ACTA
PHYSIOLOGIAE
PLANTARUM
Vol. 11. No. 2. 1989. 89–96

Effect of water saturated atmosphere on chilling injuries of maize seedlings (*Zea mays* L.)*

F. Janowiak

Department of Plant Physiology, Polish Academy of Sciences,
Podłużna 3, 30-239 Kraków, Poland

Key words: chilling sensitivity, maize inbreds, membrane permeability, relative humidity, seeding survival

Abstract

The effect of 100% relative air humidity (RH) on the extent of chilling injury in 2-week-old seedlings of three maize inbred lines was investigated. After 7 days od chilling at 5°C seedlings injuries were three times smaller at 100% RH than at 60% RH. Water vapour saturated air reduced electrolyte leakage from leaf tissues more than it reduced development of necrotic spots on leaf surfaces or the death of plants. In chilling-sensitive inbreds the decrease of chilling injury at 100% RH was greater than in chilling-tolerant inbred. In seedlings of the tolerant genotype chilled at 60% RH the least affected were the younger leaves (3rd and 4th) and in the most sensitive one the 2nd, 3rd and 4th leaves all suffered similar injuries. During chilling at 100% RM no significant differences of chilling susceptibility of leaves of experimental genotypes were observed.

Introduction

A drop of turgor and progressive wilting of leaves were the first symptoms of the effects of chilling temperatures (0°—10°C) on maize seedlings. After prolonged chilling the oldest leaves were discoloured and necrotic spots appeared on their edges, bands and tips. When chilling ceased the affected

* The research was supported by a grant from the Plant Breeding and Acclimatization Institute (IHAR) Radzików. Project No. 10.3.

areas dried out resulting in spotted and necrotic surfaces. So far no satisfactory interpretation has been advanced for the mechanism and the possible causes of these chilling injuries in maize seedlings (*cf.* Miedema 1982. Stamp 1984. Bochicho 1985). Some experimental results seem to suggest that chilling affected maize seedlings indirectly by causing a drought stress and thus disrupting the water balance of plants (Barlow *et al.* 1977, Kleinendorst and Brouwer 1970. 1972 Watts 1972a b). According to Vigh *et al.* (1981) the drastic loss of water from seedlings of a sensitive maize variety during a short exposure to chilling was associated with the shock sustained by stomata and modifications in the permeability of membranes. Prolonged chilling of two maize varieties affected their stomata differently depending on the different chilling tolerance of these varieties (Mustardy *et al.* 1982). Other experiments indicate that chilling injuries could be reduced or the appearance of symptoms could be delayed by keeping seedling in an environment with relative air humidity of 100% or by treating them with an agent blocking or reducing transpiration; these experiments were carried out with such chilling-sensitive species as *Cucumis sativus* L. (Wright and Simon 1973, Rikin and Richmond 1976), *Glycine max* L. (Musser *et al.* 1983, Markhart 1984), *Oryza sativa* L. (Kabaki and Tajima 1981), *Phaseolus vulgaris* L. and *Gossypium hirsutum* L. (Wilson 1976, Christiansen and Ashworth 1978, Mc William *et al.* 1982). But there has not been as yet any attempt at a quantitative determination of the effects of 100% RH of air on the extent of chilling injuries in maize seedlings depending on the chilling tolerance of the maize genotypes and the age of the tested leaves. The aim of the present investigation was to examine this problem using different methods for evaluating the damage to seedlings caused by chilling.

Materials and methods

The experimental plants were seedlings of two chilling-sensitive inbred lines, Co-125 and S-72 (*Zea mays ssp. indentata*) with the dent type grain and a relatively chilling-tolerant inbred line, F-7 (*Zea mays ssp. indurata*) with the flint type grain (Janowiak, Markowski 1987). The seed material came from the Breeding Station in Smolice.

Seeds treated with dressing T (50% thiuram) were planted in pots filled with 3:1 mixture of peat and soil. Each treatment consisted of 6 pots with 20 plants in every pot. Seedlings were grown in an air conditioned glass-house for the first 2 days at 25°C and subsequently for 12 days at 22/18°C in a day/night cycle of 15/9 h. in natural light and at about 80% air humidity.

Chilling treatment. After 14 days of growth, at the phase of 4—5 leaves, the number of plants in a pot was reduced to 15 and then seedlings were chilled for 7 days at $5° \pm 0.7°C$. Chilling took place in growth cabinets at low light intensity $45\,\mu E \cdot m^{-2} \cdot s^{-1}$ (400—700 nm) in a 15/9 h day/night cycle and two relative air humidities: either the saturation point (100% RH) or low air humidity ($60 \pm 5\%$ RH). 100% air humidity was obtained by enveloping the whole pot with the plants in a polyethylene bags. After 7 days of chilling plants were returned to the glass-house with the initial growth conditions.

Determinations of chilling injuries. a) Electrolyte leakage. Electrolyte leakage from leaf tissues was measured immediately before (control) and after chilling the seedlings. Discs, 0.9 mm in diameter, were cut from the middle part of the second, third and fourth seedlings leaves immersed in 20 ml of redistilled water and shaken for 24 h in darkness at $20° \pm 7°C$. Electroconductivity of the solution thus obtained was measured with a conductometer type OK 102/1 (Radelkis). The plant material was than heat killed at 100°C and after another 24 h of shaking under exactly the same conditions the electroconductivity of the solution (total content of electrolytes) was measured again. All elctroconductivity measurements were made in a thermostatic chamber at $20° \pm 0.7°C$.

All tests were performed in six replicates, each consisting of 10 discs excised from 10 leaves. The extent of chilling injuries, as determined from changes in the leakage of electrolytes, is expressed as an index (I_t) calculated according to the formula (Flint *et al.* 1967):

$$I_t = \frac{R_t - R_0}{100 - R_0} \times 100$$

where: R_t and R_0 are the amounts of electrolyte leakage from leaf tissues of chilled and control seedlings respectively, expressed in percentages of the total electrolyte content in tissue.

Mean I_t values for all the tested leaves of a genotype represent the injuries to its seedlings. Injuries of the particular leaves within the genotype are expressed as the mean I_t values for respectively the 2nd, 3rd and 4th leaves obtained in three series of experiments. The share of particular leaves in the total injuries of the whole seedling (*i.e.* in the total injury of all tested leaves) was determined on the basis of their I_t values and the percentage share of particular leaves in the total surface of a seedling (k). It was assumed that the total injury of all tested leaves of seedling within a genotype (100%) equalled the sum of the products of the expression $I_t \times k$ calculated for the 2nd, 3rd and 4th leaves separately.

b) Visual evaluation of injuries. Seven days after chilling had ended the percentage share of the injured area (necrotic spots) of leaves in the total leaf area of seedlings was determined. Also the dead plants were counted separately in every pot. The extent of seedling injury in a particular pot is expressed as **a)** the mean percentage of injured leaf area when dead seedlings are taken as 100% of injury **b)** the percentage of dead plants.

The data are presented as the means from three independent experimental series each consisting of 6 replicates. The statistical significance of differences was evaluated by the variance analysis in completely randomized design using the F-test and Duncan's multiple range test. Variance analysis for the percentage of dead plants was done with data transformed according to the formula arc sin $\sqrt{x}$.

Results

In the most chilling-sensitive genotype (Co-125) chilling at 5°C for 7 days killed 52.3% of seedlings at 60% RH and 24.5% at 100% RH whereas almost all seedlings of the chilling-tolerant genotype (F-7) survived the treatments (Table).

The percentage of injured leaf area under these chilling conditions ranged from 24.0 (F-7) to 74.1% (S-72) at 60% RH and from 3.1 (F-7)

Table

Seedling injuries and electrolyte leakage from leaves (I_t) after 7 days of chilling at 5°C and at 60% or 100% RH

Genotype	Dead plants (%)		Injured area of leaves (%)*		Electrolyte leakage I_t (%)	
	60	100	60	100	60	100
Co-125	52.3a**	24.5b (46.8)***	66.9a	37.8b (56.5)	90.9a	28.2c (31.0)
S-72	54.6a	9.6c (17.6)	74.1a	19.2c (25.9)	56.7b	11.1d (19.6)
F-7	1.4c	0.7c (50.0)	24.0c	3.1d (12.9)	35.5c	5.7d (16.1)
$\bar{x}$	36.1A	11.6B (32.1)	55.0A	20.0B (36.4)	61.0A	15.0B (24.6)

* It was assumed that in dead plants leaf injury equalled 100%

** Means within a particular chilling-tolerance trait marked with the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple range test

*** In per cent of injury at 60% RH

to 37.8% (Co-125) at 100% RH (Table). The values of the I_t index of injury determined on the basis of the electrolyte leakage from tissues ranged from 35.5 (F-7) to 90.9% (Co-125) at 60% RH and from 5.7 (F-7) to 28.2% (Co-125) at 100% RH.

In general, injuries to seedlings after 7 days of exposure at 5°C were three times smaller at 100% RH than at 60% RH. The percentage of dead plants at 100% RH dropped to 32.1%, the injuries of leaf surface to 36.4% and the injury index I_t to 24.6% of the corresponding values obtained at 60% RH (Table). A decrease of chilling injuries in water vapour saturated air was found in all the tested genotypes regardless of their chilling tolerance. The greatest difference between injuries at 60% and 100% RH was found in the sensitive inbred Co-125, though the damage to seedlings of this genotype caused by chilling at 100% RH amounted on the average to 45% of the damage caused at 60% RH, whereas in inbreds S-72 and F-7 analogical seedling damage amounted to only 21% and 26% respectively (Table).

With separate I_t determinations for the 2nd, 3rd and 4th leaves it was possible to compare the injuries to the particular leaves (Figure, A) and their share in the total chilling injury to whole seedlings (Figure, B) of the experimental genotypes at both RH levels. At 5°C and 60% RH the 2nd, 3rd and 4th leaves of the chilling-sensitive inbred Co-125 were all injured to a similar extent and the younger (upper) leaves had a higher share in the total injury of a seedling of this genotype than that of the older (lower) leaves. With increasing chilling tolerance of the genotypes

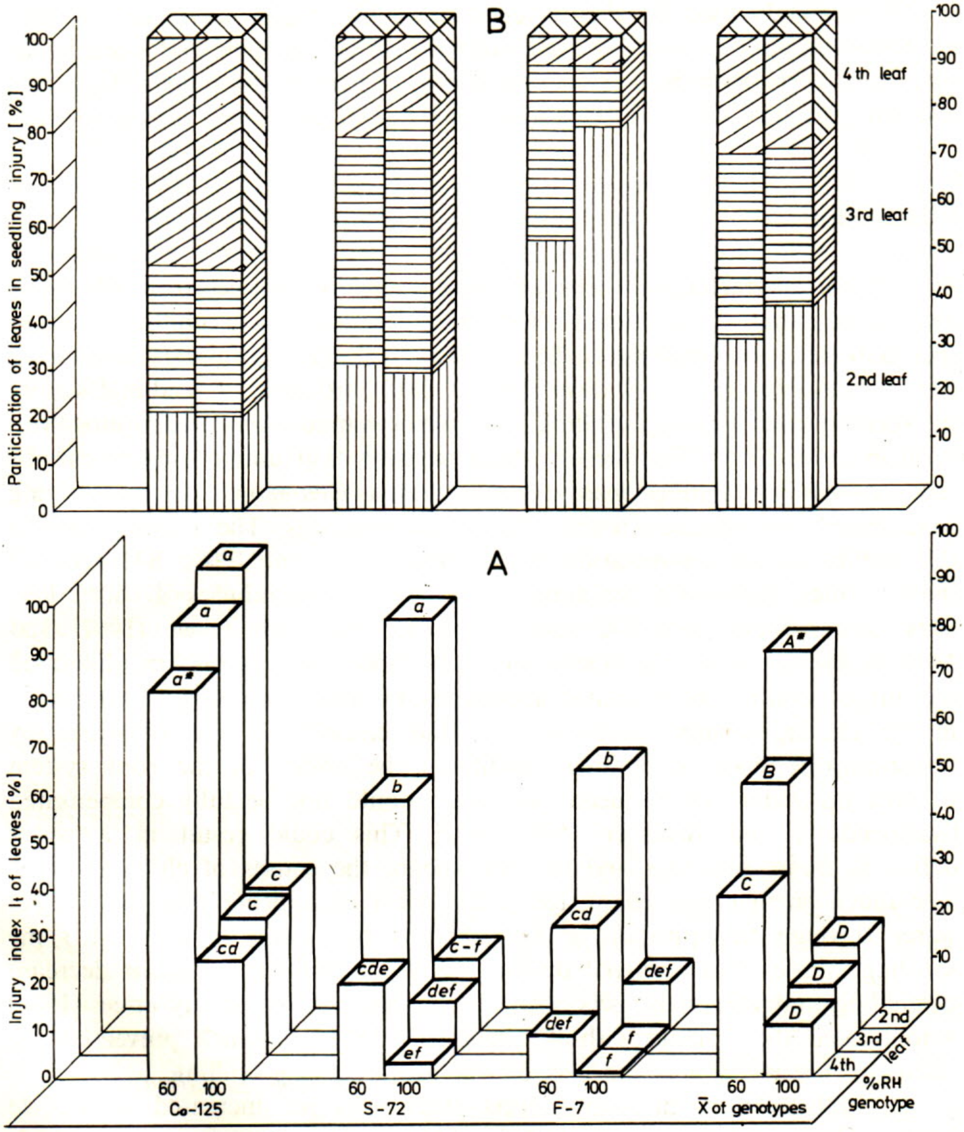

Figure. Injuries of the 2nd, 3rd and 4th leaf (A) and their proportional participation in total injury of seedling (B) in maize genotypes at 5°C at different relative air-humidity (RH).

the injury to the younger leaves diminished in the first place, and the participation of the youngest leaf in the total injury to whole seedlings definitely decreased. At 100% RH a similar tendency was observed, although the differences between the particular leaves were smaller and in the chillin-tolerant genotype the 3rd and 4th leaves were injured to a similar degree.

The methods used in this investigation gave highly consistent results in evaluating chilling injury to seedlings. These was a high and statistically significant correlation of I_t values with the percentage of injured leaf area and with the death rate of seedlings, *i.e.* 0.853** and 0.791**, respectively.

Discussion

One of the characteristic traits of chilling-sensitive species (*e.g. Phaseolus vulgaris* L.) that distinquishes them from the extremely chilling-sensitive species (*e.g. Episcia reptans*) is the fact that their chilling injuries at *e.g.* 5°C can be prevented for a few days without chill-hardening of seedling, simply by keeping plants during chilling in a water vapour saturated atmosphere (Wilson 1976, 1979). The present study suggests that chilling injury can be reduced also in a chilling-sensitive species such as *Zea mays* L. by maintaining a saturated atmosphere around the chilled seedlings. The reason may be that inhibition of transpiration of seedlings chilled at 100% RH reduced injuries, making them three times smaller than the injuries at 60% RH. This is consistent with the results reported by Vigh *et al.* (1981) and Mustardy *et al.* (1982); in the opinion of those workers chilling disturbed the turgor control of stomatal aperatures in maize seedlings, particularly in the chilling-sensitive varieties, and thus caused the loss of water by transpiration. Since in chilling conditions the ability of the root system to take up water was reduced, the losses could not be fully compensated (Kleinendorst and Brouwer 1970, 1972). This could result in a water deficit in maize seedlings and thus be one of the causes of chilling injury. The much smaller injuries at 100% RH seem to indicate that the loss of water through transpiration at 60% RH is the cause of at least some seedling injuries that occurred during 7 days of chilling. A similar decrease of chilling injury in maize seedlings was reported by Mustardy *et al.* (1984) who treated their plants with the herbicyde DCMU, which prevented the opening of stomata and the loss of leaf turgor during chilling.

At both relative humidity levels there was an increased electrolyte leakage from leaf tissues of chilled seedlings in comparison to controls (before the onset of chilling). Increased electrolyte leakage from leaf tissues of chilled maize seedlings was also observed by Creencia and Bramlage (1971). Such an increased leakage from the tissues after chilling at 5°C for 7 days, particulary at 60% RH, may be caused by greater permeability of membranes, damage to membranes or their destruction. Changes of

** Statistically significant at $P = 0.01$.

membrane permeability could be the result either of the direct effect of cold on the membranes or of dehydration caused by uncontrolled transpiration during chilling at 60% RH. The effect of RH is evidenced by the decrease in I_t value at 100% RH to as little as 24.6% of the I_t value at 60% RH as well as I_{t50} (at 50% of dead plants) equal to 70% at 60% RH and to 53% at 100% RH (Table). Similar results were reported by Wright and Simon (1973) with cucumber, where chilling at 100% RH only slightly increased the electrolyte leakage from tissues but at 85% RH it caused rapid electrolyte leakage in comparison to controls. Also Sobczyk *et al.* (1985) showed that in cucumber seedlings 100% RH delayed the occurence of the chilling-induced electrolyte leakage for at least one day as compared with 70% RH.

More injury to older leaves of maize seedlings at 60% RH, particularly in the chilling-tolerant genotype, and the lack of such differences at 100% RH confirmed the greater sensitivity of older leaves to chilling-induced water stress. Similarly chilling of roots in maize seedlings affected more strongly the older leaves (Kleinendorst and Brouwer 1972). The fact that injuries to the 2nd, 3rd and 4th leaves of maize genotypes at 100% RH were similar may indicate that these leaves differ only in their sensitivity to chilling-induced water stress and not to metabolic and structural damages caused by chilling.

References

Barlow E. W. R., Boersma L., Young J. L., 1977. Photosynthesis, transpiration, and leaf elongation in corn seedlings at suboptimal soil temperatures. Agron. J. 69: 95—100.

Bochicchio A. 1985. Zea mays L. and chilling conditions at sowing time: a review. Maydica 30: 241—256.

Christiansen M. N., Ashworth E. N., 1978. Prevention of chilling injury to seedling cotton with anti-transpirants. Crop Sci. 18: 907—908.

Creencia R. P., Bramlage W. J., 1971. Reversibility of chilling injury to corn seedlings. Plant Physiol. 47: 389—392.

Flint H. L., Boyce B. R., Beattie D. J., 1967. Index of injury — A usefull expression of freezing injury to plant tissues as determined by the elctrolytic method. Can. J. of Plant Sci. 47: 229—230.

Janowiak F., Markowski A., 1987. Effect of chilling on germination, growth, survival and membrane permeability in seedlings of different breeding forms of maize (*Zea mays* L.). Acta Physiol. Plant. 9: 77—87.

Kabaki N., Tajima K., 1981. Effect of chilling on the water balance of rice seedlings. Jap. J. Crop Sci. 50, 4: 489—494.

Kleinendorst A., Brouwer R., 1970. The effect of temperature of the root medium and of the growing point of the shoot on growth, water content and sugar content of maize leaves. Neth. J. Agric. Sci. 18: 140—148.

Kleinendorst A., Brouwer R., 1972. The effect of local cooling on growth and water content of plants. Neth. J. Agric. Sci. 20: 203—217.

Markhart A. H. III, 1984. Amelioration of chilling-induced water stress by absicisic acid--induced changes in root hydraulic conductance. Plant Physiol. 74: 81—83.

McWilliam J. R., Kramer P. J., Musser R. L., 1982. Temperature-induced water stress in chilling-sensitive plants. Aust. J. Plant Physiol. 9: 343—352.

Miedema P. 1982. The effects of low temperature on *Zea mays*. Advances in Agron. 35: 93—128.

Musser R. L., Thomas S. A., Kramer P. J., 1983. Short and long term effects of root and shoot chilling of Ransom soybean. Plant Physiol. 73: 778—783.

Mustárdy L. A., Sz-Rożsa Z., Faludi-Dániel A., 1984. Chilling syndrome in light-exposed maize leaves and its easing by low doses of DCMU. Physiol. Plant. 60: 572—576.

Mustárdy L. A., Thanh Tam Vu, Faludi-Dániel A., 1982. Stomatal response and photosynthetic capacity of maize leaves at low temperature. A study on varietal differences in chilling sensitivity. Physiol. Plant. 55: 31—34.

Rikin A., Richmond A. E., 1976. Amelioration of chilling injuries in Cucumber seedlings by abscisic acid. Physiol. Plant. 38: 95—97.

Sobczyk E. A., Marszałek A., Kacperska A., 1985. ATP involvement in plant tissue responses to low temperature. Physiol. Plant. 63: 399—405.

Stamp P. 1984. Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). Advances in Agron. and Crop Sci. Supplements to J. of Agron. and Crop Sci. No. 7, ed. by Verlag Paul Parey — Berlin and Hamburg.

Vigh L., Horváth I., Farkas T., Mustárdy L. A., Faludi-Dániel A., 1981. Stomatal behaviour and cuticular properties of maize leaves of different chilling-resistance during cold treatment. Physiol. Plant. 51: 287—290.

Watts W. R., 1972a. Leaf extension in *Zea mays*. I. Leaf extension and water potential in relation to root-zone and air temperatures. J. Exp. Bot. 23, 76: 704—712.

Watts W. R., 1972b. Leaf extension in *Zea mays*. II. Leaf extension in response to independent variation of the temperature of the apical meristem, of the air around the leaves, and of the root-zone. J. Exp. Bot. 23, 76: 713—721.

Wilson J. M., 1976. The mechanism of chill- and drought-hardening of *Phaseolus vulgaris* leaves. New Phytol. 76: 257—270.

Wilson J. M., 1979. Drought resistance as related to low temperature stress. In: "Low Temperature Stress in Crop Plants. The Role of Membrane", ed. by J. M. Lyons, D. Graham, J. K. Raison. Academic Press, New York: 47—65.

Wright M., Simon E. W., 1973. Chilling injury in cucumber leaves. J. Exp. Bot. 24, 79: 400—411.

Received February 4, 1988; accepted April 22, 1988