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Responses Associated with Low Temperature Seed Germinating Ability in Tomato[1]

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Abstract. **Factors contributing to genetic differences in low temperature seed germination of tomato (*Lycopersicon esculentum* Mill.) were examined by comparing the cold germinating (CG) accession PI 341985 the non-cold germinating (NCG) 'Centennial' and random F_4 lines with varying low temperature germinating abilities. Rate of radicle elongation at 10°C was similar for both parental genotypes indicating that differences in emergence at 10° are not due to growth rates, but rather to more rapid initiation of germination activities in CG. Preincubation of seeds in hypertonic salt solutions enhanced rate of germination at 10°C equally in both lines, but did not substitute for the genetically based cold germinating ability. Low temperature germinating ability is associated with sprouting at high osmotic concentrations, and with a several fold higher rate of increase in peroxidase activity during the first 10 days of incubation at 10°. Germination at 10° of the NCG lines is improved by activated carbon in the germination media whereas no enhancement occurred in CG lines. Inhibition and/or delay in germination at 10° in NCG lines is due, in part, to low temperature induced formation of activated carbon adsorbable inhibitors of seed germination.**

Direct field seeding of tomatoes in early spring often results in erratic or variable stands as a result of delayed germination due to low soil temperatures or soil crust formation. High vigor seeds that germinate at low temperature are required to maximize seedling emergence under such conditions.

Genetic improvement of low temperature germination can be achieved by selecting individuals from appropriate seedling populations that germinate quickly at low temperature (7, 8). Ng (7) studied the inheritance of low temperature seed germination of PI 341985 and found that the mode of inheritance was polygenic and primarily additive, with dominance for inability to germinate at sub-optimal temperatures. El Sayed (4) reported complex inheritance, whereas Cannon et al. (2) suggested single gene control of low temperature germination.

Ability to germinate at low temperatures would be expected to result from measurable physical and/or physiological differences in seed of contrasting genotypes. The purpose of this study was to detect responses associated with cold germinating ability in the tomato PI 341985.

Materials and Methods

Seed source. Seed of the cold germinating (CG) PI 341985, the non-cold germinating (NCG) 'Centennial', and partially isogenic F_4 lines of a 3rd backcross to 'Centennial' with varying abilities to germinate at 10°C, were produced at Lafayette, Indiana. Seed of all genotypes used in these studies were extracted and processed by a standard procedure involving fermentation for 24 hr followed by treatment with 10% hydrochloric acid for 5 hr. Seed were subsequently washed and dried in a forced air oven at 38° for 72 hr.

Rate of radicle elongation. Seeds of CG and NCG tomatoes were incubated in Petri dishes on moistened filter paper at 25°C for 48 hr. Unsprouted seeds were removed and dishes incubated at 10° in the dark. Radicle elongation at 10° was measured at two day intervals to 12 days.

Low temperature germination response to salt pretreatment. Seed of CG and NCG tomatoes were incubated at 25°C in Petri dishes lined with Whatman #1 filter paper moistened with 5 ml of double salt (1.8% KNO_3 + 1.8% KH_2PO_4) solutions for 1 to 8 days as described by Ells (3). Following this treatment, seed were washed in tap water and dried for 2 days at room temperature and germinated at 10° ± 1° in the dark. Germinated seeds were counted and removed daily for 21 days and the germination index (GI) at 10° calculated by the formula:

$$GI\ (10^{\circ}C) = \frac{\Sigma\ [(22 - \text{days to sprouting}) \times (\text{No. seeds sprouted})]}{\text{Total no. seeds}}$$

Germination at 25°C in hypertonic solutions. Seeds of CG and NCG tomatoes were incubated in the dark at 25°C in Petri dishes lined with Whatman #1 filter paper moistened with double salt (KNO_3 and KH_2PO_4) solutions of varying concentrations (0 to 1.8% by weight of each salt with increments of 0.2%). Four replicates of 50 seed were used for each treatment. Seed which germinated during salt treatment were counted and removed daily for 14 days.

To correlate the rate of germination at 25°C in hypertonic solutions with cold germinating ability, random F_4 lines with varying germination indices at 10°C were incubated at 25° for 14 days in the highest salt concentration (1.8%) and germination in the salt solution was correlated with GI at 10°C.

Changes in peroxidase activity. Seeds of the CG and NCG parents were incubated at 10° ± 1°C as described previously. Peroxidase activity of the seed was assayed at 1 day intervals until the 10th day of incubation by the method of Mudd et al. (6). Dry untreated seed were used to assay initial peroxidase activity levels. Peroxidase activities were also assayed at daily intervals in seed incubated at 25°C for 4 days.

To correlate peroxidase activities with cold germinating ability, F_4 lines were assayed for peroxidase activity after 0, 5 and 10 days of incubation and activity correlated with the mean G.I. at 10°C.

Germination on activated carbon substrates. Powdered activated carbon (Darco G-60) was thoroughly mixed with washed sand at rates of 0, 2, 4, 6, 8 and 10% by weight. Four replicates of 100 seed of the CG and NCG parents were incubated in the dark at 10°C for 21 days in Petri dishes filled with 20 g of the appropriate substrate. Germinated seed were counted and removed daily.

Results and Discussion

Rate of radicle elongation. The rate of radicle elongation at 10°C for the NCG 'Centennial' (0.66 mm/day) was not

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significantly different from the CG accession PI 341985 (0.43 mm/day). This suggests that germination differences between these accessions are not a result of differential low temperature growth rates, but rather to earlier or more rapid initiation of the germination process.

Low temperature germination response to salt pre-treatment. Salt pretreatment prior to incubation at 10°C hastened germination for both the CG and the NCG seed sources (Table 1). GI increased and time to 50% germination decreased with increasing length of salt treatment. Pre-incubation for 2 days in salt solution was adequate to hasten germination significantly in both cultivars. Only minor improvement was observed with salt treatment for more than 3 days for CG seeds or more than 5 days for NCG seeds.

The longest period of pre-incubation in salt solution (8 days) could not bring NCG seeds to equal the performance of untreated CG seeds. This indicates that the mechanism for hastening germination at low temperature as a result of salt pre-treatment is distinct from the genetic improvement in germination at low temperatures. Nevertheless, it raises the possibility of combining CG germplasm with salt pre-treatment to obtain rapid and uniform germination at low temperatures (7).

Table 1. Effect of pre-incubation salt treatment on germination at 10°C.

No. days in salt treatment	Germination indices at 10°C[z]		No. days to 50% germination[z]	
	PI 341985	Centennial	PI 341985	Centennial
0	12.6c[z]	0.5i	8.0ef	22.5a
1	11.8cd	1.6hi	8.3ef	20.9a
2	13.3b	3.2gh	5.0gh	17.9b
3	17.8a	4.5fg	3.0hi	17.5bc
4	17.5a	6.4f	2.4hi	15.2c
5	18.7a	10.5de	1.7i	10.6de
6	19.1a	9.2e	1.8i	10.8de
7	18.7a	11.8cd	1.7i	6.6
8	17.2a	9.6e	1.6i	12.2d

[z]Mean separation between and within accessions by Duncan's multiple range test, 5% level.

Sprouting at 25°C in hypertonic solutions. During the course of studies on salt pretreatment effects on germination, it was noted that CG seeds germinated during pretreatment at salt concentrations commonly recommended for this treatment. Germination of CG and NCG seeds at various osmotic concentrations was examined to determine the effect of osmotic concentration on germination of each genotype. Reductions in germination with increased osmotic tensions were observed in both CG and NCG seeds, however at high salt concentrations this reduction was much more pronounced for NCG than CG seeds (Fig. 1). At the highest concentration, NCG seeds showed almost no germination whereas CG seeds had 66% germination.

To determine the association between low temperature germination and germination at high osmoticum, germination indices of F_4 lines in the highest salt solution were plotted against their respective germination indices at 10°C (Fig. 2). Lines with intermediate or high CG ability appear to be less affected by tonicity of the germination media than lines lacking CG ability (Fig. 2). The lack of systematic differences in germination in hypertonic solutions between genotypes with intermediate and high cold germinating ability suggests, however, that not all factors involved in low temperature germination also control germination under high osmotic pressure.

Changes in peroxidase activity. During incubation at 10°C, three was a striking difference between CG and NCG seeds in the rate of peroxidase activity changes with time. The rate of increase was about 9 fold greater in CG than in NCG seeds (Fig. 3). Moreover, the differences in peroxidase activity between lines were significantly different as early as the 4th day of incubation, even though visible germination does not begin until the 10th or 11th day at this temperature in the CG line. No difference between lines was found during incubation at 25°C.

Fig. 4 shows the association between peroxidase activity after 0, 5, and 10 days of incubation at 10°C, and cold germinating ability of random F_4 lines as measured by germination indices. For dry seeds there was no difference in initial peroxidase activities between lines with differing CG ability. The good

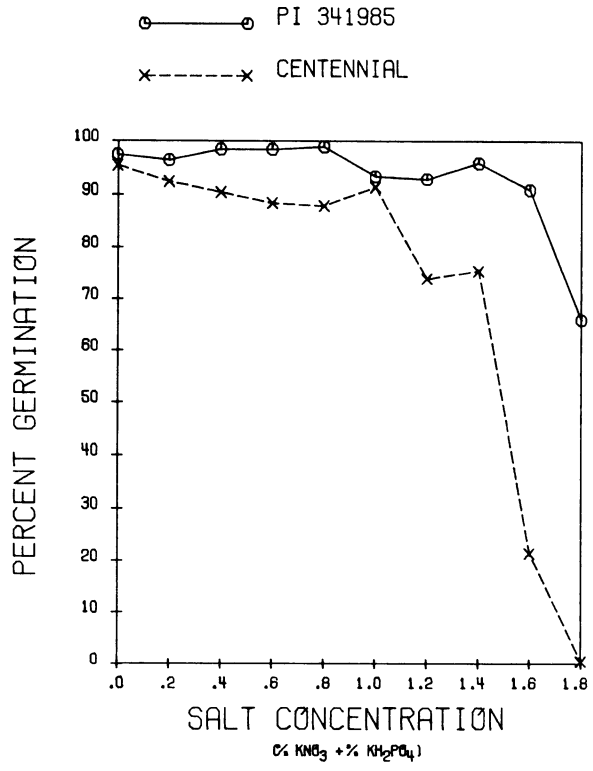

Fig. 1. Percent germination of PI 341985 and Centennial after 14 days of incubation at 25°C in hypertonic solutions.

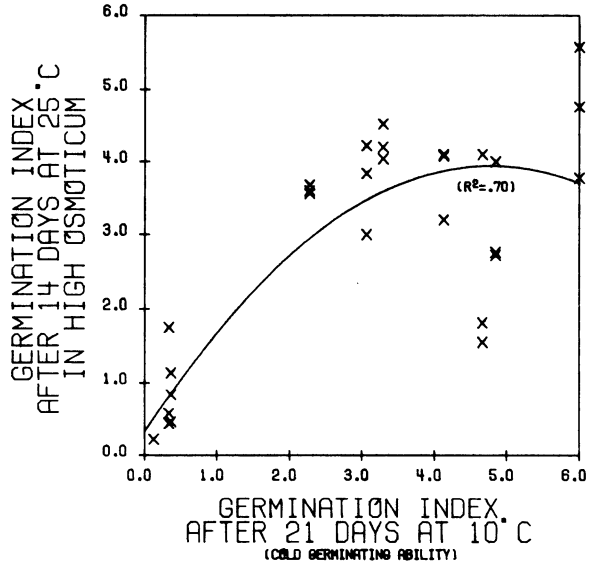

Fig. 2. Germination at 25°C in hypertonic salt solution (1.8% KNO_3 + 1.8% KH_2PO_4) as a function of cold germinating ability of random F_4 lines.

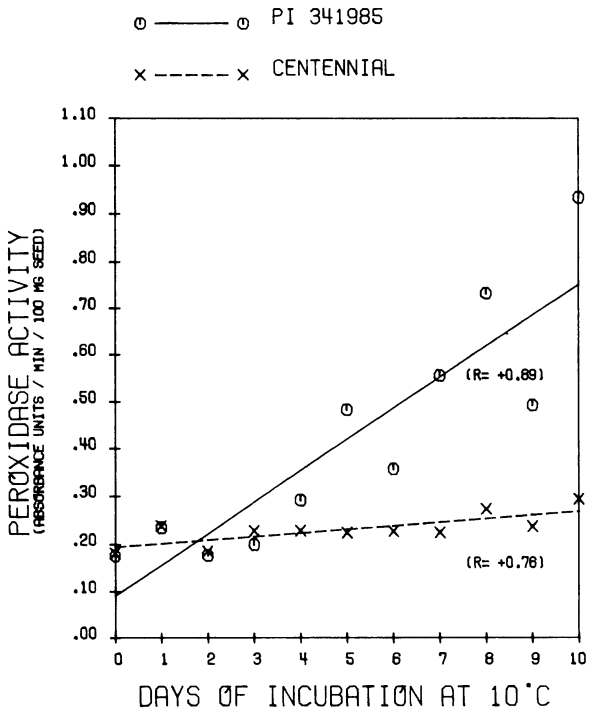

Fig. 3. Changes in peroxidase activity of PI 341985 and Centennial with time of incubation at 10°C.

fit of the asymptotic regression curves following 5 days (R^2 = .80**) or 10 days (R^2 = .72**) of incubation shows that peroxidase activity increases more slowly at 10°C for lines

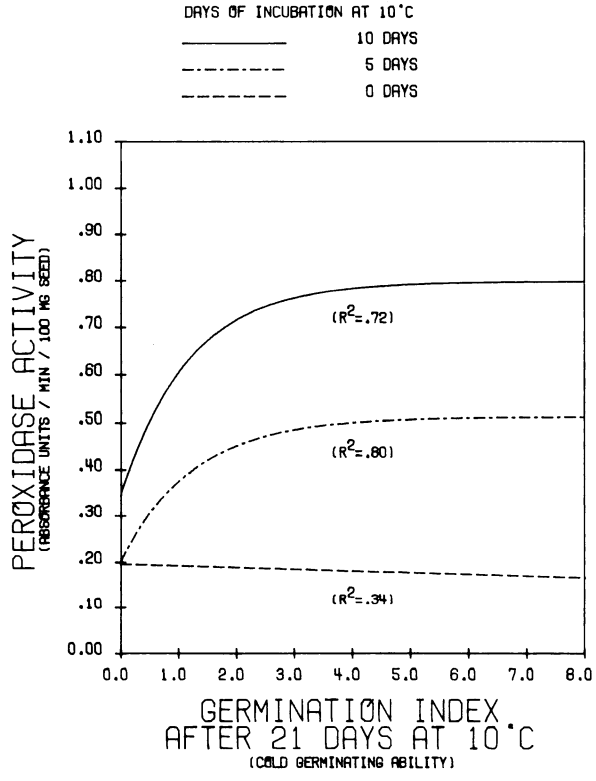

Fig. 4. Relationship between cold germinating ability of tomato lines and peroxidase activity after 0, 5 and 10 days of seed incubation at 10°C.

Table 2. Germination of PI 341985 and Centennial, incubated for 21 days at 10°C on sand-activated carbon substrates..

Carbon (%) on substrate	Percent germination at 10°C[z]		Germination indices at 10°C[z]	
	PI 341985	Centennial	PI 341985	Centennial
0	95.0ab	7.5e	10.1c	0.4e
2	98.5a	89.0bcd	11.9a	4.6d
4	87.0d	86.5d	10.5bc	4.0d
6	95.0ab	84.5d	11.6c	4.5d
8	96.0ab	88.5d	11.5ab	5.2d
10	95.0ab	84.5d	11.9a	4.6d

[z]Mean separation between and within accessions by Duncan's multiple range test, 5% level; for the % sprouting data, the arc sin $\sqrt{p}$ transformation was performed before the test.

with very low germination indices than for lines with intermediate or high indices. Elevated peroxidase activity at 5 or 10 days can be used to distinguish NCG from CG lines, however, the absence of clear-cut differences in peroxidase activity between intermediate and high CG lines at 10°C indicate that changes in this enzyme activity alone cannot be used as a sole indicator of cold germinating ability.

Germination on activated carbon substrates. The germination indices and total % germination of CG and NCG seeds as a function of activated carbon level in the germination medium are shown in Table 2. Germination indices of CG seeds were only slightly improved in the presence of activated carbon, but for NCG, seeds the increments in both GI and % germination were highly significant with as low as 2% activated carbon in the germination media. NCG seeds did not, however, achieve the same level or speed of germination as CG seeds without activated carbon.

These results support the proposal of Abdul-Baki and Stoner (1) that inhibitors formed by NCG seeds at low temperatures retard germination. They reported the presence of an inhibitor in the leachate of the NCG 'Red Rock', and a promoter of germination in the leachate of a closely related CG accession PI 341984 when these lines were incubated at 5°C. Our results suggest these inhibitors may be adsorbed by activated carbon in the germination medium, thereby accounting for the germination stimulation of NCG seed in the presence of activated carbon.

Evidence for promoters of germination of CG seed were not examined, however, the fact that activated carbon does not completely remove inhibition of germination in NCG seed provides indirect evidence that other factors also contribute to the improved low temperature germination of CG seed. Since activated carbon did not retard germination of CG, seed it would appear that activity of possible promoters are not affected by activated carbon.

The multiple physiological differences associated with ability vs. inability to germinate at low temperature raises the question of the cause and effect relationships between growth rate, absence of germination inhibitors, elevated peroxidase activity, and germination in high osmoticum. Growth rate at low temperature does not account for the observed differences in emergence. in fact NCG seeds showed slightly more rapid radicle elongation than CG seeds. Ability to germinate at low temperature is therefore not a valid indicator of improved ability to grow at low temperatures.

Low temperature inhibition of germination in NCG seeds appears to represent a temporary induced dormancy resulting from germination inhibitors which serve to delay germination until temperature conditions are suitable for emergence and growth. The transition from the dormant to non-dormant

condition in various seeds is frequently accompanied by an increased functioning of the pentose phosphate pathway (9). Hendricks and Taylorson (5) have shown that salt stimulation of lettuce and pigweed seed germination is accompanied by inhibition of the enzyme catalase. Inhibition of catalase spares hydrogen peroxide (H_2O_2) which in turn oxidizes reduced nicotinamide adenine dinucleotide phosphate (NADPH) via the enzymes peroxidase and NAD(P)H quinone oxidoreductase for use in the metabolism of glucose via the pentose phosphate pathway. Thus, peroxidase is implicated in control of seed germination and its increased activity in CG seeds at low temperature implies a function, possibly in regulating glucose metabolism via the pentose phosphate pathway.

It remains to be determined whether the observed elevated peroxidase activity in CG seeds at low temperature represents a primary or secondary effect of genes regulating low temperature germination. Likewise the association between ability to germinate at low temperature and under high osmoticum may represent pleiotropic effects of genes regulating low temperature germination. Results suggest that common mechanisms may exist which inhibit germination under these 2 forms of stress.

These studies support prior genetic analysis which indicated multigenic inheritance for ability to germinate at low temperature (4, 7). The recovery of F_4 lines with low temperature germinating abilities intermediate between the parents does not support single gene control proposed by Cannon et al. (2). The appearance of the parental phenotypes at moderately high frequencies in F_4 supports results which suggested relatively simple (3-5 genes) additive genetic control (7).

Literature Cited

1. Abdul-Baki, A. A. and A. Stoner. 1978. Germination promoter and inhibitor in leachates from tomato seeds. *J. Amer. Soc. Hort. Sci.* 103:684-686.
2. Cannon, O. S., D. M. Gatherum, and W. G. Miles. 1973. Heritability of low temperature seed germination in tomato. *HortScience* 8:404-405.
3. Ells, J. E. 1963. The influence of treating tomato seed with nutrient solutions on emergence rate and seedling growth. *Proc. Amer. Soc. Hort. Sci.* 83:684-687.
4. El-Sayed, M. N. and C. A. John. 1973. Heritability studies of tomato emergence at different temperatures. *J. Amer. Soc. Hort. Sci.* 98: 440-443.
5. Hendricks, S. B. and R. B. Taylorson. 1975. Breaking of seed dormancy by catalase inhibition. *Proc. Nat. Acad. Sci.* (USA) 72:306-309.
6. Mudd, J. B., B. G. Johnson, R. H. Burris, and P. Buchholtz. 1959. Oxidation of indoleacetic acid by quackgrass rhizomes. *Plant Physiol.* 34:144-148.
7. Ng, T. J. 1972. Inheritance and physiology of low temperature germinating ability in tomato (*Lycopersicon esculentum* Mill.). MS Thesis, Purdue Univ., West Lafayette, Ind.
8. _____ and E. C. Tigchelaar. 1973. Inheritance of low temperature seed sprouting in tomato. *J. Amer. Soc. Hort. Sci.* 98:314-316.
9. Roberts, E. H. 1973. Oxidative processes and the control of seed germination. p. 189-218. *In* W. Heydecker (ed.) Seed ecology symposium. Nottingham, Englang, 1972. Butterworths, London.

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Effect of Nitrogen Status of Dormant Rooted Lowbush Blueberry Cuttings on Rhizome Production[1]

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Abstract. **Cuttings of 2 clones of lowbush blueberry (*Vaccinium angustifolium* Ait.) treated with increasing rates of urea (0, 40, 80 kg N/ha) during rooting resulted in increased levels of total stem nitrogen in the respective treatments. Fall flowering and vegetative growth of cuttings in the propagation beds were stimulated by nitrogen treatments. Plants moved to cold storage (5°C) for 3 months and then grown under long day (16 hours) greenhouse conditions for 2 months did not produce more rhizomes in response to nitrogen treatments. However, plants which remained in the propagation beds during the winter and which were grown under field conditions for 6 months, responded to the nitrogen treatments by producing larger root systems, more aerial growth and more rhizomes.**

Kender (4) suggested that production of lowbush blueberries in a matted row culture system was feasible. However, a major problem in establishing such a system using vegetatively propagated plant material is the low rate of rhizome production (5). N has been shown to be the critical nutrient element determining length, vigor, and fruitfulness of lowbush blueberry stems (8). Kender and Eggert (6) investigated various soil management practices and substantiated the findings of Trevett (8), that blueberry plant spread could be increased by mulching and N fertilization. Kocher (7) showed a relationship between endogenous N levels in rhizome cuttings and bud activity and suggested that inhibition of lateral buds on rhizomes with low endogenous N could be attributed to the capacity of the distal buds to monopolize the nutrient supply. Thus, the N status of rooted cuttings may also influence subterranean bud release and rhizome formation.

The objective of this experiment was to determine the effect of N status of dormant rooted lowbush blueberry cuttings on rhizome initiation and development.

Materials and Methods

Two vigorous field clones were propagated on July 2, 1975 by softwood cuttings in a 1 peat:1 sand mixture under an outdoor intermittent mist system. There were 756 cuttings from each clone in separate beds, divided with wooden partitions into nine .5 × 1.0 M treatment plots, 84 cuttings per plot.

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