Theor Appl Genet (1987) 74:545–548

Pollen selection for low temperature adaptation in tomato

D. Zamir and I. Gadish

The Hebrew University of Jerusalem, The Faculty of Agriculture, Department of Field and Vegetable Crops, P.O. Box 12, Rehovot 76-100, Israel

Received October 27, 1986; Accepted April 21, 1987
Communicated by P. L. Pfahler

Summary. Pollen selection experiments were conducted in tomato to determine the effects of low temperature conditions during pollination on the rate of root elongation of the progeny. Pollen was harvested from an F_1 interspecific hybrid between a high altitude *Lycopersicon hirsutum* accession and the cultivated tomato *L. esculentum*. The pollen was applied to stigmas of male-sterile *L. esculentum* plants maintained in growth chambers set at either 12 °C/7 °C or 24 °C/18 °C. BC_1 seeds from the low and normal temperature crosses were germinated and root elongation rate was measured at either 9 °C or 24 °C. At 9 °C, the rate of root elongation for progeny of the low temperature crosses was higher than for progeny of crosses at normal temperatures; at 24 °C the rate of root elongation was similar for the two crossing treatments. To compare the temperature responses of the two backcross populations we also calculated the relative inhibitory effect of low temperature on the rate of root elongation: the ratio between the rate of root elongation at 9 °C to that at 24 °C. Root elongation of seedlings from the low temperature crosses was less inhibited by the cold than root elongation for progeny of the normal temperature crosses. These results suggest a relationship between pollen selection at low temperatures and the expression of a sporophytic trait under the same environmental stress.

Key words: *Lycopersicon* – Pollen selection – Low temperature adaptation – Root elongation rate

Introduction

Plant breeders are constantly searching for new approaches for increasing the efficiency of breeding programs; one approach is pollen selection. It is based on the expectation that selection among heterogeneous haploid male gametophytes during pollen germination and tube growth until fertilization might have a positively correlated effect on the sporophytic generation (Mulcahy 1979; Zamir 1983). This expectation arises from the finding that the sporophyte and gametophyte of higher plants rely, in part, on a common structural gene repertoire (Tanksley et al. 1981). The overlap in gene expression between the haploid and diploid phases of the life cycle provides a unique mode of evolution, particularly in cases where there is an excess of pollen relative to the number of ovules, and therefore, pollen competition is strong. With such a scheme, higher plants could make an adaptive advance at a minimal cost by selection at the microgametophytic level (Mulcahy 1979).

As a model system for pollen selection, we have been studying pollen fertilization success at low temperatures in *Lycopersicon*. Low temperature tolerant accessions of *L. hirsutum* were collected from an altitude of 3,000 m from the Peruvian Andes (Vallejos et al. 1983). Accessions of *L. hirsutum* manifest their tolerance to chilling in seed germination, seedling survival, chlorophyll development, rate of protoplasmic streaming (Paull et al. 1979), chlorophyll fluorescence (Smillie 1979) and the rate of plant growth (Miltau et al. 1986). *L. hirsutum* pollen is better adapted to complete fertilization at low temperatures than pollen of *L. esculentum*. This selective fertilization was measured in pollen mixture studies where we showed that at low temperatures the wild species is a better competitor than *L. esculentum* (Zamir et al. 1981). *L. hirsutum* can be hybridized as a male parent to the cultivated tomato; the F_1 is fertile and produces segregating generations (Zamir and Tadmor 1986). Using pollen of

the interspecific hybrid between *L. hirsutum* and *L. esculentum*, we demonstrated that the fertilization ability of the wild parent pollen is determined in part by genes expressed by the haploid genome (Zamir et al. 1982). This study addressed the following question: will progeny of crosses made at low temperatures show a better adaptation to chilling stress than progeny of crosses made at normal temperatures?

Materials and methods

Twenty male sterile (*ms10*^{35}) *L. esculentum* plants (cv Koren) and a single interspecific F_1 hybrid between *L. esculentum* and *L. hirsutum* (LA 1777) were grown in the greenhouse using culture conditions described by Miltau et al. (1986). Pollen for crosses was harvested from the interspecific hybrid and its viability, as determined by acetocarmine staining of 300 pollen grains, was 92%. A total of 80 male-sterile flowers (day of anthesis) from 10 plants were used in crosses with the hybrid pollen. Immediately after pollination, plants were transferred to a growth chamber with a day temperature of 24 °C and a night temperature of 18 °C. Day length was 12 h, light intensity was 300 μEs^{-1} m^{-2} and relative humidity 70%. After 24 h under these conditions, styles of each flower were cut at midlength to prevent further fertilizations and plants were returned to the greenhouse. Pollen from the same F_1 sample was also used to pollinate 80 flowers of the remaining 10 plants which were transferred after pollination to a growth chamber set at 12 h cycles of 12 °C/7 °C with the same light intensity. These plants were moved to the greenhouse after 72 h of the cold treatment and styles were excised. Plants were grown in the greenhouse until fruit ripened and then seeds were extracted, counted, and weighed.

Three root elongation experiments were performed: one at 24 °C and two at 9 °C. For each experiment, approximately 160 BC_1 seedlings were assayed; 80 from the 24 °C/18 °C crosses and 80 from the 12 °C/7 °C crosses. For each crossing treatment, we pooled ten BC_1 seeds from eight different fruits. In the first root elongation experiment at 9 °C (1 in Table 2), seeds were selected at random while in the second experiment at 9 °C (2 in Table 2), only seeds weighing 3.0 to 3.4 mg were selected for germination.

Seeds were sterilized with 2.5% hypochlorite for 5 min, rinsed with sterile water and placed in Petri dishes containing 2% agar in water. Germination took place at 20 °C in the dark and the germination rate, which did not differ between the crossing treatments, was 90%; under these conditions the elongating roots were straight. Root length was measured (mm) from the point of radicle protrusion to the root tip. Seedlings with roots of 3–5 mm were transferred to polyethylene bags containing wet Whatman 3 mm filter paper; approximately 90% of the germinated seedlings from each crossing treatment met this criterion. Each polyethylene bag contained six BC_1 seedlings either from the low or the normal temperature crosses. Each bag was given a random code number so throughout the experiment the identity of the seedlings in the bags was not known. The seedling bags were placed vertically (to support straight root growth) in a dark growth chamber set at either 24 °C or 9 °C. After 24 h in the chamber set at 24 °C and 72 h in the cold chamber, seedlings were taken out of the bags and root length was measured. Root elongation rate for each of the temperature treatments was calculated as the difference between the second and first root measurement for the time elapsed. The above time intervals between the first and second root measurements were selected since preliminary experiments indicated that during these periods the rate of root elongation was constant. In both experiments at 24 °C and 9 °C, 30 seedlings from each of the parents were grown under the two temperature regimes and root elongation was measured in the same manner as for the BC_1 seedlings. The differences between the means were analysed using a *t*-test.

Results

The rate of root elongation of *L. esculentum* was greater than that of *L. hirsutum* both at 24 °C and 9 °C (Table 1). At first glance this might suggest that the cultivated tomato is better adapted to low temperatures than the wild species or, conversly, we can say that adaptation to low temperatures implies slower rate of root elongation. However, when we consider that seed of *L. esculentum* is 5 times heavier than *L. hirsutum* seed (mean seed wt 4.5 mg and 0.9 mg, respectively), and that larger seeds generally produce larger seedlings (Hutchinson 1984), it appears that a comparison of the mean rate of root elongation may be a misleading measure for the degree of low temperature adaptation of the parental species. In order to compare the low temperature response of roots of the two tomato species, which have different patterns of growth at the early seedling stage (Miltau et al. 1986), we calculated for each one the ratio of root elongation at 12 °C/7 °C to that at 24 °C/18 °C. This ratio was almost twice as high for *L. hirsutum* as for *L. esculentum* – indicating a smaller inhibitory effect of the low temperature on root elongation rate in the wild species.

The mean number of BC_1 seed per fruit for the crosses made at 24 °C/18 °C was 55, with a standard deviation of ± 17 (mean of 38 fruit) and 27 ± 17 (mean of 30 fruit) for crosses made at 12 °C/7 °C ($P_{(t)}$ for the difference < 0.001). There was no significant difference

Table 1. The effect of two temperature regimes on the rate of root elongation of *L. esculentum* and *L. hirsutum*

Species	Root elongation rate		Ratio[b]
	At 24 °C (mm/24 h)	At 9 °C (mm/72 h)	
L. esculentum	17.6 ± 0.3[a]	5.2 ± 0.2	0.30
L. hirsutum	8.7 ± 0.6	4.5 ± 0.3	0.52

[a] Values given are means ± standard errors

[b] The ratio between mean root elongation rate at 9 °C to that at 24 °C

Table 2. Root elongation rate for BC_1 progeny of crosses at low and normal temperatures

Pollination temperature	Root elongation rate			Ratio[b] (1)	Ratio[c] (2)
	At 24 °C (mm/24 h)	At 9 °C (1) (mm/72 h)	At 9 °C (2) (mm/72 h)		
24 °C/18 °C	17.0 ± 0.5[a]	5.2 ± 0.2	5.3 ± 0.2	0.31	0.31
12 °C/7 °C	16.5 ± 0.4	6.0 ± 0.2	6.1 ± 0.2	0.36	0.37
Difference	0.5	0.8	0.8		
P value	0.4	0.001	0.01		

[a] Values given are means ± standard errors
[b] The ratio between mean root elongation rate at 9 °C to that at 24 °C for experiment 1
[c] The ratio between mean root elongation rate at 9 °C to that at 24 °C for experiment 2

in the mean weight of the seed produced in the low temperature crosses (3.3 ± 0.2 mg) and the normal temperature crosses (3.2 ± 0.2 mg); therefore we compared the mean values of the rate of root elongation at normal and low temperatures. Table 2 shows that there was no significant difference in the rate of root elongation at 24 °C between BC_1 progeny from the normal and low temperature crosses. In the first experiment at 9 °C (1), the rate of root elongation at 12 °C/7 °C was significantly higher than for progeny of crosses at 24 °C/18 °C. This was also the result of the second root elongation experiment (2) at 9 °C where seeds of similar weight were selected in order to minimize possible effects of seed size on the rate of root elongation. The limitation of these results is that they cannot be compared to the values of the parents because of the differences in seed size between the species. Therefore the higher rate of root growth in the cold could be derived from *L. esculentum* with its higher mean value for root elongation rate in the cold or from *L. hirsutum* with the lesser low temperature inhibition of root elongation. Bearing this limitation in mind, we would like to emphasise that root elongation rate in the cold, for progreny of the low temperature crosses, was higher than that of the normal temperature crosses and this effect was independent of seed size.

The ratio of the rate of root elongation at low temperatures to that at normal temperatures was higher for the BC_1 seedlings from the low temperature crosses than for BC_1 seedlings from the normal temperature crosses (Table 2). This suggests a smaller inhibitory effect of low temperature on root elongation as a result of pollen selection in the cold. The direction of the difference in ratio of root elongation rates between the two temperature crosses is consistent with that obtained for the parents (Table 1), however, the actual values differed; pollen selection in the cold improved the ratio in the low temperature backcrosses only to a quarter of the potential available in *L. hirsutum*.

Discussion

How does pollen selection affect the performance of the progeny? The first explanation is a physiological one which may result from the lower number of seed per fruit in the low temperature crosses as compared to the number of seed per fruit in the normal temperature crosses. Although seed weight did not differ significantly between the two crossing treatments it is possible that undetermined components with limited availability might be distributed at higher concentrations to the fewer seed per fruit in the low temperature crosses. There are two observations which seem to preclude the physiological explanation. (1) The difference in the rate of root elongation between the crossing treatments was detected only at low temperatures. (2) Each male sterile plant from the two treatments carried an average of four fruit which is a much lower number than that which normally ripens on this variety. We therefore think that it is not likely that resource availability to the developing seed differed between the treatments in a way which would influence the rate of root elongation.

The more likely explanation is genetic, where two models are suggested. (1) Selection of individual gene/s affects both gametophytic and sporophytic responses to low temperatures. Selection of specific alleles at one or more loci during the controlled crosses would improve the response of the sporophytic generation if the same loci are active in both phases of the life cycle. (2) Selection at linked loci independently affects pollen fertilization ability and root elongation at low temperatures. Selection for one allele during fertilization in the cold would affect allele frequency at the linked locus.

Studies in a number of plant species indicate that gametophytic selection can modify traits of the sporophytic generations (Lewis 1954; Mulcahy 1974; Mulcahy and Mulcahy 1975; Ter-Avanesian 1978; Ottaviano et al. 1982). In these studies the intensity of gametophytic competition was regulated by changing

the number of pollen grains on the stigma or by varying the length of the stylar tissue. Few studies examined the effects of gametophytic selection under a particular environmental stress on the degree of tolerance of the progeny: Searcy and Mulcahy (1985) compared the number of metal tolerant progeny from crosses using pollen which was harvested from plants grown in the absence or presence of toxic concentrations of zinc or copper. They found that the proportion of tolerant progeny increased significantly if pollen came from metal-treated plants, indicating that gametophytic selection occurred during microsporogenesis. The degree of tolerance of the plants was evaluated by their ability to form new roots and by measuring root length under the different conditions. Maisonneuve et al. (1986) applied pollen selection in a breeding program for adaptation of tomato to low temperatures. Using hybrids between different *L. esculentum* varieties that did not differ dramatically for growth capacity, they concluded that low temperature treatments during pollen formation or pollen germination did not appear to aid sporophytic selection. The sporophytic traits that they measured were associated with shoot development. Our experience with the genotypes presented in this paper has also shown that pollen selection in the cold did not affect traits of the shoot as measured by the plastochron index duration (the time interval between corresponding stages of development of successive leaves) (unpublished data). It appears that pollen tube growth correlates better with root than with shoot taits; a similar observation was made by Ottaviano et al. (1982) who found a good correlation between pollen competitive ability and root growth *in vitro*.

Acknowledgement. This research was supported by grant no. I-487-82 from BARD, The United States – Israel Binational Agricultural Research and Development Fund.

References

Hutchinson ES (1984) Seed size and quantitative characters in *Avena barbata*. Heredity 52:25–33

Lewis D (1954) Pollen competition and sporophytic characters. Annu Rep John Innes Hortic Inst 45:16–17

Maisonneuve B, Hogenboom NG, Den Nijs APM (1986) Pollen selection in breeding tomato (*Lycopersicon esculentum* Mill.) for adaptation of low temperature. Euphytica 35:983–992

Miltau O, Zamir D, Rudich J (1986) Growth rates of *Lycopersicon* species at low temperatures. Z Pflanzenzücht 96: 193–199

Mulcahy DL (1974) Correlation between speed of pollen tube growth and seedling height in *Zea mays* L. Nature 249: 491–493

Mulcahy DL (1979) The rise of angiosperms: a genecological factor. Science 206:20–23

Mulcahy DL, Mulcahy GB (1975) The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. Theor Appl Genet 46:277–280

Ottaviano E, Sari Gorla M, Pe E (1982) Male gametophytic selection in maize. Theor Appl Genet 63:249–254

Paull RE, Patterson BD, Graham D (1979) Chilling injury assays for plant breeding. In: Lyons JM, Graham D, Raison JK (eds) Low temperature stress in crop plants. Academic Press, New York, pp 507–519

Searcy KB, Mulcahy DL (1985) Pollen selection and gametophytic expression of metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). Am J Bot 72:1700–1706

Smillie RM (1979) The useful chloroplast: a new approach for investigating chilling stress in plants. In: Lyons JM, Graham D, Raison JK (eds) Low temperature stress in crop plants. Academic Press, New York, pp 187–202

Tanksley SD, Zamir D, Rick CM (1981) Evidence of extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicon esculentum*. Science 213:453–455

Ter-Avanesian DV (1978) The effect of varying the number of pollen grains used in fertilization. Theor Appl Genet 52: 77–79

Vallejos CE, Lyons JM, Breidenbach RW, Miller MF (1983) Characterization of a differential low temperature growth response in two species of *Lycopersicon:* the plastochron as a tool. Planta 159:487–496

Zamir D (1983) Pollen gene expression and selection: application in plant breeding. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding (Part A). Elsevier, Amsterdam, pp 313–330

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz Chicago 147:355–358

Zamir D, Tanksley SD, Jones RA (1981) Low temperature effect on selective fertilization by pollen mixtures of wild and cultivated tomato species. Theor Appl Genet 59: 235–238

Zamir D, Tanksley SD, Jones RA (1982) Haploid selection for low temperature tolerance of tomato pollen. Genetics 101: 129–137