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Pollen germination in vitro at low temperature in European and Andean tetraploid potatoes

I.S. Kristjansdottir

Department of plant Breeding, Swedish University of Agricultural Sciences, P.O. Box 7003, S-750 07 Uppsala, Sweden

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Summary. Confined design combined with use of tolerance ratio was used to compare pollen germination capacity at low and high temperature in Andean and European potato material. Four clones of *Solanum tuberosum* from the European gene pool were compared with four Andean potato clones derived from the breeding program for frost resistance at the International Potato Center (CIP), Lima, Peru. For each clone, the same pollen lot was used throughout each replication. Pollen were germinated at 9 °C and 21 °C. Fortification of media with potato starch and 14 min preincubation at 25 °C were used as variables. The Andean material maintained its germination capacity better than the European material when temperature was decreased. It was possible significantly to distinguish potato clones with low temperature requirement for pollen germination if incubation proceeded germination at 21 °C, but not at 9 °C. Fortification with starch had no significant effect.

Key words: *Solanum* sp. – *Solanum tuberosum* – Low temperature tolerance – Tolerance ratio – Incubation period

Introduction

Tetraploid *Solanum* hybrids of Andean origin are found to be less affected at low temperature during the growing season than European standard cultivars. Some of the Andean lines are able to accumulate as much dry matter at 10 °C day and 4 °C night as at 20 °C day and 10 °C night (Kristjansdottir 1989). In order to explore such valuable material in breeding for northerly marginal areas, the aim of the present investigation was to try to find a convenient selection method.

Comparable difference in reaction to temperature is reported for high altitude accessions of *Lycopersicon hirsutum* and cultivars of *L. esculentum* (Miltau et al. 1986). In addition, it was shown that pollen from *L. hirsutum* is also better adapted to low temperature than pollen from *L. esculentum* (Zamir et al. 1981). Since Tanksley et al. (1981) have found considerable similarity (60%) between the gametophytic and the sporophytic enzymatic system, pollen might be convenient in the search for potato genotypes adapted to low temperature regimes.

Germinability and tube elongation of pollen are well-defined features to measure. Since the pollen tube wall is proved to be a structure with no close analogue in somatic tissue (Heslop-Harrison 1987), correlations between gametophytic and sporophytic reaction to low temperature may not be clear enough. Pollen germinability was thus preferred as selective criterion, as it is also easier to measure.

Materials and methods

With the aim of finding a simple method capable of discriminating between *Solanum* types able or unable to grow equally well at low temperature as at temperature found optimal for certain potato cultivars (Borah and Milthorpe 1963), material known to have this kind of difference was chosen. The cultivar Stina (E1) and the three clones, S. 22 (E2), S. 23 (E3), and I. 124 (E4), were selected to represent the frost-sensitive European gene pool of *Solanum tuberosum*. In contrast, four 4× hybrid clones, 194.31 (A1), 199.13 (A2), 201.12 (A3), and 201.23 (A4), were chosen from the breeding program for frost resistance at the CIP. This material, henceforth called the Andean material, has the following tuber-bearing *Solanum* species in its background: *S. phureja*, *S. stenotomum*, *S. curtilobum*, *S. ajanhuiri*, *S. tuberosum* ssp. *andigena* as well as *S. tuberosum* L. from South America. For details as to ancestry and pedigree, see Kristjansdottir (1989).

Flowers with newly opened anthers were collected at about 10:00 a.m. and immediately transferred to a temperature of 4 °C, where germination was considered negligible.

At the onset of the experiment, anthers from each of the eight potato clones were shaken over a well (Castor, 48-well multibox), so that the pollen became evenly spread as a thin layer over the medium. The basic medium was made up of 12.5% sucrose, 70 ppm H_2BO_3, 15 ppm $Ca(NO_3)_2$, 100 ppm $MgSO_4$, and 170 ppm KH_2PO_4. Since pollen from the European material showed a germination optimum at pH 5.8 and the Andean lines at pH 5.4, the pH of the medium was adjusted to the mean 5.6.

Besides two temperature regimes, one at 9 °C representing low temperature conditions and one at 20 °C giving more favorable conditions, two other variations were introduced, both with the intention of influencing germinability.

One of these variables was to insert a warm incubation period between the resting condition in the storage at 4 °C and the germination test at low or higher temperature. The incubation temperature was set to 25 °C. Preliminary studies showed that the incubation had no effect if the period was shorter than 10 min and was not discriminating enough if longer than 18 min. Consequently, a period of 14 min at 25 °C was chosen with the intention of stimulating water uptake and increasing respiration/metabolism, i.e., to prepare for pollen tube growth (cf. Heslop-Harrison 1987). This treatment was introduced in order better to prepare the pollen for germination, if mere onset of the metabolism should be essential.

The other variable had a similar intention. According to, e.g., Billings (1974) or Levitt (1980), the rhythm of carbohydrate storage utilization plays an important role in adaptation of plants to cool environments. This reaction has also been found relevant for *S. tuberosum* (Chen and Liu 1980). In *Dactylis glomerata*, Eagles and Othman (1978) found that northern ecotypes had more water-soluble carbohydrates in leaf blades and sheaths at 8 °C than at 20 °C. At the higher temperature, there was no real difference between ecotypes from different climatic zones. In addition, van Herpen (1984) found that *Petunia* styles developed at low temperature had more low-molecular-weight carbohydrates than styles developed at higher temperature. The reverse proved, however, to be true for pollen.

All these kinds of information justified finding out whether addition of starch to the medium could influence pollen germinability with or without the above-mentioned incubation pretreatment. Preliminary investigations indicated that the European and the Andean material reacted somewhat differently as to the amount of starch added as a filtrate to the distilled water of the medium. It was found that filtrate from an amount of starch of 10 g/l gave a somewhat improved germination at 9 °C for the Andean clones without depression in germination of the European clones at 20 °C.

The conclusive experiment was thus designed with two temperatures, one incubation pretreatment and one enrichment of the medium with starch which, according to Table 1 makes up altogether eight combinations. They are briefly named Bas. low and Bas. high (no extra and a kind of control), Inc. low and Inc. high (incubation pretreatment only), Sta. low and Sta. high (fortification with starch only), Com. low and Com. high (incubation plus starch in combination). Each treatment was replicated three times. Within each clone and replication, pollen from the same flower was used over all treatments in order to minimize effects of minor physiological differences between pollen lots.

The treatments, where the pollen was allowed to germinate at 9 °C, were kept at this temperature for 5 h before germinability was recorded. Treatments at the higher temperature of 20 °C were examined already after 2.5 h. The germination process was immediately stopped by adding 30 µl of a solution with formalin, acetic acid, and 50% ethanol in the ratio 10 : 5 : 85 (Weinbaum et al. 1984). The percentage of germinated pollen was determined by examining 250 pollen grains per well. A pollen grain was considered to have germinated when its tube length was at least equal to the grain diameter, which is ca. 50 µm (Maisonneuve and Den Nijs 1984).

Table 1. Denomination of the different treatments at screening for temperature tolerance at pollen germination

Abbreviations used	Potato starch	Incubation period	Germination temperature
Bas. low	–	–	9 °C
Bas. high	–	–	21 °C
Inc. low	–	25 °C/14 min	9 °C
Inc. high	–	25 °C/14 min	21 °C
Sta. low	10 g/l	–	9 °C
Sta. high	10 g/l	–	21 °C
Com. low	10 g/l	25 °C/14 min	9 °C
Com. high	10 g/l	25 °C/14 min	21 °C

Results and discussion

Pollen germinability is usually found to differ widely depending on the physiological status and age of the plant and flower (Jansen and Hermsen 1976). This experience could also be made from the present experiment. A highly significant difference in germinability ($P = 0.001$ ***) was not only found between temperatures, origins, and clones but also between replications. Even the interaction between replication, on the one hand, and origin ($P = 0.012$ **) or clones ($P = 0.030$ *) on the other, indicates that sampling is biased. It is obvious that each pollen lot has its own characteristic germination capacity.

It is thus important to use pollen taken from the same flower at the same time when germination capacity at low and high temperature is to be compared. As mentioned above, this precautionary measure was also taken in the present experiment, i.e., the same pollen lot was used for all treatments over one replication. Under such circumstances, the ratio between germinability at low and high temperature will function as a more unbiased measure (Table 2). In addition, clones with different germination capacity will be easier to compare (Table 3). In the present case, the Andean clones proved consistently to have lower germination capacity than the European clones.

Accordingly, the ratio between the percentage of germinated pollen at low (9 °C) and high (20 °C) temperature, hereafter called pollen germination tolerance ratio or simply tolerance ratio, was chosen as variable. Since a ratio based on a certain treatment at low and another at high temperature appeared to offer the best discrimination between origins, the 16 different possibilities of how to combine treatments in the present experiment were examined. The probabilities (*P*-values at three replications for discriminating between the Andean and the

European pollen material for each of these tolerance ratios are presented in Table 4. Figure 1 offers a more direct way of demonstrating how the Andean and European clones are reacting in treatments where the groups show maximal deviation. Following the standard Box and Whisker test for 95% confidence intervals, the lengths of the bars indicate difference in reaction at low versus high germination temperature. The position of the bars around the dotted line (1.00) indicates whether low (above 1.00) or high temperature favors germination. Each bar is divided into two sections, the upper part refers to the confidence interval at low, the lower part at high germination temperature.

It is evident from such a survey that the germinability obtained after incubation followed by growth at low temperature gives tolerance ratios that are unable to discriminate between origins. The reason evidently is that, by incubation, the European pollen material obtains a stimulation to grow at low temperature, while such a treatment does not influence the Andean pollen material. The temperature tolerance ratio will tend to be similar. An incubation prior to the germination test at high temperature, however, tends consistently to distinguish the pollen growth pattern of the two groups. Figure 2 shows perhaps the best way to illustrate this reaction pattern. For the European pollen lots, the difference in germination is negligible when subjected to Bas. high or Inc. high, which indicates a certain temperature level before the

Table 2. Analysis of variance between origins, clones, replications, and treatments when tolerance ratio is used

	df	*F* value	Significance
Origins	1	99.27	0.0001***
Clones	6	13.88	0.0001***
Replications	2	10.83	0.0001***
Treatments	15	1.58	0.0804NS
Origin* treatments	15	2.16	0.0092**
Origin* replications	2	1.62	0.2008NS
Clones* replications	12	2.17	0.0148**

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 3. Analysis of variance between the eight potato genotypes, based on the ratio between germination at 9 °C (sub) and 21 °C (opt) temperature level for each treatment

Denominator	Numerator			
	Bas. low	Inc. low	Sta. low	Com. low
Bas. high	0.450NS	0.701NS	0.024**	0.021**
Inc. high	0.015**	0.267NS	0.009***	0.004***
Sta. high	0.296NS	0.155NS	0.059NS	0.021**
Com. high	0.181NS	0.499NS	0.162NS	0.156NS

* $p<0.05$, ** $p<0.01$, *** $p<0.001$
For abbreviations, cf. Table 1

Table 4. Analysis of variance between the Andean and the European material groups, based on ratio between germination at 9 °C (sub) and 21 °C (opt) temperature level for each treatment

Denominator	Numerator			
	Bas. low	Inc. low	Sta. low	Com. low
Bas. high	0.033*	0.938NS	0.003***	0.066NS
Inc. high	0.000***	0.091NS	0.009***	0.000***
Sta. high	0.019**	0.832NS	0.004**	0.083NS
Com. high	0.009***	0.911NS	0.004***	0.045*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$
For abbreviations, cf. Table 1

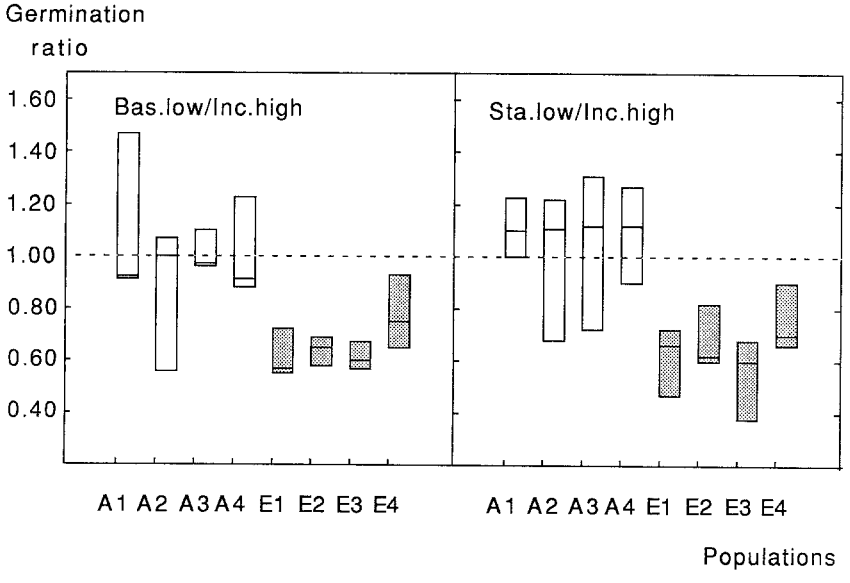

Fig. 1. Standard Box and Whisker test illustrating the reaction of the Andean (*open bars*) versus the European group (*dotted bars*) at the most discriminating treatment combinations, where significant differences were found both between clones and between origins. For aberrations, cf. Table 1

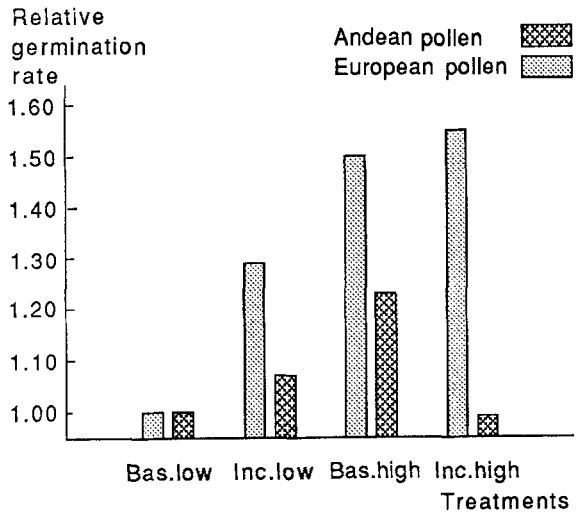

Fig. 2. The effect of incubation on Andean and European pollen at different germination temperatures. The figures are means related to Bas. low for each material group. For aberrations, cf. Table 1

pollen starts to germinate properly (Blum 1988). On the other hand, germination of the Andean material significantly decreased ($P = 0.0001$ ***) if incubation period was added before germination at 20 °C. Apparently, at least the onset of the germination process of Andean pollen must have another temperature optimum than that of European pollen. There may be qualitative differences in the properties of key constituents of the metabolic apparatus that lead to different temperature response curves (Hochachka and Somero 1973). The primary biosynthetic processes appear to be more sensitive to extreme temperatures than growth that has already begun (Raison and Berry 1981). The incubation temperature of 25 °C may thus be beyond the optimum for enzymes inducing the pollen germination process in the Andean material (cf. Alexandrov 1977).

Enrichment of the basic medium with starch did not improve the discriminating effect even if it had some influence (cf. Fig. 2). Irrespective of whether starch was present or not in the medium at low or high temperature, germination was much the same. Even in combination with incubation, improved discrimination was not obtained.

In conclusion, a method has been found that is able to distinguish potato genotypes with low demands on temperature for pollen growth. It is important to be aware of variations in pollen metabolism depending on flower development and environment at sampling. A comparison as to reaction at low and high temperature must always be made with the same pollen lot from the same flower and sampling time. In addition, the ratio betwen germinability at the two temperature regimes is a better characteristic than the mere percentages per se. The present study indicates that the pollen should be incubated at 25 °C for 14 min before germinability is checked, preferably at high rather than at low temperature. A definite recommendation of the method proposed at potato breeding for cool marginal areas can only be made when a firm enough correlation between temperature tolerance of pollen and plant growth is established.

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