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Effect of chilling on germination, growth, survival and membrane permeability in seedlings of different breeding forms of maize (*Zea mays* L.)*

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Abstract

Cold tolerance of 15 inbred lines and 10 hybrids of maize was evaluated on the basis of seedling survival and membrane permeability changes of leaf cells after 7 days of chilling at 5 C, growth rate of seedlings at different temperatures as well as on the basis of seed germination rate under various chilling temperature regimes. The great differentiation in seedling survival allowed to identify cold sensitive and cold resistant genotypes. A high correlation between seedling survival and membrane permeability of leaf cells ($r = 0.848$***) was found for the genotypes studied. These results were, however, inconsistent with the sensitivity of seed germination for chilling temperatures. Seedling growth at 10°C and 15°C did not allow for a clear-cut evaluation of the cold tolerance of the experimental genotypes but chilling (5 C)-induced modifications of seedlings growth at 20 C corresponded well to the cold tolerance of genotypes, as estimated by the seedling survival and electric-conductivity tests.

Introduction

In the Polish climatic zone maize cultivation, particulary for seed crops, is limited by the often unfavourable temperature conditions during germination, seedling growth and also the maturation of plants. Therefore, the evaluation

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of possible differences in the sensitivity of various breeding forms to chilling temperatures prevailing at the time of germination and emergence of seedlings is of great importance. The differences in the cold tolerance of older seedlings are of special importance, because favourable weather at the time of germination and seedling emergence may be followed by much colder spells inducing the damage of leaves or even of the whole crops. Another problem is the sensitivity of growth processes of maize to low temperatures, as a close relationship between growth rate of seedling and the grain yield has been demonstrated (Mock and McNeill 1979).

The studies presented below were aimed at testing the cold tolerance of fifteen inbred lines and ten hybrids of maize under controlled conditions and at comparing different cold-tolerance traits. The seedling survival rate and membrane permeability changes in leaf cells of twelve-day-old seedlings exposed to severe chilling (5°C), dry weight accumulation and height increments in seedlings grown at different suboptimal temperatures (10°C, 15°C) or pretreated with chilling (5°C) and then transferred to a higher (20°C) temperature were measured. Seed germination rates at different temperatures (7°C, 10°C, 12°C) were also estimated for different breeding forms of maize.

Materials and methods

Seeds treated with dressing T (50% thiuram) were planted in pots filled with 3:1 mixture of peat and soil.

In the first experiment the germination, emergence and growth of seedlings occurred in an air-conditioned glass-house in natural light at 25°C (for the first two days), and then at 20°C (for the next ten days), at relative air humidity 70%±5%. Every treatment consisted of five pots, each containing 15 plants. The temperature and the time of chilling were chosen on the basis of preliminary experiment, in which seedlings of chilling sensitive hybrid (S-72xCo-125) were chilled at several cold temperatures affecting different time. In the present experiment, the twelve-day-old plants were chilled for seven days at 5°±0.7°C in air-conditioned chambers, in a day/night cycle of 15/9 h, respectively; light intensity 50 W/m^2 (metal-halide ROF 400 W lamps) and relative air humidity 60%±5%. After chilling, they were transferred back to the initial conditions of growth and a week later dead plants were counted and the plant survival was calculated in percentage of the original plant number.

Membrane permeability changes were determined by measurements of electrolyte leakage from leaves before and after chilling. Disks (0.9 cm in diameter) were cut from the middle parts of the first three seedling leaves, immersed in 20 ml redistilled water and shaken for 24 h in darkness at 20°C. Electroconductivity of the solution thus obtained was measured with a conductometer (OK 102/1 Radelkis, cell constant of electrode 1.03). The plant material was then heat-killed, shaken for 24 h at 20°C in darknes and the total content of electrolytes was measured. All electroconductivity measurements were made in a thermostatic chamber at 20°C±0.7°C, with five replicates in every treatment, every replicate being composed of five disks excised from five plants.
The results were calculated according to the formula:

$$I_t = \frac{R_t - R_o}{100 - R_o} \times 100$$

where: R_t and R_o are the electrolyte leakage from chilled and control seedlings, respectively, expressed in per cent of total electrolyte content in tissue (Flint *et al.* 1967).

In the second experiment, dry weight and height of seedlings were determined at first after 12 days of growth at 20°C and then after 9 days at 5°, 10°, 15° and 20°C. All other conditions of seedling growth were exactly the same as in the first experiment. Additionally, plants chilled at 5°C were returned to the initial 20°C and grown for further nine days before measuring their final dry weight and height. Temperature-induced changes in dry weight and height increments of seedlings grown at 10° and 15°C are shown as per cent of growth observed at 20°C. However, increments of dry weight and height in 20°C of seedlings prechilled at 5°C are expressed as per cent of these parameters for seedlings 21-days-old growing continuously at 20°C.

The third experiment was aimed to determine germination rate at different temperatures. Seeds were placed on wet paper in Petri dishes in five replicates with 25 seeds in each and kept at 7°, 10°, 12° and 20°C±0.2°C for 7 days, and then germinated seeds were counted. A seed was considered as germinated when its germ had emerged through the seed coat and was visible to the naked eye. The results are shown as per cent of seeds germinating at 20°C.

The data in Tables 1, 2 and 3 come from one series of experiments, whereas for the determination of the rank summation indexes for cold tolerance (Table 4) all the experiments were repeated twice (with the exception of the part of the second experiment concerning the effect of chilling pretreatment on seedlings growth). The values of the index were obtained by: a) arrangement of means, for inbred lines and hybrids separately, in the ascending order of the sensitivity to chilling within all variants of every cold-tolerance trait investigated in both series of experiments; b) giving every breeding form ranking number according to the position: from 1 to 15 for inbred lines and from 1 to 10 for hybrids; c) summing the ranking numbers for each breeding form within every cold-tolerance trait investigated — for instance the index for the breeding form with the greatest increments of dry weight and height at 10°C and 15°C in both series of experiments would amount to 8 and, similarly, the index for the form with the lowest increments of dry weight and height at both temperatures and in both series of experiments would amount to 120 in the case of inbred lines and 80 in the case of hybrids.

The statistical significance of differences was evaluated by the variance analysis using the F-test and Duncan's multipie t-test. The variance analysis in the case of per cent of dead plants after seven days at 5°C and per cent of germinated seeds after seven days at 7°, 10° and 12°C was carried out with data transformed according to the formula arc sin $\sqrt{x}$.

Results

Chilling conditions, *i.e.* 5°C during 7 days, which had been established in the preliminary experiment, allowed to demonstrate the great differentiation of cold tolerance among experimental breeding forms in the range from 4% to 100% of injuries (Table 1). It was thus possible to identify four cold resistant lines (EP-1, F-7, SR-10, S-25) and three cold sensitive ones (Co-125, Co-151, S-72) among the 15 inbred lines, and two cold resistant (SR-10x EP-1, F-7xF-2) and two cold sensitive (S-72xCo-125, S-72xCo-151) forms among the ten hybrids. Moreover, it was found that inbred lines with flint type grain and their hybrids were more resistant to cold than form with dent type grain.

Table 1

Plant injuries and electrolyte leakage from leaves of maize seedlings after 7 days of chilling at 5°C

No.	Breeding form	Type of grain	Plant injury (%)*	Electrolyte leakage (%)
		Inbred lines		
1	EP-1	Ft**	7.8 a***	19.2 a
2	F-7	Ft	24.8 bc	32.0 abc
3	SR-10	Ft	20.2 ab	30.1 abc
4	S-25	Ft	31.0 bcd	41.5 abcd
5	F-2	Ft	27.5 bc	46.2 bcd
6	S-3	Ft	48.5 de	25.0 ab
7	F-115	Dt**	53.8 e	53.2 cde
8	Cm-7	Ft	42.2 cde	27.9 ab
9	S-61	Dt	38.8 cde	30.5 ac
10	W-33	Dt	57.7 e	27.7 ab
11	F-83	Ft	55.8 e	59.4 def
12	S-37	Dt	90.0 f	74.5 efg
13	S-72	Dt	100.0 f	76.2 efg
14	Co-151	Dt	100.0 f	78.2 fg
15	Co-125	Dt	100.0 f	90.8 g
x̄			53.2	47.5
		Hybrids		
1	SR-10xEP-1	FtxFt	4.0 a	49.3 ab
2	F-7xF-2	FtxFt	15.5 abc	49.6 ab
3	S-72xS-61	DtxDt	8.8 ab	35.7 a
4	Cm-7xCo-125	FtxDt	41.2 de	42.7 a
5	S-72xCm-7	DtxFt	8.2 ab	42.4 a
6	F-7xCo-151	FtxDt	26.2 bcd	66.1 bc
7	SMTC-278	DtxDtxFt	10.3 ab	49.9 ab
8	S-72xF-2	DtxFt	36.6 cd	72.7 c
9	S-72xCo-151	DtxDt	63.7 ef	84.9 c
10	S-72xCo-125	DtxDt	67.7 f	76.9 c
x̄			28.2	57.0

* Dead plants as percentage of the number of plants before chilling.
** Ft — flint, Dt — dent.
*** Means within each column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple t-test.

Changes in membrane permeability of leaf cells as indicated by electrolyte leakage showed a similar differentiation among breeding forms as that shown by the seedling survival (Table 1). The smallest increase of membrane permeability at low temperatures was observed in genotypes defined as resistant according to their survival percentage, whereas the highest increase of permeability occured in genotypes defined as cold sensitive. The relation between seedling survival and the changes in membrane permeability under chilling conditions was confirmed by the correlation coefficient for these values, which was 0.848***.

Dry weight and height of seedlings of cold sensitive inbred lines seemed to be higher than those of more resistant inbred lines (Table 2). The correlation index of cold sensitivity of seedlings with their dry weight and height before chilling was r = 0.511 ***, however, similar relationship was not found in hybrids (Tables 2 and 4). The experimental forms differred only to a small degree in the rate of seedling dry weight and height increments at 10°C or 15°C (Table 2). Lowered temperature seemed to inhibit the extension growth (the height increments) to a greater extent than the accumulation of dry weight in seedlings of both inbred lines and hybrids. On the whole, the growth of the resistant inbred lines was less affected by these temperatures than that of the cold sensitive forms (r = 0.512**). No similar relationship, however, was found in hybrids; *e.g.* the sensitive hybrid S-72xCo-125 was one of the fastest growing form at 10°C and 15°C (Tables 2 and 4).

The increments of dry weight and height of seedlings chilled for nine days at 5°C were small and plants of the inbred lines S-72 and Co-151 did not survive. Nine days later, however, after plant transfer from 5°C back to 20°C, the studied genotypes of maize showed differences in the rate of dry weight accumulation and the height increments (Table 2). The slowest was the growth of seedlings from inbred lines S-61 and Co-125 and of hybrids S-72xCo-125 and S-72xCo-151. On the other hand, the most rapid dry weight and height increases occurred in seedlings of inbred lines EP-1, S-25 and SR-10, and of hybrids F-7xF-2, SR-10xEP-1 and F-7xCo-151. It seems, therefore, that forms classified as cold tolerant, on the basis of seedling survival and electrolyte leakage from leaf tissue after seven days of chilling at 5°C (Table 1), also proved to be inhibited to the least extent in their growth. The level of correlation between the extent of injuries in plants treated with 5°C and the effect of this low temperature on the subsequent seedling growth at 20°C was high, r = 0.839***.

Germination rates at 7°C, 10°C and 12°C expressed in per cent of germinations at 20°C revealed significant differences among the genotypes studied (Table 3). The differences are not, however, consistent with the differences in the cold tolerance of the genotypes tested at the seedling growth stage. Only in the case of the cold resistant inbred line SR-10, the germination rate at lowered temperatures was also high (67%). In contrast, the forms EP-1

,* Statistically significant at $P = 0.05$ and 0.01, respectively.

and F-7xF-2, classified as resistant ones at the seedling stage, appeared to be sensitive to chilling during germination (their average germination rate

Table 2

Dry weight and height of seedlings as affected by different temperature conditions

No	Breeding form	Initial		Increments as percentage of control (20 C)					
		dry weight mg/plant	height mm/plant	dry weight		height		dry weight	height
				10 C	15 C	10 C	15 C	(chilling pretreatment) 20 C	
				Inbred lines					
1	EP-1	27	56	34	51	17	39	52	46
2	F-7	24	76	31	47	13	42	27	26
3	SR-10	40	90	32	55	13	40	38	37
4	S-25	32	84	28	51	14	40	49	44
5	F-2	32	88	25	52	10	39	29	36
6	S-3	28	80	25	63	14	50	12	33
7	F-115	30	77	29	56	13	41	13	21
8	Cm-7	41	123	32	54	15	44	24	21
9	S-61	56	116	25	58	13	46	4	9
10	W-33	33	101	31	51	12	40	11	14
11	F-83	33	93	22	47	12	43	31	31
12	S-37	46	91	26	51	12	40	12	11
13	S-72	47	91	34	55	14	37	plants were killed	
14	Co-151	38	87	28	52	13	43	" "	"
15	Co-125	49	107	30	54	11	42	6	13
x		37	91	29	53	13	42	21	23
LSD, P = 0.05		5.6	13.4	10.5	12.9	6.6	7.6	16.9	11.3
				Hybrids					
1	SR-10xEP-1	59	118	40	65	18	50	32	24
2	F-7xF-2	35	100	34	57	19	49	39	31
3	S-72xS-61	63	118	31	50	18	45	19	22
4	Cm-7xCo-125	41	119	28	54	17	44	20	20
5	S-72xCm-7	60	134	31	50	20	46	21	22
6	F-7xCo-151	45	111	30	51	18	42	29	25
7	SMTC-278	51	107	32	57	23	47	9	8
8	S-72xF-2	48	101	35	55	19	46	21	20
9	S-72xCo-151	65	117	29	51	17	45	14	13
10	S-72xCo-125	58	115	31	56	20	53	11	17
x		52	114	32	55	19	47	22	20
LSD, P = 0.05		8.9	14.3	7.0	11.1	4.9	8.1	17.4	6.8

for temperature tested was 15 and 19%, respectively) and forms S-72 and S-72xCo-125, sensitive to cold at the seedling stage, showed a good germination rate at low temperatures (57% and 64%, respectively).

Discussion

The studies performed showed a great differentiation of cold tolerance among inbred lines and hybrids, which is consistent with the data of other authors indicating the large differentiation in this trait in *Zea mays* (Mock and McNeill 1979; Bojarczuk 1972; Mock and Skradla 1978; Segeta 1964). Exposure of plants to 5°C during seven days caused extensive injuries or death of maize seedlings (Table 1). Not all cold related indirect causes

Table 3

Seed germination rate of maize after 7 days of chilling at 7°, 10° and 12°C as percentage of seeds germinating at 20°C.

No	Breeding form	Germination at 7°C	Germination at 10°C	Germination at 12°C	x
		Inbred lines			
8	Cm-7	55.8 a*	86.7 ab	94.8 abc	79.1 A
3	SR-10	13.0 c	88.8 a	100.0 a	67.2 AB
9	S-61	21.6 b	81.6 ab	96.0 abc	66.4 ABC
10	W-33	12.0 c	73.1 bc	98.2 ab	61.1 BC
15	Co-125	6.4 de	76.0 b	93.6 abc	58.6 CD
13	S-72	10.6 cd	76.4 b	84.6 abc	57.2 CD
11	F-83	5.1 e	55.9 cd	87.3 abc	49.4 DE
12	S-37	0 f	40.8 de	96.8 ab	45.8 E
4	S-25	1.0 f	27.2 ef	92.0 abc	40.0 EF
14	Co-151	0 f	10.3 g	84.2 bc	31.5 F
2	F-7	0 f	14.5 fg	75.8 c	30.1 F
1	EP-1	0 f	0 i	45.2 d	15.1 G
5	F-2	0 f	1.0 hi	25.0 de	8.6 G
7	F-115	0 f	9.8 gh	15.5 e	8.4 G
6	S-3	0 f	4.0 hi	18.7 e	7.5 G
	x	8.4	43.1	73.8	41.7
		Hybrids			
3	S-72xS-61	21.6 ab	95.2 a	97.6 a	71.4 A
4	Cm-7xCo-125	19.2 ab	88.0 ab	94.4 abc	67.2 A
10	S-72xCo-125	12.9 a	83.1 ab	97.6 a	64.5 A
5	S-72xCm-7	2.4 cd	71.2 bc	96.0 a	56.5 B
8	S-72xF-2	6.4 bcd	50.4 cd	81.6 bcd	46.1 BC
1	SR-10xEP-1	2.4 cd	37.6 d	94.4 ab	44.8 BC
9	S-72xCo-151	0 d	44.2 d	73.3 d	39.1 C
7	SMTC-278	1.6 cd	35.5 d	78.2 cd	38.4 C
6	F-7xCo-151	4.8 bc	31.5 d	65.3 d	33.8 C
2	F-7xF-2	0 d	0 e	57.4 d	19.1 D
	x	7.1	53.7	83.6	48.1

* Means within each column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple t-test.

Table 4

Rank summation indexes of cold tolerance* of the experimental breeding forms of maize

No	Breeding form	Rank summation index for				
		Plant injury	Electrolyte leakage	Increments of dry weight and height at		Germination at
				10°C and 15°C	20°C**	7°, 10° 12°C
			Inbred lines			
1	EP-1	5.0	8.0	32.5	2.0	77.5
2	F-7	5.0	8.0	68.5	13.0	70.5
3	SR-10	4.0	10.0	58.0	6.0	19.0
4	S-25	7.0	10.0	56.5	4.0	47.5
5	F-2	9.5	13.0	73.0	9.0	71.5
6	S-3	16.5	11.0	54.0	15.0	65.5
7	F-115	14.5	16.0	63.0	17.0	75.5
8	Cm-7	19.0	14.0	56.0	15.0	14.0
9	S-61	14.5	19.0	69.0	26.0	26.0
10	W-33	21.5	14.0	75.5	21.0	25.0
11	F-83	17.0	19.0	64.5	10.0	46.5
12	S-37	22.5	15.0	79.5	21.0	47.5
13	S-72	28.0	25.0	59.5	29.0	26.0
14	Co-151	28.0	28.0	73.5	23.0	69.5
15	Co-125	28.0	30.0	77.0	29.0	38.5
			Hybrids			
1	SR-10xEP-1	2.5	8.0	28.0	5.0	36.5
2	F-7xF-2	6.5	6.0	44.0	2.0	52.5
3	S-72xS-61	10.0	6.0	49.5	12.0	8.0
4	Cm-7xCo-125	11.0	5.0	45.0	12.0	16.0
5	S-72xCm-7	10.5	9.0	52.0	9.0	32.5
6	F-7xCo-151	10.0	10.0	48.5	5.0	47.5
7	SMTC-278	9.0	14.0	43.0	20.0	50.5
8	S-72xF-2	13.0	14.0	40.0	11.0	32.5
9	S-72xCo-151	17.5	19.0	64.0	17.0	39.0
10	S-72xCo-125	20.0	19.0	26.0	17.0	15.0

* The values of the index assigned for every particular cold-tolerance trait ranged from 1 to 15 for inbred lines and from 1 to 10 for hybrids and they were established according to the position (ranking number) of a genotype on a list arranged in the ascending order of sensitivity to cold. The ranking numbers were then summed up to obtain the rank summation indexes in this table.

** seedlings chilled for 9 days at 5°C, then transferred to 20°C.

and mechanisms of physiological injuries in plants have so far been explained (Miedema 1982). In the opinion of many authors the main cause of injuries in severe chilling may be phase transitions in membranes causing their greater permeability or total disfunction (Lyons 1973; Bagnall and Wolfe 1978; Wilson and McMurdo 1981). The high correlation between the leakage of electrolytes from leaf tissue of seedlings and the survival of maize seedlings after seven days of chilling at 5°C ($r = 0.848$***, Tables 1 and 4) reported here confirms the previously outlined suppositions. It also confirms the usefulness of electrolyte leakage measurements as a method informing adequately about the extent of chilling injuries in maize seedlings.

The growth of maize seedlings at 10°C and 15°C did not reflect any clearly marked differentiation among the experimental genotypes (Table 2). Many authors have used the growth rate of maize seedlings in chilling temperatures (in the laboratory or after very early sowing dates in the field) as a criterion of the cold tolerance of genotypes. Many claimed that the greater the accumulation of dry matter and the extension growth under chilling conditions the higher the cold resistance of genotypes (Cal and Obendorf 1972; Stamp 1981; Stamp 1982). In our investigation, such a correlation was observed in inbred lines only. It seems that the phenomenon of heterosis may modify the effect of chilling on growth in hybrids. The stronger inhibition of elongation growth than of the accumulation of dry matter at 10°C and 15°C (Table 2) is consistent with the findings of Stamp (1981), who demonstrated that chilling had a stronger effect on the area growth of the second leaf than on the accumulation of dry matter in maize seedlings. Also Barlow *et al.* (1977) reported that a drop of soil temperature from 28°C to 10°C reduced the leaf elongation rate in maize seedlings.

The effect of the chilling (5°C) pretreatment on the further growth of seedlings at 20°C seems to be of some significance. Since the modification of growth under these conditions is consistent with the cold tolerance of seedlings determined by other methods ($r = 0.839$***), it can be used as a suitable way of evaluation of the genotype sensitivity to low temperature.

The considerable differentiation in seed germinability at low temperature observed in our investigation confirms the reports of others authors (Segeta 1964). However, no correlation was observed between the cold tolerance of seedlings and the seed germinability at chilling temperatures. It may be, thus, assumed that the cold tolerance of plants at the two stages of development (*i.e.* germination and seedling growth) is under control of different genetic factors. Another factor also affecting the cold tolerance of seed may be the seed vigour, which depends on the conditions of growth and seed maturation of the parental plants. If so, the actual ability of seeds to germinate at low temperature may result from both genetic and somatic factors that are difficult to discern. A similar conclusion was reached by Gupta and Kovacs (1976) who compared the effects of recurring spells of colds on maize seedlings with data obtained in laboratory tests. It seems, therefore, that for a full evaluation of the utility of a maize genotype in the climatic conditions of North and Central Europe it is necessary to test the effects of cold on plants both at the stage of their germination and at a later stage of seedling development.

The greater chilling resistance of inbred lines with the flint type of grain and of their hybrids (*Zea mays* L. ssp. *indurata*) than that of the forms with the dent type of grain (*Zea mays* L. ssp. *indentata*) was also reported by Bojarczuk (1972) and Bojarczuk *et al.* (1972). In our investigations, hybrids on the whole proved to be more resistant to cold than inbred lines. These differences, however, were not large and depended on the temperature applied and the process under consideration. Different authors also reported greater cold resistance of hybrids (Bojarczuk 1972; Bojarczuk *et al.* 1972), but others, on the contrary, found that hybrids were more sensitive to chilling that inbred lines (Hussein 1982; Hussein and Pozsar 1982). These inconsistent results may have been caused by the use of different methods for evaluation of cold tolerance in maize genotypes and of the different developmental stage of plants at the time of testing.

The inbred lines SR-10, EP-1, F-7, S-25 and Cm-7 identified in our investigation as cold resistant may have a set of genes determining their greater cold tolerance and as such can be used for breeding hardy forms of maize, since some authors have shown that cold tolerance is genetically controlled and may be heritable (Bojarczuk 1972; Gupta and Kovacs 1976; Mock and Eberhart 1972). At the same time Mock and Bakri (1976) improved the cold tolerance of a breeding population of maize by recurrent selection.

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