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LOW TEMPERATURE SEED GERMINATION OF *LYCOPERSICON* SPECIES EVALUATED BY SURVIVAL ANALYSIS

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SUMMARY

Low temperature germination responses were evaluated for 18 high altitude accessions representing five wild *Lycopersicon* species and 19 accessions of *L. esculentum* which have reputed ability to germinate in the cold. Survival analysis indicated that one accession of *L. chilense* germinates better at 10 °C than PI 120256, the fastest-germinating *L. esculentum* genotype, and that PI 120256 germinates as well as PI 126435 (*L. peruvianum*). Additional wild ecotypes exhibiting rapid germination at 10 °C were identified from *L. peruvianum* and *L. hirsutum*. These ecotypes may possess genetic potential for introgressing cold germination ability into *L. esculentum* cultivars.

INTRODUCTION

Seeds of cultivated tomatoes germinate slowly if at all at temperatures of 10 °C or below. This characteristic prevents early direct seeding of tomatoes in the field and necessitates expensive heating for greenhouse production of transplants. During the past twenty years, plant breeders have not developed horticulturally acceptable tomato cultivars that germinate and grow at low temperatures. Reasons for this may include 1) lack of sufficient genetic variability within *Lycopersicon esculentum*, 2) ineffective selection procedures and/or 3) lack of concerted effort.

Genetic variability for low temperature seed germination in *L. esculentum*, first reported by SMITH & MILLET (1964), has since been the subject of numerous studies (CANNON *et al.*, 1973; DE VOS *et al.*, 1981; EL SAYED & JOHN, 1973; NG & TIGCHELAAR, 1973; THOMPSON, 1974; and WHITTINGTON *et al.*, 1965). Estimates of broad sense heritability range from 97% for germination (NG & TIGCHELAAR, 1973) to 25–40% for emergence (EL SAYED & JOHN, 1973). Narrow sense heritability has been estimated at 67–69% by NG & TIGCHELAAR (1973) and recently confirmed by DEVOS *et al.* (1981). Narrow sense heritability for emergence was estimated to be 25% by EL SAYED & JOHN (1973). The number of genes involved in germination has been estimated as one (CANNON, 1973) to three to five (NG & TIGCHELAAR, 1973). The number of genes involved in emergence was estimated to be twenty-four loci by EL SAYED & JOHN (1973).

The search for genetic diversity for temperature adaptation logically leads one to examine extreme environments for adapted cultivars or ecotypes. The potential for

this geographic approach in identifying genotypes tolerant to low temperatures was examined by THOMPSON (1970) and reviewed by VALLEJOS (1979). PATTERSON *et al.* (1979) studied altitudinal ecotypes of *L. hirsutum* and reported variable tolerance to chilling temperatures at the whole plant level. Equivalent germination rates of *L. hirsutum* AF (native to 3100 m altitude in Peru), however, occurred at 3 °C lower temperature than *L. esculentum* cv. Rutgers. This marginally better performance suggests that *L. hirsutum* AF lacks sufficient potential to improve low temperature seed germination in tomato.

Methods frequently reported for analyzing seed germination data include: 1) time to 50% germination, 2) a sprouting index such as:

$$\text{S.I.} = \frac{\Sigma\,(\textit{days to sprouting})\,(\textit{number sprouted})}{\textit{total number of seeds}}$$

and 3) cumulative percent germination. These statistics do not satisfactorily describe germination responses nor provide for detailed comparisons. Time to 50% germination ignores germination rates and the final percent germination. The sprouting index fails to distinguish between the effects of germination rates and the percent germination. It also provides no information about the distribution of germination rates over time or the final percent germination. The third method does not allow robust statistical comparisons of treatments.

The authors propose the use of survival analysis to evaluate seed germination and other developmental events such as time to flowering and time to maturity. Survival analysis has general applicability for analysis for developmental responses that occur over time with the following conditions: 1) A starting event (e.g. imbitition) must occur. 2) The terminal event (e.g. germination) cannot occur before the starting event. 3) The terminal event need not occur in every case, but cannot occur more than once. Events occurring within specified time intervals are recorded. Analysis by the life table method allows seed germination responses within an experimental unit or treatment to be summed for each observation interval. Responses may be recorded as normal germination, abnormal germination, or nongermination. Seeds lost through accident or fungal contamination may also be accounted for in survival analysis as censored observations. A low percentage of developmentally abnormal germinations may be considered as censored; a high percent may be treated as a competing risk to normal germination. Survival analysis computes several statistics which describe the distribution of germination responses over time. In the present study, survival analysis is applied to the cold germinability of 37 accessions of *Lycopersicon* spp.

MATERALS AND METHODS

Seeds of 19 accessions of *L. esculentum* which were reported to germinate well at low temperatures and two controls were increased in the field during the summer of 1980. PI 280597 segregated for two plant types; fruits of these were harvested separately. Mature fruits were crushed in a Millet Wet Vegetable Seed Separator and the brei fermented for 24 hours. Seeds, cleaned by floating off debris then washing in a strainer, were placed in nylon bags and tumble dried for 6 h at 25 °C.

Fifteen plants of each of 18 high altitude accessions of wild *Lycopersicon* species and one *L. esculentum* control were greenhouse-grown for seed increase. Pollen from all plants of each accession were bulked, and flowers from each plant in the population were then hand pollinated. Mature fruits were crushed in a hand crank type nut chopper and fermented 24 hours. Seeds were separated from the skins by washing them through a conical colander, and debris was floated off. The seeds were collected in a fine mesh strainer and dried on paper towels at room temperature. All seeds were stored in paper envelopes at 25 °C with low humidity until used.

Germination screen. Germination tests were conducted at 10 ± 1.0 and 20 ± 0.5 °C in a Precision model 815 incubator. The inside opening was covered with a double layer of polyethylene with flaps cut to allow access to individual shelves with minimal temperature fluctuation. Seeds of each accession were surface sterilized at 10 °C with chilled 10% Chlorox for 10 minutes then rinsed with chilled deionized water. Fifty seeds were placed in each sterile petri dish containing 0.8% agar and maintained at 10 °C. The 22 accessions of *L. esculentum* tested as one experiment are listed in Table 2. In a separate experiment, 18 accessions of wild *Lycopersicon* species were tested with five *L. esculentum* genotypes and greenhouse-grown seed of one cultivar selected from a preliminary screen. These genotypes are listed in Table 4. Petri dishes were arranged in three randomized blocks on two shelves. Germination responses at 20 °C were scored every 8 hours for 7 full days, and at 10 °C were scored daily for 30 days.

Statistical analysis. Germination responses were analyzed by survival analysis (LEE, 1980) which measures the distributions of several parameters over time. Results of each treatment are summarized in a life table, illustrated in Table 1. Each row presents analysis of events occurring within a specified interval of time. Events recorded at the end of an interval are assumed to occur, on the average, in the middle of that interval. It is therefore important that intervals between measurements be sufficiently small to provide meaningful information.

Several of the parameters in the life table shown in Table 1 are self-explanatory. Information provided by other parameters such as probability density and hazard rate may not be as readily apparent. The following definitions, modified from GEHAN (1969), are presented to aid interpretation of survival analysis of seed germination.

Number entering this interval: The number of nongerminated seeds at the beginning of the time interval.

Number withdrawn during this interval: The number of seeds lost from the petri dish, abnormal germinations, or the number of nongerminated seeds at the end of the last interval. These are considered censored observations.

Number exposed to risk (r): The number entering the interval minus one half of those withdrawn.

Number of terminal events: The number of seeds germinated in the interval.

Proportion of terminal events (q): An estimate of the probability of germination within an interval, computed as the number of terminal events in the interval divided by the number exposed to risk.

Table 1. Life table for seed germination of PI120256 at 20°C.

Intvl start time (days)	Number				Proportion			Proba-bility density	Hazard rate	Standard error of		
	entrng this intvl	wdrawn during intvl	exposed to risk	of termnl events	termi-nating	survi-ving	cumul. surv.			cumul. surv-iving	prob-ability dens.	hazrd rate
0.000	150.0	0.0	150.0	0.0	0.0000	1.0000	1.0000	0.0000	0.0000	0.000	0.000	0.000
0.334	150.0	0.0	150.0	0.0	0.0000	1.0000	1.0000	0.0000	0.0000	0.000	0.000	0.000
0.668	150.0	0.0	150.0	0.0	0.0000	1.0000	1.0000	0.0000	0.0000	0.000	0.000	0.000
1.002	150.0	0.0	150.0	0.0	0.0000	1.0000	1.0000	0.0000	0.0000	0.000	0.000	0.000
1.336	150.0	0.0	150.0	0.0	0.0000	1.0000	1.0000	0.0000	0.0000	0.000	0.000	0.000
1.670	150.0	0.0	150.0	47.0	0.3133	0.6867	0.6867	0.9381	1.1124	0.038	0.113	0.159
2.004	103.0	0.0	103.0	77.0	0.7476	0.2524	0.1733	1.5369	3.5742	0.031	0.122	0.327
2.338	26.0	0.0	26.0	17.0	0.6538	0.3462	0.0600	0.3393	2.9085	0.019	0.077	0.617
2.672	9.0	0.0	9.0	5.0	0.5556	0.4444	0.0267	0.0998	2.3031	0.013	0.044	0.951
3.006	4.0	0.0	4.0	1.0	0.2500	0.7500	0.0200	0.0200	0.8554	0.011	0.020	0.847
3.340	3.0	0.0	3.0	1.0	0.3333	0.6667	0.0133	0.0200	1.1976	0.009	0.020	1.173
3.674	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
4.008	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
4.342	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
4.676	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
5.010	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
5.344	2.0	0.0	2.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
5.678	2.0	0.0	1.5	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
6.012	1.0	0.0	1.0	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000
6.346	1.0	1.0	0.5	0.0	0.0000	1.0000	0.0133	0.0000	0.0000	0.009	0.000	0.000

* The median survival time for these data is 2.13.

Proportion surviving (p): One minus the proportion of terminal events.

Cumulative proportion surviving (S_i): Estimates the cumulative survival (nongermination) rate as the product of the probabilities of survival up through the present interval. The cumulative germination curve is therefore one minus the cumulative proportion surviving.

Probability density (f.): Estimates the probability per unit time of germination in the *i*th interval. The density function is computed as:

$$f_i = (S_{i-1}\, q_i)h_i$$

where h_i is the interval width. This may be considered an estimate of the germination rate.

Hazard rate (λ_i): Estimates the probability per unit time that an individual which has not germinated before the beginning of a given interval will germinate during that interval. Estimates of the hazard function are appropriate for hypothesizing a theoretical parametric distribution for right-censored data (as when all seeds do not germinate). It is computed as the number of germinations per unit time in the interval, divided by the average number of nongerminated seeds at the mid-point of the interval. The hazard rate is calculated as:

$$\lambda_i = 2q_i/h_i(1 + p_i)$$

Median survival time: The time in days to 50% germination, determined by linear interpolation.

Comparison of germination responses was by a nonparametric test of whether the survival curves of individual accessions come from the same survival distribution (MANTEL, 1966; BRESLOW, 1970). For each seed of an accession, a score U is computed by comparing the survival time (time to germination) of that seed with all other seeds. The value of U is initially zero and is incremented by one for each other seed whose germination time is less than that of the present individual seed, and is decremented by one for each case whose germination time is longer than the particular case or seed. For accession j and seed i, $W_j = \Sigma U_i$. A statistic D is then calculated by an algorithm of LEE & DESU (1972) so that: $D = (N-1)B/T$ where N = the sum of weights of all cases (total number of seeds), $B = \sum^{g} \frac{W_j^2}{N_j}$ and $T = \Sigma\, U_j^2$. The statistic D is asymptotically distributed as chi-square with g-1 degrees of freedom under the null hypothesis that the g subgroups (accessions) are from the same survival distribution (LEE & DESU, 1972). An overall test of all accessions if first performed, then all possible pairwise comparisons.

RESULT AND DISCUSSION

Nonparametric comparisons. The statistic D for overall comparisons of germination distributions among *L. esculentum* genotypes at 10 °C was significant at the 0.0001 level. D was equally significant for overall comparisons of genotypes at 20 °C. Genotypes were ranked by their scores for U at 10 °C. More negative values indicate faster

Table 2. Germination responses for *L. esculentum* genotypes tested. Rank is by mean scores for U at 10°C.

Rank	PI#	Seed source**	Origin	10°C			20°C		
				mean score ($\overline{U}$)	days to 50% germin.	final % germin.	mean score ($\overline{U}$)	days to 50% germin.	final % germin.
1	120256	1	Turkey	−2650.2	8.43	98.7	−2566.0	2.13	98.7
2	341988	2	USA	−2074.6	10.28	100.0	−1769.2	2.40	100.0
3	174261	3	USA	−1934.0	10.65	100.0	−859.9	2.79	99.3
4	341985-2*	2	USA	−1800.9	10.87	100.0	−1748.0	2.45	100.0
5	174263	1	Turkey	−1320.0	10.25	90.3	−2252.9	2.11	98.7
6	237132	3	–	−1297.4	12.48	99.3	−738.6	2.84	100.0
7	341985-1	2	USA	−1290.3	11.59	95.5	−796.5	2.79	99.3
8	341984-1	2	USA	−482.4	13.74	96.0	−878.4	2.79	89.0
9	341984-2	2	USA	−390.3	14.15	98.4	−779.7	2.80	99.3
10	120256-C	3	–	−244.8	14.80	99.3	−107.1	3.04	99.3
11	T6	3	USA	−56.8	14.95	92.2	505.0	3.28	96.7
12	370088	1	Canada	73.1	15.00	98.6	−1739.8	2.48	97.3
13	280597-1	1	USSR	87.7	14.94	96.6	1307.7	3.75	99.3
14	T5	3	USA	221.5	15.78	92.0	38.9	3.05	98.7
15	303726	1	Canada	953.1	17.67	92.8	209.3	3.06	100.0
16	280597-2	1	USSR	1038.1	17.42	96.6	1392.6	3.68	98.0
17	370080	1	Canada	1497.0	20.51	65.4	1901.5	4.34	76.9
18	298633	1	USSR	1507.0	19.99	71.0	1750.4	4.31	89.7
19	370087	1	–	1745.1	20.49	77.5	1465.1	3.80	93.4
20	201773	1	Canada	1799.2	21.42	65.6	1515.5	3.87	97.0
21	339914	1	USA	2210.7	26.45	72.4	2436.4	5.48	73.3
22	288069	1	U.K.	2399.2	29.89	50.4	1713.7	3.89	86.7

* Hyphenated numbers indicate populations segregating for obvious horticultural characteristics.
** Seed sources: 1 = Northcentral regional plant introduction center, Ames, Iowa; 2 = G. Dickinson, Vegetable Crops, U.C. Davis; 3 = A. Millet, Vegetable Crops, U.C. Davis.

germination. These are listed in Table 2 along with their origin, seed source, *U* scores, days to 50% germination and final percent germination at 10 and 20°C. Seeds of PI 120256 germinated most rapidly, followed by PI 341988, then PI 174261. PI's 341985-2, 174263, 237132 and 341985-1 were ranked fourth, fifth, sixth and seventh, respectively.

In the absence of an appropriate multiple range test, all possible pairwise comparisons of genotypes were performed. Pairwise comparisons of germination distributions at 10°C are listed in Table 3 for the top eleven genotypes. Values listed are the tail probabilities for D which indicate the probability that *U* scores of the two cultivars represent the same germination distribution. Probabilities smaller than 0.01 were interpreted as significant. PI 120256 had a significantly smaller germination time than all other genotypes. Germination of PI 341988 was not significantly different from PI 174261, although the probability was only 0.012. The germination distribution for PI 341988 was significantly smaller than all other genotypes ranked below it except PI 174263. Comparison of PI 174261 with PI 341985-2 and PI 174263 were not significant. PI 341985-2 and PI 174263 were not significantly different. The germination distribution of PI 120256-C was not significantly different from the control, T6. It

Table 3. Pairwise comparisons of *L. esculentum* genotypes at 10°C.* Values are the tail probabilities that two genotypes share the same germination distribution. Rank corresponds to genotype identity in Table 2.

Rank	1	2	3	4	5	6	7	8	9	10
2	0.000									
3	0.000	0.012								
4	0.000	0.002	0.332							
5	0.000	0.614	0.454	0.325						
6	0.000	0.000	0.000	0.000	0.003					
7	0.000	0.000	0.000	0.002	0.009	0.089				
8	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.397		
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.210	0.446	
11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.032	0.402

* Pairwise comparisons for additonal genotypes are available on request.

should be pointed out that PI 120256-C was a cherry-fruited type that bore little resemblance to PI 120256 from the P.I. station. It evidently originated as either a volunteer or cross-pollination in the field, or a contaminant in the seed packet.

The statistic D for overall comparisons of germination distributions among accessions of *Lycopersicon* species were highly significant (0.0001 level) at 10 and 20°C. *Lycopersicon* ecotypes and cultivars tested together are listed in Table 4 in order of their rank scores for U at 10°C. Included in the table are species, native elevation, $\overline{U}$ scores at 10 and 20°C, days to 50% germination at 10 and 20°C, as well as the final percent germination at 30 and 6.6 days after sowing. Note that ranking germination by mean U score ($\overline{U}$) is generally consistent with time to 50% germination. Inconsistencies between the two parameters are accounted for by the germination distribution of the second 50% of each sample. Seed of LA 460 (*L. chilense*) germinated most rapidly, while PI 126435 (*L. peruvianum*) ranked second. *L. esculentum* genotypes PI 120256, PI 174263 and PI 341988 ranked third, fourth and sixth, respectively. PI 127831 and LA 1474 (*L. peruvianum*) ranked fifth and seventh, while PI 127826 (*L. hirsutum*) ranked eight. The fieldgrown *L. esculentum* control (T5) ranked 17th while seed of the same culitvar increased in the greenhouse ranked 19th. If this environmental effect of more rapid germination of seed increased in the field and tumble dried is consistent among genotypes, then our estimates of differences between greenhouse increased seed of wild species and field increased seed of PI 120256 may be conservative.

Results of all possible pairwise comparisons of germination distributions among the top twelve-ranked accessions of *Lycopersicon spp.* are presented in Table 5. Genotypes are ordered by their rank in the overall comparison, as listed in Table 4. Values listed in the matrix are the tail probabilities for the statistic D in each pairwise test. The germination time for LA 460 was significantly less than all other genotypes. Germination times for PI 126435 and PI 120256 were not significantly different, but both germinated faster than other genotypes listed below them in rank. PI 127831, PI 341988, and LA 1474 were not significantly different at the 0.01 or 0.05 level. PI 127831 and LA 386 differed at the 0.05 but not the 0.01 level.

Table 4. Germination responses for *Lycopersicon* spp. Rank is by mean scores for U at 10 °C.

Rank	Accession #	Species	Native elevation (m)*	10 °C			20 °C		
				mean score ($\bar{U}$)	days to 50% germin.	final % germin.	mean score ($\bar{U}$)	days to 50% germin.	final % germin.
1	LA460	*L. chilense*	2500	−2955.7	7.71	99.3	−1567.1	2.31	98.7
2	PI126435	*L. peruvianum*	2430	−2399.1	9.27	100.0	−2695.8	1.99	100.0
3	PI120256	*L. esculentum*		−2236.0	9.45	96.7	−1744.5	2.27	99.3
4	PI174263	*L. esculentum*		−1646.4	10.09	95.3	−2322.2	2.07	100.0
5	PI127831	*L. peruvianum*	2500	−1328.7	11.06	94.0	−596.4	2.62	97.3
6	PI341988	*L. esculentum*		−1326.9	11.59	97.7	−1336.6	2.35	100.0
7	LA 1474	*L. peruvianum*	1300	−1294.8	10.69	89.3	319.9	2.95	93.9
8	PI127826	*L. hirsutum*	3000	−1265.6	11.25	92.0	−355.3	2.72	99.3
9	LA 386	*L. hirsutum*	3000	−1207.1	11.52	98.7	1252.0	3.31	97.4
10	PI127832	*L. peruvianum*	2500	−512.0	13.50	89.1	−2376.3	2.03	99.3
11	PI126434	*L. peruvianum*	2400	−331.8	12.40	70.7	−298.0	2.64	99.0
12	LA 1316	*L. chmielewskii*	2920	−270.5	14.28	98.7	1783.7	3.48	97.3
13	PI390660	*L. hirsutum*	2300	311.9	16.24	96.0	485.3	3.02	96.7
14	PI390663	*L. hirsutum*	2300	400.1	14.44	64.7	−1825.1	2.24	99.3
15	LA 1317	*L. chmielewskii*	2610	629.4	17.92	100.0	2414.0	3.82	98.0
16	T6	*L. esculentum*		795.2	19.00	96.7	1227.9	3.20	92.7
17	T5 (field)	*L. esculentum*		821.0	17.83	94.5	812.5	3.09	94.7
18	PI390662	*L. hirsutum*	2300	1158.8	19.83	59.3	488.4	2.90	82.0
19	T5 (greenhouse)	*L. esculentum*		1346.5	20.92	85.9	2110.5	3.76	85.9
20	LA 1305	*L. peruvianum*	2900	1822.7	29.00+	32.0	1236.8	3.30	89.5
21	PI390661	*L. hirsutum*	2300	1940.3	29.00+	46.5	−37.3	2.83	99.3
22	PI126949	*L. pimpinellifolium*	3000	2227.3	29.71	52.1	871.0	3.19	100.0
23	PI390513	*L. hirsutum*	2700	2620.8	29.00+	4.7	185.2	2.93	100.0
24	PI390659	*L. hirsutum*	2300	2700.6	29.00+	5.3	1919.2	3.62	100.0

* (HOLLE et al., 1979)

Previous studies have not specifically attempted to compare genotypes reputed to germihate well in the cold. DEVOS *et al.* (1981) reported that PI 341985 germinated more rapidly at 10 C than PI 120256 or PI 341988, while PI 280597 ranked fourth. However, sprouting indices reported for the first three P.I.'s were all very close. The discrepancy in rank between the results reported by DEVOS *et al.* (1981), and those reported here may be due to the different methods of analyzing the data. KEMP (1968) reported high germination percentage after two weeks at 8.5 °C for PI 120256 (65%) and PI 280597 (79%), while Cold Set germinated 52%. NG & TIGCHELAAR (1973) used PI 341985 as a cold-germinating cultivar but did not compare performance with other cold-tolerant material.

The very poor performance of *L. pimpinellifolium* (PI 126949), which ranked 22nd out of 24 accessions, is not consistent with earlier reports. WHITTINGTON *et al.* (1965) reported faster germination for *L. pimpinellifolium* 'Red Currant' than other cultivars at 15, 20 and 26 °C. THOMPSON (1974) also found faster germination of *L. pimpinellifolium, L. e. pyriforme* and *L. e. cerasiforme* than other *L. esculentum* cultivars. However, they did not examine the very rapidly germinating P.I.'s of *L. esculentum* reported here nor did they test germination at 10 °C.

Table 5. Pairwise comparisons of *Lycopersicon spp* accessions at 10°C. Values are the tail probabilities that two accessions share the same germination distribution. Rank corresponds to accession identity in Table 4.*

Rank	1	2	3	4	5	6	7	8	9	10	11	12
2	0.000											
3	0.000	0.853										
4	0.000	0.004	0.009									
5	0.000	0.000	0.000	0.007								
6	0.000	0.000	0.000	0.000	0.206							
7	0.000	0.000	0.000	0.005	0.867	0.052						
8	0.000	0.000	0.000	0.000	0.333	0.393	0.155					
9	0.000	0.000	0.000	0.000	0.036	0.419	0.005	0.079				
10	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.004			
11	0.000	0.000	0.000	0.000	0.001	0.016	0.002	0.015	0.056	0.631		
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.118	0.019	
13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

* Pairwise comparisons of additional accessions tested are available on request.

Probability density. Germination responses can be further characterized by their probability density functions. These functions indicate the probability per unit time that a seed will germinate in a given time interval. Plots of this function show the distribution of germination rates over time. Germination rates for the top three-ranked *L. esculentum* genotypes and a control are presented in Figure 1A as probability density functions. Peaks in the density function indicate high germination rates, while the distribution over time is indicative of the length of time over which a population of seeds germinated. Note that the plots of the density functions are frequently skewed to the right. The nonparametric comparisons performed by survival analysis are not dependent on normally distributed data, thus reiterating the advantage of this method of analysis.

Distribution of the probability density function for PI 120256 is substantially earlier than other genotypes. Its highest germination rate (probability of 0.32) occurs on day seven, while the maximum rate for PI 341988 occurs about day 10, as did PI 174261. The breeding line T6 was delayed considerably, in comparison, with a maximum rate near day 15.

Germination rates at 10°C for all accessions tested in the screen of *Lycopersicon spp.* and selected cultivars, expressed as probability density functions, are presented in Figures 1B and C, 2 and 3. Plots are grouped by species. The maximum germination rate for *L. chilense* LA 460 (Figure 2A), was observed on day 7. This probability of 0.340 was the highest observed at 10°C among all genotypes. The two accessions of *L. chmielewskii* (Figure 2A) had maximal germination rates at day 13 and 18. *L. esculentum* genotypes are presented in Figures 1B and C. PI 120256 again had the highest rate (0.267) among these five genotypes. Differences between Figures 1A and B are due to location within the incubator; seeds in Figure 1B were at a slightly colder location. Small differences in temperature may result in moderate changes in the germination. Density functions of control cultivars of *L. esculentum* (T5 and T6) were shifted substantially to the right.

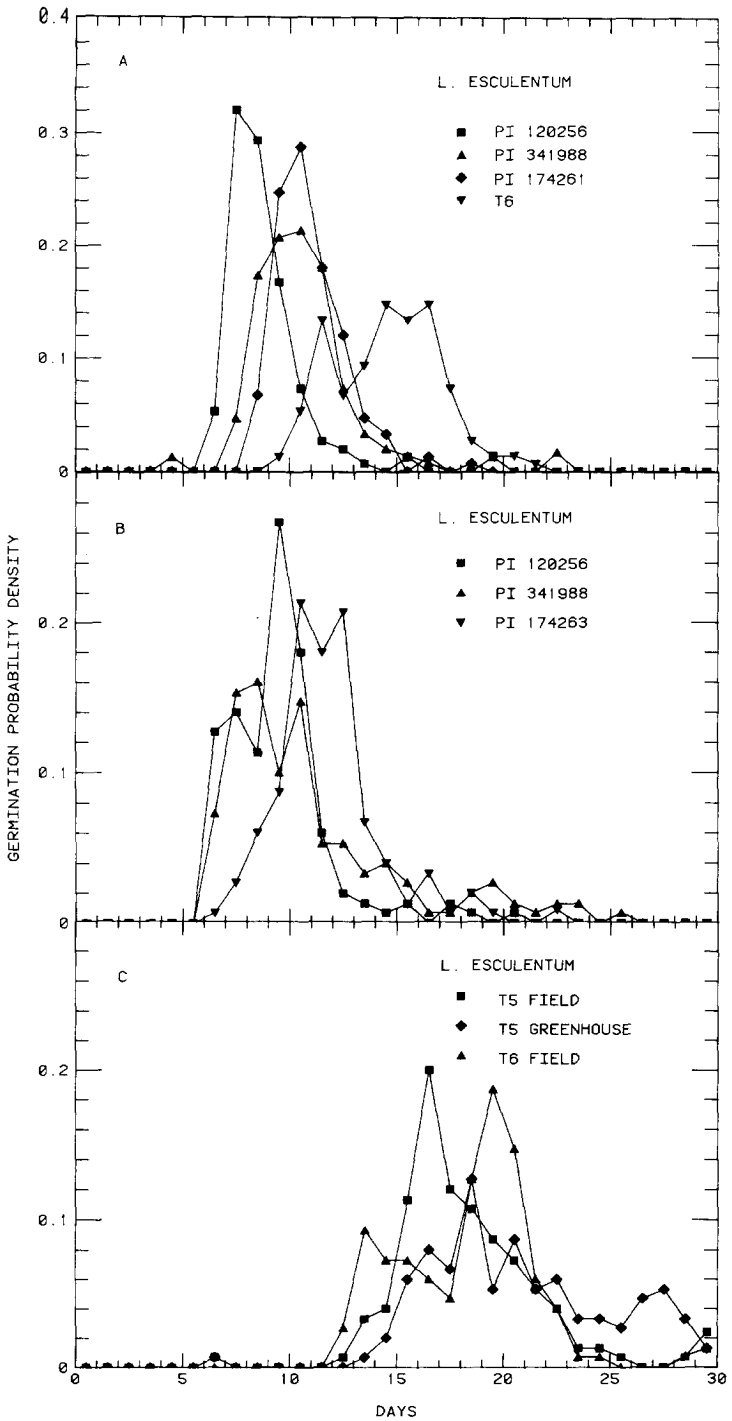

Fig. 1. Germination rates at 10°C, expressed as probability density functions of three superior *L. esculentum* accessions and control in the initial screening experiment (A); *L. esculentum* accessions included as known cold germinators (B) and normal *L. esculentum* controls (C) in the screening of wild *Lycopersicon spp.*

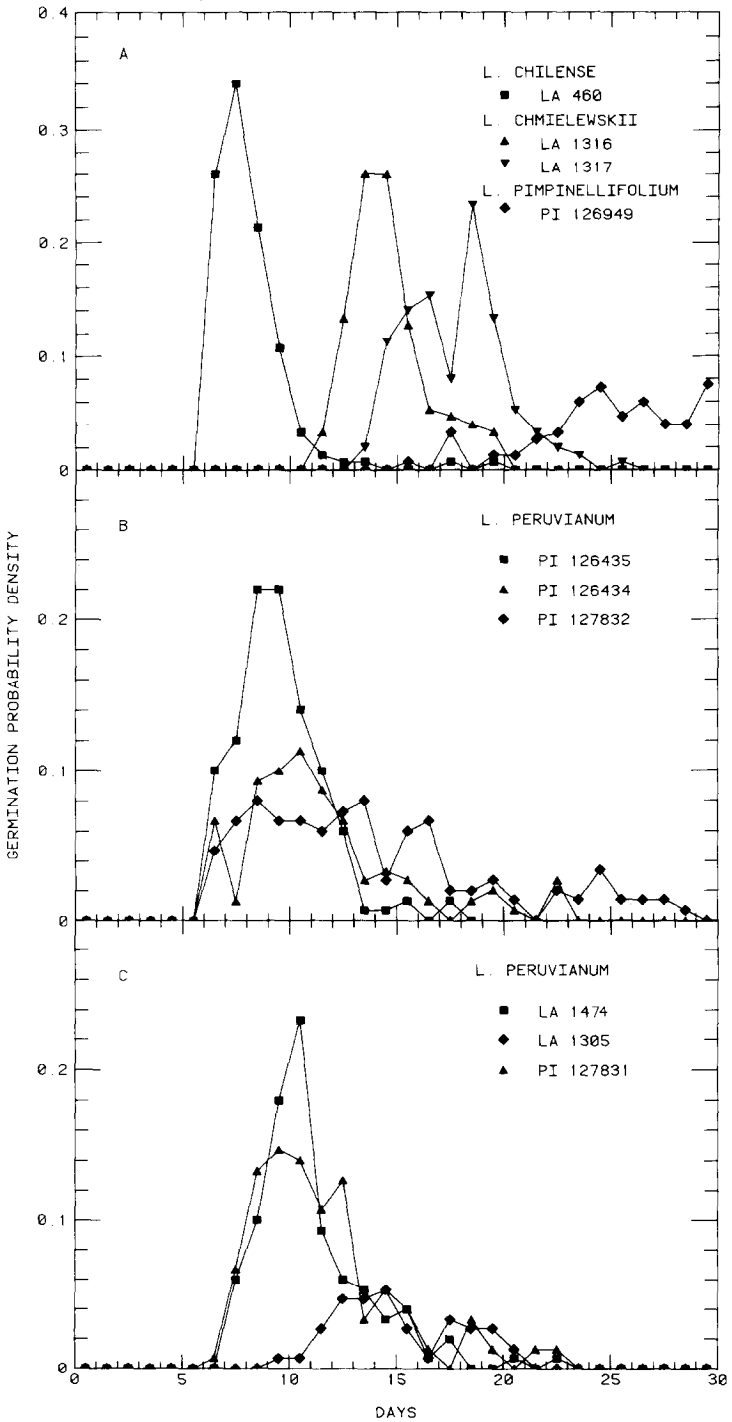

Fig. 2. Germination rates of high altitude accessions of *Lycopersicon spp.* at 10 °C, expressed as probability density functions. A) *L. chilense*, *L. chmielewskii* and *L. pimpinellifolium*; B) *L. peruvianum*; C) *L. peruvianum*

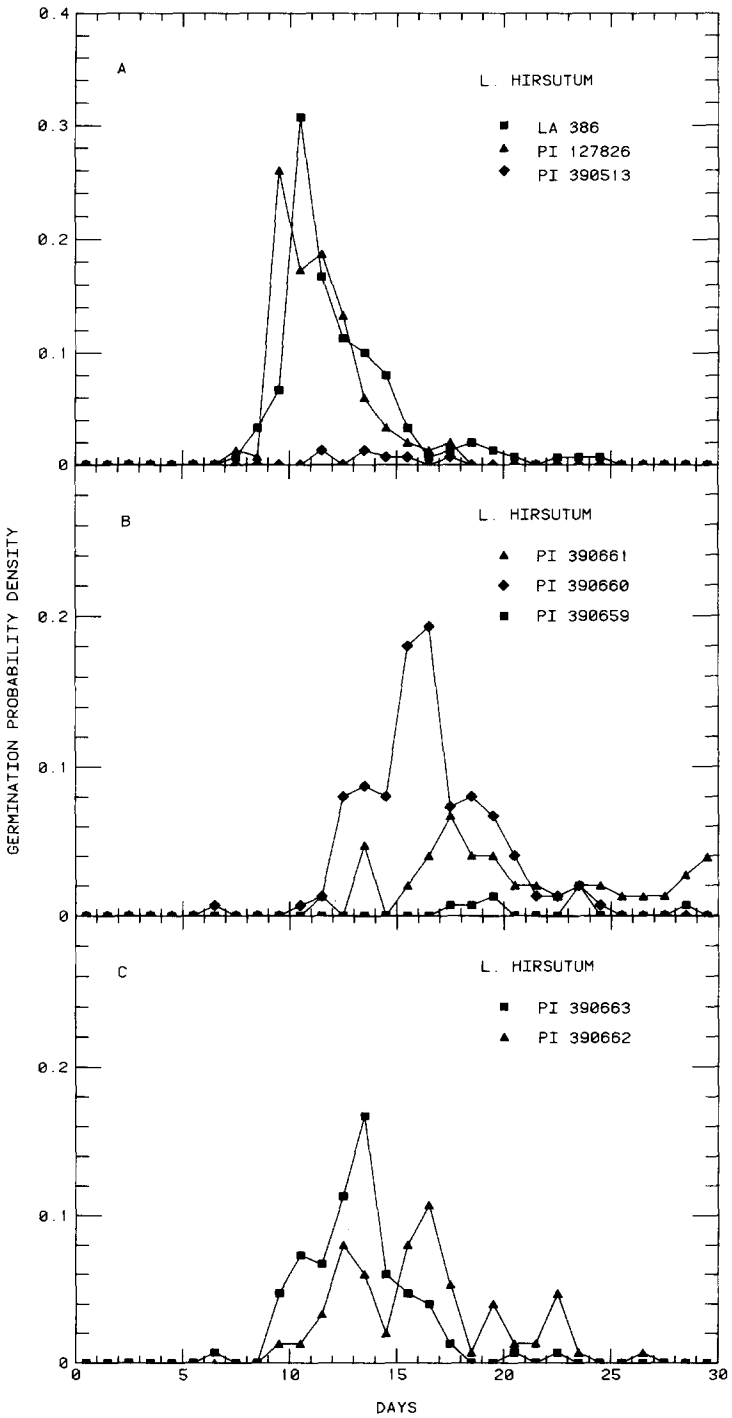

Fig. 3. Germination rates of high altitude *L. hirsutum* accessions at 10 °C, expressed as probability density functions.

Germination rates for *L. hirsutum* ecotypes tested are plotted in Figures 3A, B and C. The highest rate was observed for L.A. 386 (0.307) and second for PI 127826 (0.26). The peak for PI 127826 occurred on day 9.5, as compared to day 10.5 for LA 386. Germination rates and times of other accessions were rather poor. Probability density functions of *L. peruvianum* accessions are illustrated in Figures 2B and 2C. The superior performance of PI 126435 is readily apparent in Figure 2B, with a maximal germination probability of 0.220 occurring on days eight and nine. The width of the germination distribution suggests a large variance. Genetic diversity may be maintained by populations of self-incompatible individuals. This suggests potential for selecting superior individual genotypes within populations of PI 126435. The maximal germination rate for PI 126434 was half that of PI 126435. The highest probability observed for LA 1474 was 0.233 on day 10.5. PI 127831 had a peak germination probability of only 0.147 at day 9.5. Germination rates of *L. pimpinellifolium* PI 126949 were very low (0.073) and very late in time.

Comparison of temperatures. Genotypes that germinate rapidly at 10 °C also tend to germinate rapidly at 20 °C, although this trend was not always consistent. In the wild *Lycopersicon* screen, PI 126435 germinated most rapidly at 20 °C while PI 127832 was ranked second by its $\overline{U}$ score. *L. chilense* (LA 460) ranked fifth, and PI 120256 ranked fourth. Linear correlation between $\overline{U}$ scores at 10 and 20 °C in the screen of wild material was 0.688; correlation between ranking at 10 and 20 °C was 0.649. This trend suggests that some of the same genetic and physiological parameters that allow rapid germination in the cold may also permit rapid germination at ambient temperatures, but that additional components may also be involved at low temperatures.

One may infer that processes required for germination are very temperature dependent in genotypes that germinate poorly at 10 °C but germinate very well at 20 °C. Processes required for germination are evidently progressively less temperature dependent for the intermediate to rapidly germinating genotypes at 10 °C. Ideally, the best genotypes for use as germplasm to introgress genes into *L. esculentum* would be those exhibiting rapid germination over a wide temperature range.

Elevation. It is interesting to note that the fastest-germinating accessions were not collected at the highest elevations (Table 4). The authors had hypothesized a negative correlation of $\overline{U}$ scores with elevation. However, this correlation is 0.128. Assuming the estimates of elevation are correct, then several hypotheses may be presented to account for this apparent disparity. First, the size of plant populations grown to maintain germplasm since it was orginally collected could affect the degree of inbreeding, and thus loss of some genetic variability. Second, seed increase at 20–25 °C over a number of years may select for certain gene combinations not conferring low temperature tolerance (ZAMIR *et al.*, 1981). Also, we have no information about the microclimate where each collection of wild material was originally made. Microclimates at the highest elevations of collections might have been more protected than locations where LA 460 and PI 126435 were collected.

Another hypotheses is that different species and ecotypes may have evolved different strategies for adapting to their native environment. Rapid germination at low temperatures may not be necessary if temperatures in the day are warm enough for germination and growth. These species grow as perennials in their native habitat, so a plant growing

at a high elevation could have originated as a seed germinating during a period of mild temperatures. Other ecotypes may adapt by selection for cold tolerance even during germination. These ecotypes might have resistance to chilling at later development stages but this has not yet been demonstrated.

CONCLUSIONS

In this study, 18 high elevation ecotypes of wild *Lycopersicon* species were tested for germination at 10 °C. These represent almost all of the known and available high altitude accessions of *Lycopersicon* species. At least one of these, LA 460 (*L. chilense*) was superior to all 22 genotypes of *L. esculentum* reported to germinate well at low temperatures. PI 126435 (*L. peruvianum*) and PI 120256 (*L. esculentum*) were also very promising material. Performance of PI 120256 was generally consistent with earlier reports, but it is disappointing that this characteristic has been known but not exploited. The *L. peruvianum* accessions PI 127831, LA 1474 and PI 127832 as well as *L. hirsutum* PI 127826 and LA 386 may have potential for further selection. This material may provide new sources of germplasm for introgression of genes for low temperature seed germination in tomato. This study represents the most complete attempt at screening diverse *Lycopersicon* germplasm with presumed (due to native habitat) or reported ability to germinate at low temperatures.

This paper proposes use of survival analysis to evaluate low temperature seed germination of *Lycopersicon spp.* Survival analysis of seed germination responses has numerous advantages over previously reported methods because 1) It computes several statistics which describe the distribution of germination responses over time. These are the cumulative survival, probability density and hazard functions. 2) Standard errors are computed for these estimates. 3) It utilizes information such as seeds lost to contamination, seeds that germinate abnormally or fail to germinate. 4) It computes nonparametric statistical comparisons to determine if two (or more) survival curves are from the same survival distribution. 5) Germination responses need not be normally distributed. 6) Time to 50% response is computed.

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