.

3. **Effect of sucrose concentration** : Sucrose with different concentrations of 0, 5, 10, 15, 20 and 25% (w/v) were prepared and each was added to medium solution. Pollen collected from dehiscent anther was placed on a petridish in a closed chamber of 95% relative humidity for 30 min. Subsequently, following the hanging-drop technique, pollen was mixed with a few drop of culture medium. After 3 hours, the germination percentages were recorded.

4. **Effect of developmental stage of anther** : Pollens from indehiscent, young dehiscent and from old dehiscent anther were collected from mother plants in the field at 10.00 am. and were transferred directly to the culture medium with 15% sucrose (w/v) for germination test as described in 1.

5. **Pollen tube structure** : One drop of pollen solution cultured in medium with 15% sucrose (w/v) for 60 min. was mixed with 0.2% aniline blue fluorochrome. Callose formation was observed under Zeiss fluorescence microscope with a combination of filter sets No. 487702. As light source, an Osram HBO 200 W High Pressure Mercury Lamp was used.

RESULTS AND DISCUSSIONS

1. **Effect of Prehydration** : The T-test analysis revealed a significant difference of germination percentage between the prehydration and the control (Table 1). The pollen as shed without prehydration had very low germination. Compared to the control, the prehydration promoted germinability from 7.5 to 48.0%.

This experimental result confirmed the work of Stanley and Linkskens (1974). They also reported that maximum germination was obtained only when it had been conditioned for a period of time in a humid atmosphere. Heslop-Harrison (1987) informed that pollen of some species showed low germinability in culture media because during the final period of maturation in anther pollen grains underwent rapid dehydration. In that condition

Table 1 Germination percentage of pollen of Chili cv. Kheenuu (Capsicum *frutescens L.* cv. Kheenuu) after 30 min. prehydration in an atmosphere with 95% relative humidity and subsequently cultured in medium with 15% sucrose for 3 hours in dark.

Treatment	Germination (%)
Prehydration	48.00
Without prehydration (control)	7.50
t-test(cal.)	12.68**
t-test(0.01,98)	2.63

membrane of dehydrated grains were ineffective as osmotic barriers. Prehydration caused the stabilization in dehydrated grains and protected the pollen cell from osmotic shock. Shivanna and Heslop-Harison (1981) showed that prehydration of *Carex ovalis* pollen with 95% relative humidity for 30 min. induced germination of pollen upto 90%. This requirement is considerable important for breeder, and practice of storing pollen before use in humid chambers to improve germination.

2. **Sucrose concentration** : The germination of pollen in the medium containing 0, 5, 10, 15, 20 and 25% sucrose were 6.5, 17.2, 26.2, 31.0, 4.5 and 1.7%, respectively (Fig. 1). The variance analysis showed a statistical difference between treatments ($P<0.001$). Maximum germination of pollen cultured in medium with 15% sucrose was recorded.

Effect of sucrose on germination of pollen is still discussed. However, in this experiment the evidence of bursting indicated that pollen germinated readily in medium with appropriate osmotic balance of 15% sucrose. Sucrose concentration lower than 15% resulted in the bursting of pollen. Moreover, plasmolysis of the pollen was found in medium containing sucrose more than 15%. Furthermore, germination of pollen varied among the species of *Capsicum*. The

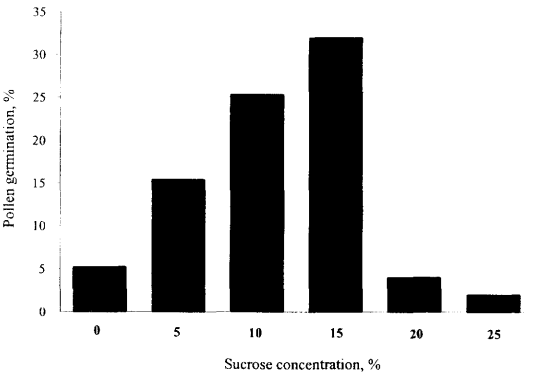

Figure 1 Germination percentage of pollen of Chili cv. Kheenuu (*Capsicum frutescens L. cv. K*heenuu) after prehydration in an atmosphere of 95% relative humidity for 30 minutes. and subsequently cultured in Brewbaker and Kwack medium with different sucrose concentrations for 3 hours in dark.

maximum percentage of pollen germination of Chili cv. Cheefaa was observed at 25% sucrose (Juntawong,1994), whereas, in this experiment it attained maximum germination at 15%. As for energy source, Vasil (1987) reported carbohydrate content in pollen was particularly variable. Although the starch content in pollen of *Typha latifola, Pinus*

thunbergii and *Lilium auratum* was 13, 2.6 and 1.4%, respectively, these reserves are not adequate for pollen tube growth. Effect of sucrose is not only on germination percentage but also on tube length. Pollen of *Hippeastrum aulicum* formed tube length 17-22 mm. in medium with 1% sucrose without bursting of the tube. In contrast, with 25-0.50% sucrose tube length was 7-8 mm. It was suggested that the favorable effect of sucrose, in comparison to other sugars, was due to the presence of bound β-D fructofuranose in the sucrose molecule (Vasil, 1987).

3. **Effect of developmental stage of anther :** Pollen grains from three developmental stages of anther were studied for germination. The result in Fig. 2 showed that the developmental stage of anther had a great influence on pollen germination. The germination of pollen from indehiscent, young dehiscent and mature dehiscent anther were 25, 7 and 5%, respectively. Analysis of variance revealed that there were statistically difference between the treatments ($P<0.001$). The germination of pollen from indehiscent anther was found to be significantly higher than those from young dehiscent and old dehiscent anther ($P<0.001$).

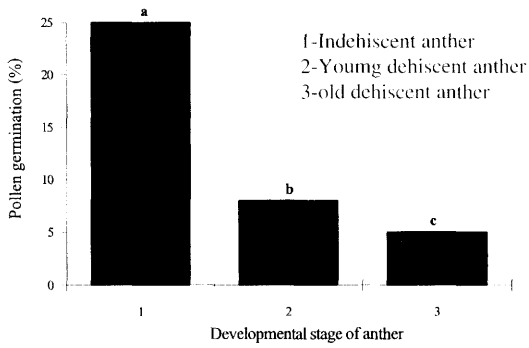

Figure 2 Germination percentage of pollen of Chili cv. Kheenuu (*Capsicum frutescens L.* cv. Kheenuu) collected from indehiscent (1), young dehiscent (2) and old dehiscent anther (3) and cultured in Brewbaker and Kwack medium comprising 15% sucrose for 3 hours in darkness. Different letters on histogram indicates the values are significantly different by Duncan's New Multiple Range test at confidential level 99%.

The result that young pollen germinated more than mature pollen can confirm the hypothesis that developmental stage of anther throughout the day has effects on pollen germination. As stated by Linkskens (1967), under dry condition mature pollen from dehiscent anther underwent hydration rapidly. Moreover, light also has effect on the pollen germination of some plant species (Vasil, 1987). As shedding, exposure of pollen to light decreases its viability (Stanley and Linskens, 1974). In Chili cv. Cheefaa the germinability of is also strongly restricted by light, (Juntawong 1994). Collecting time and radiation could also exert a negative influence on pollen germination. Apart from genetic and physiological differences in plant themselves, the time of collection of pollen grains and temperature and humidity in the field are largely responsible for variations. On a warm day, if the pollen is collected even a few hours after dehiscence, it becomes desiccated and loses viability (Heslop-Harrison, 1987).

4. **Pollen tube structure :** By its affinity for aniline blue fluorochrome, callose layer of the tube was defined (Fig. 3). It was found that the zonation of callose located from the oldest part to the tube apex. This showed that the insertion of callose occurred in wall-thickening area.

Heslop-Harrison (1987) reported that older pollen-tube walls from proximal to an extending tip of grasses consistently showed a three-layered structure. They constituted (1) an outer microfibrillar pectic coating, (2) a cellulosic middle layer and (3) an inner callosic sheath. At the older part of the tube the callose of the wall appeared homogeneous in fluorescence microscope,

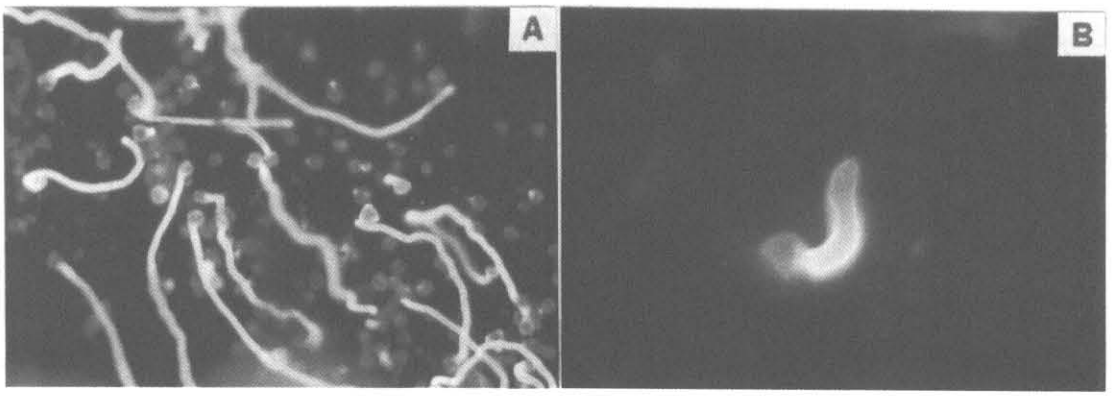

Figure 3 Fluorescence micrograph of pollen tubes of Chili cv. Kheenuu (Cap*sicum frutescens L. cv.* Kheenuu) with decolorized aniline blue staining for callose (A). The callosic layer is defined along the tube to an apex area (B).

generally in the part of the tube where the wall was thickening. Since the transmitting tracts of most stigmas and styles had little or no callose in their wall, this staining technique provided a simple and very effective way of detecting pollen tubes in situ.

CONCLUSION

From the study results it can be concluded that in Chili cv. Kheenuu (*Capsicum frutescens* L. cv. Kheenuu) prehydration technique, sucrose concentration and developmental stage of anther had great effects on germinability of pollen. After prehydration for 30 minutes. the germination of pollen was increased from 7.58 to 48.03%. They attained maximum germination in culture medium of Brewbaker and Kwack (1963) with 15% sucrose. Moreover, the germination of pollen from indehiscent anther increased significantly upto 25%. Callose layer along the tube, particularly at the wall-thickening areas was also found.

ACKNOWLEDGEMENT

The author wishes to thank the Kasetsart University Research and Development Institute for providing the grant to carry out this research work (grant no. 5.38).

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