GERMINABILITY AND VIABILITY OF RAPESEED POLLEN UNDER THE EFFECT OF TEMPERATURE

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INTRODUCTION

This work lies within the general scope of the study of climatic determinism of the seed number per pod, yield component which explains most winter rapeseed yield variations among cultivars as well as variations from one year to the next (MERRIEN and TRIBOI, 1988).

The transformation from buds to flowers, the subsequent transformation from flowers to pods and the grain number per pod will depend on : the intensity of photosynthetate, of water and of nutrient (N, S) fluxes which enter the floral peduncle ; the number of sinks (pods) simultaneously present on the plant ; the climatic conditions (rain, temperature, radiation) during the flowering.

This number of grains per pod (NG/P) depends on : the fertility of ovules (for the female part) or the proportion of ovules which develops an embryo sac, determined during meiosis or early megaspore differentiation (BOUTTIER, 1990) ; the presence and the viability of pollen (for the male part) on the stigma (MESQUIDA and RENARD 1981, 1984), the stigma-pollen recognition (DUMAS et al., 1984b) and the pollen tube growth (germination) in the style ; and finally the conditions in which the actual fecondation occurs (DUMAS et al., 1984a).

The objective of our study, which lies before the fecondation, is to quantify the effects of the climate, or more particularly of the temperature, on the pollen tube growth rate, and the germinability and viability of "free" pollen from the time of anther dehiscence until the pollen arrives on the stigma.

MATERIALS AND METHODS

Materials

In order to obtain pollen rapidly, short cycle spring rape, clone CRGC 5.11., was used.

In a growth chamber, at 20°C constant temperature, with a photoperiod of 16 hours, and a PAR of 350-400 $\mu mol^{-2}\ s^{-1}$, the plants in pots, with a peat-compost mixture, watered regularly and enriched with mineral nutrients, flower about 30 days after sowing.

Few pots were placed in a greenhouse, the plants flower after 5 months (in February), at a mean temperature of about 10°C, and with a mean photoperiod of 9 hours.

Winter rapeseed (DARMOR and SAMOURAI cultivars) grown in the field, sown early September and which flowers after about 8 months (in April), was also used in our study.

Characteristics of the flowers. The flowering takes place from the base of the branch to the top. We generally numbered 0, the highest open flower, and -1 to -n, older flowers situated below. In order to facilitate comparisons between plants, in the study of the evolution of pollen germinability during ageing, we defined different types of flowers according to their age, in terms of changes of petal habit or shape :

- type 1 = flower not yet open (Y-shape),
- type 2 = last opening flower (T-shape), horizontal petals,
- type 3 = "normal" flower, fully open but Y-shape, flat petals
- type 4 = closed flower, non-wilted petals,
- type 5 = withered flower, wilted and folded up petals.

Methods

Pollen germinability. The method used was a slightly modified version of the "in vitro" germination test of MESQUIDA et al. (1987). The germination medium adopted was that of ROBERTS et al. (1983) : for 1 liter, 100 mg KNO3, 180 mg boric acid, 250 mg Tris (pH 8.8) and 20 % sucrose (freshly prepared). Polyethylen-Glycol (10 % PEG 4000) was added to the medium, in order to modify the osmotic pressure and to induce a slower watering rate of pollen grains. This modification clearly improved the germination percentage, while limiting the rupture of pollen grains when they arrived in the liquid medium. The pollen grains are deposited in 2 drops of germination medium, placed in a watch glass, covered with parafilm and left to incubate at 25°C, for 3 hours.

Pollen Characteristics after "in vitro" germination test. In each watch glass representing the pollen of one anther, three successive counts were carried out in 3 different microscopic fields (X 160). At each reading, we counted between 150 and 250 pollen grains divided into different categories : "germinated grain", when the pollen tube is longer than the grain diameter ; "slightly germinated", when the pollen tube is shorter than the grain diameter ; "ruptured grain" when a globular accumulation is emitted in place of the tube ; "intact grain" when there is no pollen tube emission ; "dead grain" when the grain is round (instead of oval) and smaller.

Pollen Viability. Pollen viability, equivalent, in the present case, to membrane integrity has been estimated in certain cases by a cytological technique called fluorochromatic reaction (FCR test), put forward by J. and Y. HESLOP-HARRISSON (1970) and routinely used on rape pollen by DUMAS et al., (1983). The pollen is laid on a drop of fluorescein diacetate (DAF) disolved in acetone, with sucrose added. The positive results of membrane integrity (fluorescent green pollen grain) were achieved using a OLYMPUS BH2-RFL epifluorescent-microscope.

Temperature effect.

- Pollen having a maximal germinability was submitted to "in vitro" germination test carried out at different incubation temperatures between -4°C and 35°C.

- Freshly opened flowers were submitted to different conservation temperatures for 24 hours (in Petri dishes) between -20°C and 35°C. Next, the pollen germinability was tested using the "in vitro" germination test, at 25°C, the optimal incubation temperature.

Statistical Analyses. The percentages of the different categories of pollen grains, represent the mean of 3 counts per microscope test. Each experiment was repeated at least 3 times. When the analysis of the variances revealed significant effects (F test), we compared the means by the least significant difference (LSD). In all tables, means followed by the same letter within a column were not significantly different at 0,05 probability level.

RESULTS AND DISCUSSION

1. Germinability, Viability and ageing of pollen.

Before measuring the effects of temperature on the quality of pollen, it was necessary to carry out a preliminary study on the natural evolution of the pollen germinability, during ageing, in flowers of different ages. This is the process we performed on plants grown in different climatic conditions (Fig. 1).

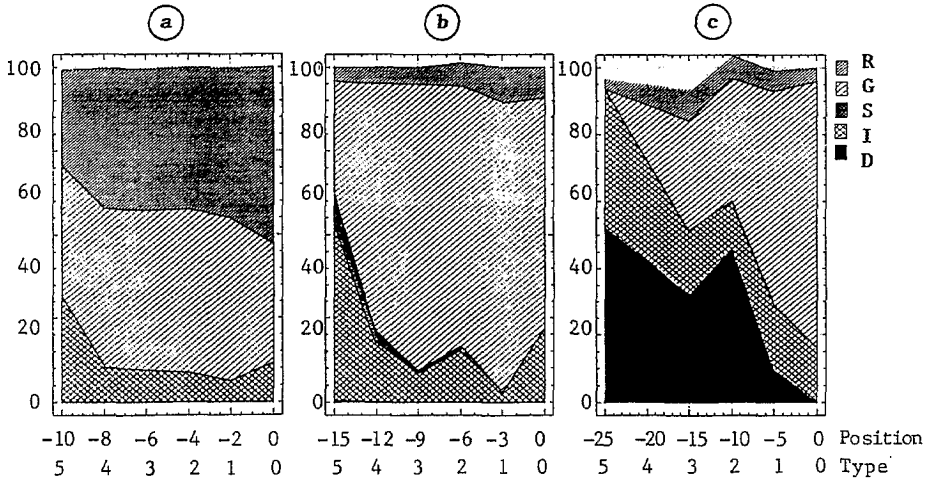

Fig. 1. Pollen Germinability and Flower Ageing, for plants grown in growth chamber (a), greenhouse (b) and field (c).
The relative percentages of ruptured (R), germinated (G), slightly germinated (S), intact (I) or dead (D) grains are indicated.

The percentages of "germinated" or "ruptured" pollen grains (the ruptured grains have a good germinability, but are sensitive to osmotic pressure of the medium) is the highest in the flowers at position 0, or just below, corresponding to type 2 (T-shape). The flowers at position 0 (Y-shape), in growth chamber and greenhouse, possess a little more "intact" pollen than the T-shape flowers, which confirms the protogyne characteristic of rapeseed : pollen maturity delay, compared with flower dehiscence.

When we move towards older flowers, the pollen germinability decreases. This is not very evident in the growth chamber, but here, the 20°C temperatures lead to a very short life span of flowers (2.5 days compared with 5 days in greenhouse). In the greenhouse, approximately 60 % of the pollen lost its germinability in the flower at position -15. In the field, pollen ageing is more evident : the percentage of germinated grains diminishes considerably, and the proportion of intact and dead grains, increases in older flowers (position 0 to -25).

Conclusion. The germinability of pollen grains, in the growth chamber, in the greenhouse and in the field are only slightly different and close to 80 % for flowers at anthesis. The percentage of intact grains (which do not emit a pollen tube) and dead grains (small, circular and showing a negative response to FCR viability test), increases during flower ageing, while the germinability (germinated or ruptured grains) diminishes.

Moreover, we notice that these percentages do not vary in relation to the position of the flower on the branch, according to the type of branch, or the cultivar.

2. Incubation Temperature and Pollen Tube Growth

Table 1. Effect of Incubation Temperature of "in vitro Germination" on Percentages of different categories of Pollen Grains : ruptured (R), germinated (G), slightly germinated (S), intact (I) or dead (D) grains.

Incubation Temperatures	% R	% G	% S	% I	% D
– 4°C	0.0 a	0.0 a	0.0 a	100 c	0
3	3.7 a	0.0 a	48.6 b	47.6 b	0
10	6.5 a	82.7 b	5.3 a	5.0 a	0
25	11.7 a	87.7 b	0.0 a	0.7 a	0
35	84.8 b	1.5 a	12.0 a	0.0 a	2

Means followed by the same letter within a column were not significantly different at 0.05 probability.

At -4°C, there is no pollen tube growth and at 3°C, half of the pollen grains sprout a little pollen tube. Incubation temperatures of 10 or 25°C induce the best pollen germination rate. At 35°C, the grains rupture.

Conclusion. The pollen tube growth is optimal at 10-25°C. Therefore, this temperature zone seems to be more favorable to fecondation.

3. Storage Temperature of Flowers and Germinability

Table 2. Effect of Conservation Temperatures of flowers (24h) on Percentages of different categories of Pollen Grains after "in vitro Germination" : ruptured (R), germinated (G), intact (I) or dead (D) grains.

Conservation Temperatures	% R	% G	% I	% D
− 20°C	8.8 ab	3.1 a	86.8 b	1.3 a
3	42.2 c	37.3 b	19.0 a	1.1 a
10	19.0 b	48.9 b	25.0 a	2.3 a
25	18.0 b	53.0 b	20.0 a	4.3 a
35	0.0 a	0.0 a	0.0 a	100.0 b
Control	43.3 c	53.6 b	3.0 a	0.3 a

Means followed by the same letter within a column were not significantly different at 0.05 probability.

The treatment of flowers at -20°C leads to a loss of pollen germinability. The treatment at 35°C kills the pollen grains. The treatments at 3, 10 and 25°C only slightly modifies the germinability : 20-25 % of the pollen remains "intact", approximately 50 % "germinate" and the others "rupture" in the medium.

Only the "dead" grains showed a negative result to the FCR viability test. The "intact" grains, after the treatment at -20°C were FCR (+). This indicates that pollen germinability is not irreversibly lost ; a rewatering could be sufficient to allow pollen germination.

Conclusion. Temperatures from 10 to 25°C do not affect the germinability or the viability of the pollen. A treatment at 3°C only slightly modifies the pollen quality, but we noted in the previous experiment that these temperatures close to 0°C prevent the pollen tube growth.

The pollen treated at low temperature (-20°C) loses its germinability without any loss of membrane integrity (FCR +). On the other hand, the high temperatures (35°C), cause the pollen to lose its germinability, its viability (or membrane integrity, FCR - test) and its shape changes from oval to circular.

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