

# RECEPTIVENESS OF THE STIGMA AND *IN VITRO* GERMINATION OF ORANGE POLLEN, SUBMITTED TO DIFFERENT TEMPERATURES

## Receptividade do estigma e germinação *in vitro* do pólen de laranjas submetido a diferentes temperaturas

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### ABSTRACT

The study was carried out in order to evaluate the effect of temperature and *in vitro* stigma receptivity on regeneration of orange cultivar (Valência, Pêra and Natal) pollen. Two experiments were carried out, in the first the ideal temperature of germination was assessed. Pollen was obtained from flowers at the balloon stage and inoculated in culture medium with 10 g L<sup>-1</sup> agar, 200 mg L<sup>-1</sup> boric acid, 100 g L<sup>-1</sup> sucrose, 800 mg L<sup>-1</sup> calcium nitrate, pH adjusted to 6,5 and incubated in a BOD chamber at temperatures of 23, 24, 25, 26 and 27°C during a 24-hour photoperiod. After 12 hours of incubation, the best temperature for pollen grain germination was 25°C for all varieties. In a second experiment, in order to test the receptivity of the stigma, flowers were collected at different flowering stages: small bud, balloon and open flower. The stigmas were by immersion exposed to hydrogen peroxide (peroxidase reaction), 3% for 3 minutes. Through the technique of Zeisler (1938), better results were detected at the balloon stage with 80 to 100% receptivity, while for the open flower the receptivity presented maximum indexes.

**Index terms:** *Citrus sinensis*, Palinology, tissue culture.

### RESUMO

Objetivou-se determinar a melhor temperatura para a germinação de grãos de pólen e avaliar a receptividade do estigma das cultivares cítricas Valência, Pêra e Natal. Para avaliar a temperatura ideal de germinação, grãos de pólen foram obtidos de flores em estágio “balão” e inoculados em meio de cultura constituído por 10 g L<sup>-1</sup> de ágar, 200 mg L<sup>-1</sup> de ácido bórico, 100 g L<sup>-1</sup> de sacarose, 800 mg L<sup>-1</sup> de nitrato de cálcio, pH ajustado para 6,5 e incubado nas temperaturas de 23, 24, 25, 26 e 27°C em estufa tipo D.B.O. e fotoperíodo constante de 24 horas. Após 12 horas de incubação, observou-se que a melhor temperatura de germinação de grãos de pólen é de 25°C para todas as variedades. Para avaliar a receptividade do estigma, foram coletadas flores em diferentes estádios de florescimento: botão pequeno, balão e flor aberta. Os estigmas foram retirados e imersos em água oxigenada a 3% por 3 minutos. A liberação de bolhas de ar indica que o estigma está receptivo. Melhores resultados foram obtidos com a técnica de Zeisler (1938), no estágio de balão com 80 a 100% de receptividade, ao passo que a receptividade para a flor aberta apresentou os índices máximos.

**Termos para indexação:** *Citrus sinensis*, Palinologia, Cultura de tecidos.

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### INTRODUCTION

Brazil is the first world producer of citric fruits. Orange production, mainly for the concentrated and frozen orange juice industry (SLCC) has always been greater compared to the other citrus varieties. In breeding programs for this species, manual pollination is normally carried out in the field. To perform this work, it is necessary to know whether the stigma is receptive at the moment of this pollination.

For successful pollination, the pollen must be transferred to the stigma at the moment that it is receptive. In some cases, the pollen is deposited before the receptive period; and the pollen should remain viable for a period long enough to germinate (STÖSSER et al., 1997). For this to happen the period of receptiveness of the stigma must be known, but in many cases it is easily determined (THOMPSON and BARRET, 1981).

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Chemical tests have been developed to determine the stigma receptiveness and one involves the enzymatic reactions of the peroxidase enzyme, based on the hypothesis that the presence of this enzyme reflects in the receptiveness of the stigma (GALEN et al., 1985). The test is based on staining the stigma with hydrogen peroxide (peroxide water), if the peroxidase enzyme is present many oxygen bubbles form, released by the chemical reaction of the peroxide with the enzyme.

Several studies using the enzyme method to assess the stigma receptiveness were found in the literature. JAWALE et al. (1999) reported that stigma receptiveness in sunflower lasted from 22 hours to three days. JAWALE et al. (1990) studied the garden rose and detected that stigma receptiveness was very low on the first day when the flower opened, and reached maximum between the 4th and 6th day after the flower opened, depending on the cultivar. In quinces, the stigma receptiveness was 19.99% one day before anthesis, 34.99% on the day of anthesis and 12.49% one day after anthesis (SINGH and SIRIVASTAVA, 2000). Harikarunakar and Haripriya (2000) reported that onion stigmas were receptive for six days after anthesis. The same authors observed that the stigma receptiveness in pomegranate varied from two days before to six days after anthesis. No studies on receptiveness in citrus stigma were found.

Several studies have been performed to determine quantitatively and qualitatively the components necessary for the best composition of culture medium in pollen grain germination. However, temperature is another very important factor in the control of the environmental conditions and influences pollen grain germination significantly (Pio, 2002).

*Pinus ponderosa* germination was observed after eight days at 3°C, while at 15°C it germinated in two days and at 30°C it began to germinate in six hours (WORSLEY, 1959). However, Dorman (1976) observed, in studies on *Pinus*, that temperatures between 25 and 26°C were more indicated for pollen germination. The temperatures of 25°C and 30°C would be more suitable for germination of various *Eucalyptus* species (Boden, 1958).

Silva (1996) studied different temperatures for passion fruit pollen germination, 20, 24, 25 and 28°C and obtained best results at 28°C. Ruggiero et al. (1976) observed that yellow passion fruit pollen grains kept at 24 and 25°C began germination 30

minutes after placing in the artificial medium and six hours afterwards there was no more pollen tube formation.

When assessing pear tree (*Pyrus communis*) pollen, Vasilakakis and Porling (1985) observed a reduction in germination below 15°C, while the growth rate of the *in vitro* pollen increased when the temperature was between 5 and 25°C.

Rosell et al. (1999) observed that the optimum temperature for cherimoya (*Annona squamosa*) pollen grain germination ranged from 20 to 25°C. In experiments performed with kiwi (*Actinidia chinensis*) pollen grains, Boden (1958) observed that the ideal temperature was 27°C. Tuinstra and Wendel studied sorghum (*Sorghum bicolor*), and reported that pollen germination was not affected in the interval between 20 and 40°C, but germination was significantly reduced at 10°C. No report was found in the literature regarding temperature for citrus pollen grain germination.

The objective of this study was to determine the best temperature for pollen grain germination and stigma receptiveness in the citrus cultivars Valência, Pêra and Natal.

## MATERIAL AND METHODS

To carry out the stigma receptiveness experiment, flowers were collected at different stages of flowering: small buds (less than 1 cm), balloon (larger than 1 cm) and recently opened flowers, of the orange tree cultivars Valência, Pêra and Natal, in the orchard at EPAMIG, Lavras-MG. The collection was made in September 2002, from five-year-old plants. Fifty flowers were collected, at each stage, in all the quadrants of the plant, using two plants for each variety.

The flowers were taken to the Plant Tissue Culture Laboratory at the Department of Agriculture, UFPA, to detect the receptiveness of the stigma. The flower structures were removed, leaving only the stigma that was immersed in a solution with oxygenated water at 3% for three minutes, following the technique described by Zeisler (1938). By this technique, the direct action of the oxygenated water with the enzyme present is detected in the receptive stigmas. The release of oxygen bubbles indicates that the stigma is receptive. The size, color and presence of droplets was also observed on the upper part of the stigma and the permanence of pollen on it.

In the experiment to assess the best germination temperature, a completely randomized block design was used, consisting of four replications, and each replication was represented by one Petri dish and 100 pollen grains counted per replication, with the help of an optical microscope with a 10x lens. Pollen grains were considered germinated whose pollen tube length was greater than the diameter of the pollen grains.

The cultivars used were the same as in the previous experiment. Flowers were collected at the balloon stage and taken to the Tissue Culture Laboratory where the anthers were removed and placed on petri dishes with filter paper and kept at 26°C for 24 hours to complete the dehiscence. The culture medium used for germination was 10 g L<sup>-1</sup> agar, 200 mg L<sup>-1</sup> boric acid, 100 g L<sup>-1</sup> sucrose, 800 mg L<sup>-1</sup> calcium nitrate and pH adjusted to 6.5, Pio (2003). The culture medium was heated in a microwave oven to 95°C, then 10ml was poured on to a Petri dish. The pollen from each cultivar was sprinkled on the surface of the culture medium using a paint brush, so the material was uniformly distributed. The temperatures of 23, 24, 25, 26°C were tested, obtained in a BOD chamber with a 12 hour light period.

After 12 hours incubation, the percentage of germinated pollen grains was counted. The data observed were submitted to statistical analysis using the Sisvar software.

## RESULTS AND DISCUSSION

At the balloon stage, 80 to 100% of the flowers were receptive, observed by the presence of bubbling verified visually after contact of the stigma with oxygenated water, indicating the presence of the enzyme. This value was 100% For the open flowers. The small buds did not show receptiveness (Table 1).

For successful fertilization, it is desirable that the pollen is transferred to the receptive stigma of another flower. In many cases, fertilization can occur when the pollen grain is deposited before the receptive period as long as it remains viable long enough to be able to germinate as soon as the flower becomes receptive (THOMSON AND BARRET, 1981).

**TABLE 1** – Percentage of receptiveness of the stigma in flowers of citric varieties in three stages of development. UFLA, Lavras, 2002.

Cultivars	Small Bud (%)	Balloon Bud (%)	Open Flower (%)
Natal	0	80	100
Valência	0	100	100
Pêra	0	100	100

**Mean of 50 flowers analyzed for each treatment.**

The enzymatic activity of the peroxidase, in this study, was greater in open flowers, and can increase fertilization success. The flowers at the balloon stage also presented activity of this enzyme. This data is important for the breeder, because it allows him to make the crossings in this phase, when the flowers do not need to be bagged to prevent previous contamination by undesired pollen. The index of self pollination at this phase is not significant, because the anthesis occurs only after flower opening.

Table 3 shows the table of analysis of variance for the factors studied, variety and germination temperature of pollen grains. There was a significant effect for variety in temperature at the level of 1% probability, and there was no interaction among the factors studied.

In Figure 1A, the best percentage of germinated pollen grains (11.7%) was obtained with the Valencia cultivar. The other cultivars, Pêra and Natal, presented significantly inferior results. This difference among the varieties studied is related to the differences in their genotypes.

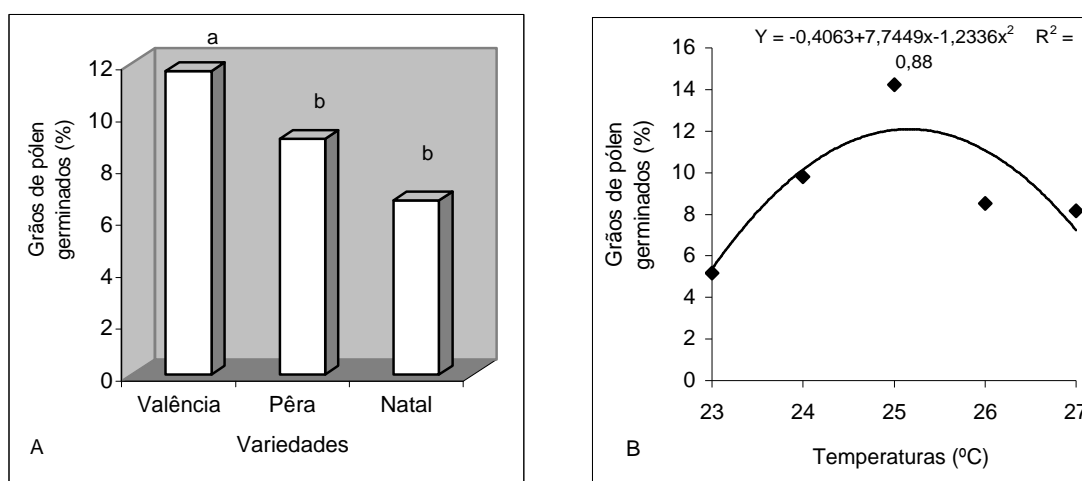
These results are in line with those reported by Vasilakakis and Porling (1985), who obtained greater indices of pollen tube growth at 25°C, in studies carried out with Pêra orange tree pollen.

The optimum temperature for citrus pollen grain germination was 25°C, similar to most of the species quoted in the literature, where the best germination indices occur in the interval of 20 to 30°C. (FARMER JÚNIOR and HALL, 1974; RUGGIERO et al., 1976; VASILAKAKIS and PORLING, 1985).

**TABLE 2** – Stigma characteristics in flowers of the Valência, Pêra and Natal varieties at three stages of development. UFLA, Lavras, 2002.

Parâmetros	Botão Small	Botão balão	Flor Aberta
Size	Small	medium	large
Color	Dark green	Greenish-yellow	yellow
Presence of droplets	absent	present	present
Permanence of pollen on the surface	absent	permanent	permanent

Mean of 50 flowers analyzed for each treatment.

**FIGURE 1** – Mean of germinated pollen grains of the orange tree cultivars (1A) presented and percentage of germinated pollen grains of the orange tree cultivars when submitted to different temperatures (1B). UFLA, Lavras, 2002**TABLE 3** – Analysis of variance for the variable germinated pollen grains of different citrus cultivars submitted to various temperatures. Lavras – MG 2003.

Causes of variation	GL	Mean square
		Germinated (%)
Cultivars	3	109.994**
Temperatures	5	120.661**
Var. X Temp.	7	0,354 ns
Residue	44	
C.V. (%)		24,68
General Mean (%)		9,178

\*\*Significant at the level of 1% probability.

## CONCLUSION

For the Valencia and Pêra cultivars, manual pollination can be performed at the balloon stage. For the Natal cultivar, receptivity was greater in open flowers.

The greater percentage of pollen grain germination was obtained at 25°C for all the cultivars tested. The Valencia cultivar presented a greater percent of germinated pollen grains.

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