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PRELIMINARY RESULTS OF RESEARCH ON STORAGE AND IN VITRO GERMINATION OF LETTUCE POLLEN AS AN AID IN LETTUCE BREEDING

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SUMMARY

Lettuce pollen can be stored at −18 °C for over 30 days without serious loss of viability. This viability was checked through in vitro germination in a liquid nutrient medium containing 40% sucrose, 100 ppm H_3BO_3 and 100 ppm $Ca(NO_3)_2 . 4H_2O$ after rehydration of the pollen for 15–20 minutes in an atmosphere of 100% RH.

INTRODUCTION

Interspecific and intraspecific crossing in lettuce is frequently hindered by asynchronous flowering of the plants. Synchronization can be achieved by growing the genotypes to be crossed under different temperature and light regimes. This procedure, however, is laborious, time consuming and expensive.

Storage of lettuce pollen to be used for pollination of late flowering seed parents might be a solution for the problem of asynchronous flowering. To this end, optimal storage conditions need to be investigated and in addition a reliable in vitro germination test for the viability of stored pollen is required.

In several plant species investigations have been conducted on storage and in vitro germination of pollen (VISSER, 1955; VISSER et al., 1977; HOEKSTRA & BRUINSMA, 1975a; 1975b). HOEKSTRA & BRUINSMA (1975a and b) found that Compositae pollen had often a characteristically limited longevity, frequently occurring in plants with trinucleate pollen. A rapid decrease of viability was found, influenced by plant genotype and environmental conditions during pollen formation, dehiscence and storage.

This paper contains preliminary results from research on in vitro germination and storage of the trinucleate lettuce pollen.

MATERIALS AND METHODS

Lettuce plants of various breeding lines, carrying genes from cultivars such as Plenos, Iceberg, Taiwan and Edgar were grown in a glasshouse in July and August of 1976, 1977, 1978 and 1981 and flowered at a temperature fluctuating between 25 °C (D)

and 15 °C (N), a relative humidity of ⩽ 70% and under natural light conditions. Some experiments were performed in a phytotron at constant temperatures of 14 °C, 17 °C, 20 °C, 23 °C and/or 26 °C, at 70% relative humidity and under natural light conditions.

In the majority of the experiments, unless otherwise stated, flower heads were collected soon after pollen dehiscence when the maximum number of pollen grains is present on the stigma of the composite lettuce flower heads at about 10.30 h. Pollen was collected by vibrating the flower heads above glass slides.

In vitro and in vivo pollen germination. In vitro germination experiments were made in liquid medium with approximately 3 mg pollen, from 25 flower heads, per 300 mg of nutrient medium. Immediately after incubation, the tubes (length 5.0 cm, diameter 2.0 cm) containing the germination medium and pollen were shaken for 15 minutes to improve aeration of the pollen suspension. Similar experiments were performed with aeration via oxygen, guided through the pollen suspension. The two methods gave similar results, therefore the former method was preferred for convenience. Comparable but slightly reduced germination percentages were also obtained by scattering pollen grains over a thin layer of 0.5 mm of nutrient medium with 5% agar on a glass slide. Without aeration of the suspension, germination percentages in vitro were usually extremely poor, e.g. similar to results obtained from the 'hanging-drop' culture in a Van Tieghem-cell.

Rehydration of pollen grains prior to germination is of vital importance to prevent leakage of valuable pollen metabolites (HOEKSTRA & BRUINSMA, 1975a). For this reason each pollen sample was exposed to a RH of 100% by placing the pollen on a slide in a petri dish with moist filter paper.

To establish the optimal conditions for in vitro germination tests, the following treatments were applied:
- Different temperatures before and during dehiscence;
- Pollen collection at different time periods;
- Incubation of pollen grains with and without anthers in nutrient medium;
- Different temperatures during rehydration, and periods of rehydration;
- Different compositions of the nutrient medium with varying quantities of sugars (sucrose, glucose, raffinose, maltose and fructose), H_3BO_3, $Ca(NO_3)_2 . 4H_2O$ and with tap or distilled water as a solvent.

In the majority of the germination experiments three pollen samples were taken to determine the germination percentage per treatment. Each determination consisted of three counts with 100 pollen grains per count. Pollen grains were considered as germinated if the pollen tube length was equal to or greater than the pollen diameter at 30 minutes after the beginning of the germination experiment. The majority of the tubes had twice the length of that diameter. After reaching that length, they stopped growing which is characteristic for trinucleate Compositae species (HOEKSTRA & BRUINSMA, 1975a).

In addition, in vivo pollen germination on stigmas at temperatures of 17 °C, 20 °C, 23 °C and 26 °C was investigated.

Stigmas and pollen were stained with aniline blue and examined with a UV-microscope; 10–40 pistils each containing approximately 30 pollen grains per stigma were examined per treatment.

Storage of pollen. Collected pollen was stored in small glass tubes (length 5.0 cm, diameter 2.0 cm) with approximately 3 mg of pollen per tube. Tubes were placed in glass pots (length 9.0 cm, diameter 4.5 cm) which contained 20 gram of NaOH pellets for dessication and were hermetically sealed The following storage treatments were applied:

- at room temperature (± 20 °C);
- in a refrigerator (± 4 °C);
- in a deep-freezer (± −18 °C) after precooling for 8 h in a refrigerator at 4 °C.

1, 2, 4, 8, 16 or 32 days after storage, pollen grains were rehydrated for 20 minutes at 100% RH prior to incubation in the germination medium of 40% sucrose + 100 ppm H_3BO_3 + 100 ppm $Ca(NO_3)_2.4H_20$.

RESULTS AND DISCUSSION

In vivo pollen germination. In vivo pollen germination, assessed in different years, was usually poor. It usually varied between 0 and 20% of the pollen grains present on the stigmas and never exceeded 50% of all pollen grains on single stigmas (Fig. 1).

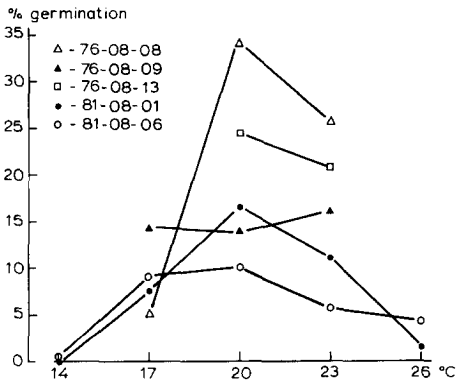

Fig. 1. In vivo germination of lettuce pollen at different temperatures and in different years. Each point is based on 10–40 stigmas.

Table 1. Effect of various concentrations (in ppm) of H_3BO_3 and $Ca(NO_3)_2.4H_20$ in a nutrient medium with 45% sucrose in water on the percentage of in vitro germination in an experiment in 1978. Means are based on 3 samples and 9 counts.

$Ca(NO_3)_2.4H_20$	H_3BO_3 (ppm)				
	25	50	75	100	mean
0	16	10	16	20	16
50	19	27	23	29	25
100	25	24	31	40	30
200	22	30	22	24	25
Mean	21	23	23	28	

These low percentages suggest that in vitro germination percentages may also be poor. The optimum temperature for in vivo germination was approximately 20 °C. It is not yet known whether the non-germinated pollen grains are inviable.

In vitro pollen germination. A part of the in vitro germination experiments suffered from a large error or environmental variation. This interfered with the comparison of the results from similar pollen samples which were collected at different periods of time. Therefore, at least part of the results should be considered with some reservations. A short summary of some experiments with such a relative large variation is given.

In vitro pollen germination was not clearly affected by:

- The sugar type (sucrose, glucose, raffinose, maltose or fructose);
- Distilled or tap water as a solvent;
- The period of shaking of the incubated nutrient solution, which was varied between 15 and 60 minutes;
- Incubation of the nutrient medium with pollen grains only or with pollen including anther tissue. In some experiments the germination percentage increased slightly when anther tissue was present;
- Different periods of rehydration, varying between 15 and 60 minutes at temperatures of 17 °C and 20 °C.

Germination experiments in 1977 and 1981 indicated that sucrose concentrations should be relatively high, varying between 40% and 50% (Fig. 2). This figure also illustrates the large differences in germination percentages between pollen samples taken from the same plants on successive days. Omitting H_3BO_3 and $Ca(NO_3)_2.4H_20$ resulted in a very poor in vitro pollen germination, which was usually 30–75% lower than after application of these components, partly shown in Table 1. The addition of 25–100 ppm H_3BO_3 and 50–200 ppm$Ca(NO_3)_2.4H_20$ to a 45% sucrose solution led to a percentage of in vitro pollen germination comparable to the percentage of in vivo germination. Table 1 shows that the best results were obtained when 45% sucrose, 100 ppm boric acid and 100 ppm $Ca(NO_3)_2.4H_20$ was used.

Figure 3 shows the effect of temperature before, during and after anthesis and the effect of sampling time on pollen germination. Pollen collected at 17 °C, always germinated better than pollen collected at 26 °C. The delay of sampling from 11.00 hrs to

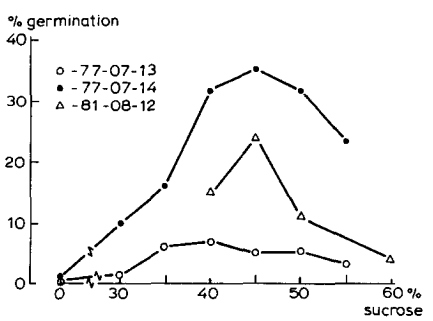

Fig. 2. In vitro germination of lettuce pollen at different sucrose concentrations and pooled for various concentrations of H_3BO_3 and $Ca(NO_3)_2.4H_20$ in two years. Each point is based on 10–20 samples.

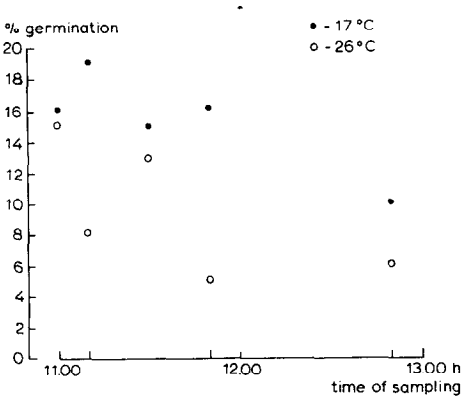

Fig. 3. Influence of time of sampling and temperature during pollen formation and dehiscence on in vitro germination of lettuce pollen in a nutrient medium containing 50% sucrose, 50 ppm H_3BO_3 and 50 ppm $Ca(NO_3)_2.4H_20$. Each point is based on 3 samples, collected at the same day.

13.00 hrs resulted in a significant decrease in germinability, indicating the rapid loss of viability of the trinucleate lettuce pollen.

Storage of pollen. Vitality of pollen after storage was derived from the percentage of in vitro germination as shown in Fig. 4. Each point in the curves is the overall mean of 6 samples per storage temperature. Storage at room temperature resulted in a dramatic decrease in germinability within two days of the beginning of storage while after 8 and 16 days practically no pollen grains germinated anymore.

Vitality of pollen persisted longer when pollen grains were stored in the refrigerator at 4 °C, compared with storage at room temperature, though here too, within eight days after collection and the beginning of storage, the percentage of germinated pollen grains had become less than 50% of the original germination percentage.

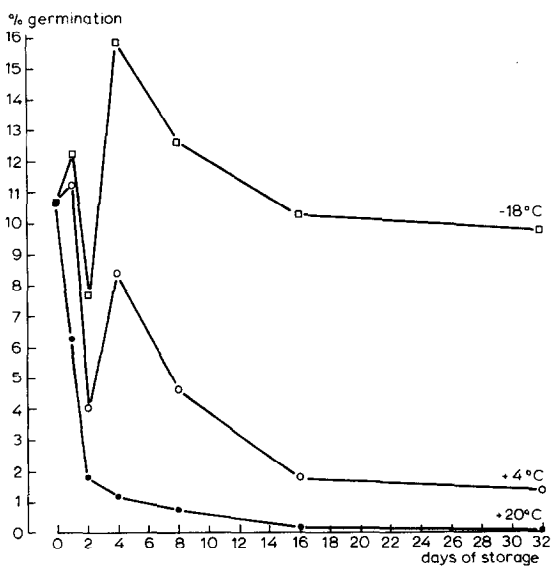

Fig. 4. Influence of storage temperature and storage period on in vitro germination of lettuce pollen. Each point is based on 6 samples.

The curve path for germination percentages after storage at −18 °C suggests that storage for 1, 4 and 8 days results in an increase of pollen in vitro germination compared with the same, non-stored, control samples. This phenomenon applies to all 6 samples after storage in the deep freezer. Perhaps a very low temperature neutralizes germination inhibitors or positively influences germination stimulants. Further investigations will have to elucidate whether the above phenomenon is attributable to an artefact or not.

After storage in the deep freezer the decrease of pollen vitality was very limited compared with the percentage of geminated pollen grains of samples, immediately set to germinate after collection and functioning as a standard. Even 32 days after the start of the storage experiments, no significant decrease in vitality was observed, which suggests that pollen can be stored even for a longer period at −18 °C without significant detrimental effects.

CONCLUSIONS

In vitro germination of lettuce pollen, up to a maximum of 40%, occurred in a nutrient medium containing 40% sucrose, 100 ppm H_3BO_3 and 100 ppm $Ca(NO_3)_2.4H_20$. This percentage is relatively high if one takes into consideration that in vivo germination on single stigmas never exceeded 50%. Pollen vitality and germinability were significantly influenced by internal and external factors especially around anthesis and therefore showed large variation. The nutrient medium enabled to check pollen vitality after storage. Vitality remained at an acceptable level after storage at −18 °C during 32 days. This storability may facilitate the crossing of asynchronously flowering lettuce genotypes.

In later experiments after storage some samples were used for in vivo pollination of lettuce flowers which then yielded seeds. However, more extensive studies on storability at extremely low temperatures and on the correlation between in vitro and in vivo pollen germination have to be performed.

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