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The effect of vesicular-arbuscular mycorrhizae and chilling on two hybrids of *Zea mays* L.

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Abstract. In order to investigate the effect of vesicular-arbuscular mycorrhizae on the chilling resistance of *Zea mays*, seeds of two hybrids (Pioneer 3902 and Pride 5) were grown in soil inoculated with *Glomus mosseae*. Germination tests at 10° C and 25° C showed that Pride 5 was more resistant to chilling than Pioneer 3902. Plants grown at 25° C for 6 weeks were given a 1-week chilling treatment at 10° C and the responses of mycorrhizal and nonmycorrhizal plants of the two hybrids were compared. At 10° C, the mycorrhizal plants had greater biomass, carbohydrate, and protein content than the nonmycorrhizal plants.

Key words: Chilling – *Zea mays* hybrids – *Glomus mosseae* – Germination – Metabolites

Introduction

The growth and development of crops which are sensitive to chilling, such as *Zea mays* L., can be hindered when environmental temperatures reach 10–15° C (Stamp 1988). Low temperatures reduce plant growth by affecting physiological and metabolic processes (Charest and Phan 1990; Guy 1990). Numerous attempts to improve yield have been made through breeding, cultural practices, and selection of cold-resistant hybrids (Hope et al. 1992; Tollenaar and Dwyer 1990). Growth variation at different temperatures depends on vesicular-arbuscular mycorrhizal (VAM) fungi species. Some of these may develop denser hyphal networks thus increasing the kinetics of nutrient transport, including phosphorus, and resulting in better plant growth (Raju et al. 1990).

VAM fungi are known to stimulate the growth of many plants and in some cases to increase plant tolerance to drastic environmental conditions such as drought (Nelsen 1987), disturbance and nutrient deficiency (Miller 1987), and diseases (Caron 1989). Relatively little is known about the protective effect of mycorrhizae on cold-stressed plants. Anderson et al. (1987) indicated that VAM fungi stimulated growth of green ash *Fraxinus pennsylvanica* Marsh. at root temperatures as low as 7.5° C. Mycorrhizal colonization was slow at low root temperatures in barley *Hordeum vulgare* L. but still resulted in an increased root:shoot ratio (Volkmar and Woodbury 1988). In wheat *Triticum aestivum* L. little or no colonization by VAM occurred at low temperatures, and at 10° C the mycorrhizae were apparently no longer beneficial to the plant (Hetrick and Bloom 1984). No benefit from mycorrhizae at low temperatures was found in cotton *Gossypium* sp. and growth may even be suppressed by the fungi (Smith and Roncadori 1986). The effect of chilling on inoculated and noninoculated maize was tested in the following experiment.

Materials and methods

Plant material

Two maize hybrids were used, Pride 5 and Pioneer 3902. The former has been recommended by the Ontario Corn Committee (1959–70) for its high yield and resistance to high density, but is sensitive to chilling. The latter was recommended (1988) for its high yield and is less sensitive to cold. The selected VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (specimen DAOM # 214744) was originally isolated from the rhizosphere of *Poa eminens* Presl. growing on a fixed sand dune at the sea shore level of Côte-Nord Québec (50° 16′ N 65° 35′ W). A single spore culture was isolated and subsequently cultivated in a growth chamber on leek *Allium porrum* L. in vermiculite substrate.

Germination test

For each hybrid, four replicates of 100 seeds were germinated in petri dishes (20 seeds/dish) on wet Whatman no. 1 filter paper at 10° C and 25° C, 98% relative humidity, in the dark, in a controlled growth germinator (Conviron Model E15). Germination

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was initiated by adding sterile distilled water to each dish, and further additions were made as needed. Observations on germination were made daily during 1 week at 25° C, and 2 months at 10° C, or until no further seeds germinated. Germination was determined to have occurred when the seeds reached a 1-cm coleoptile height (Hope et al. 1992), and the seedlings were then removed from the petri dish. Percentage germination and the average time of germination were recorded. Statistical analysis of the germination tests was conducted using an SAS package, Version 6.1, using PROC ANOVA and PROC GLM.

Mycorrhizal colonization of roots and plant growth

Sixteen plants of each hybrid, one plant per pot, were grown in the greenhouse for 6 weeks in a mixture of vermiculite:montmorillonite clay:peat:sand (1:1:1:1), at 25° C:15° C (day:night) with an 18-h photoperiod. Half of the pots were inoculated with leek roots previously colonized with *G. mosseae* (Dalpé 1992) and watered once a week with low-phosphorus Hoagland's solution. Mycorrhizal root colonization was verified 4 weeks after planting, and weekly for 2 further weeks.

After 6 weeks, half of the pots were given a 1-week chilling treatment by placing pots in a growth chamber at 10° C with an 18-h photoperiod. Both control and 10° C plants were then harvested and the biomass recorded. Plant material was dried in an oven at 60° C until the dry mass remained constant.

Metabolite analysis

Leaf protein was extracted by the method of Charest and Phan (1990), and determined according to the method of Bradford (1976). Sugars were analyzed by the classical method of Nelson as adapted by Potvin and Charest (1991). The starch content was determined according to the method of Potvin and Charest (1991). Chlorophyll was extracted in 100% ethanol, and assayed by the method of Bruinsma (1963). Results were statistically analyzed by a three-way analysis of variance (ANOVA). The starch data had to be square-root transformed to normalize their distribution.

Mycorrhizal root colonization

Roots from each plant inoculated with *G. mosseae* were randomly selected and analyzed for VAM fungi colonization. Roots were cleaned in water, cleared in KOH 2.5%, acidified in 1 N HCl and stained with trypan blue (Koske and Gemma 1989) or with aniline blue (Grace and Stribley 1991) before mounting on slides in polyvinyl alcohol–lactic acid–glycerol (PVLG) media (Omar et al. 1979). Approximately 200 root sections from each treated plant were screened for the presence of mycorrhizae and the percent of colonized roots was recorded. Only root sections showing intraradical vesicles and/or intracellular arbuscules were considered to be colonized.

Results

Germination and root colonization

Pride 5 showed a higher ($P<0.05$) percentage germination than Pioneer 3902 at 10° C, 98% and 75%, respectively (Fig. 1). Pride also germinated faster ($P<0.05$) than Pioneer, 25.1 days for the former compared to 32.5 days for the latter (Fig. 1). No differences were detected at the control temperature (25° C); percentage germination (100 and 95%) and the average time of germination (4.6 and 4.5 days) were similar for both hybrids (Fig. 1). Thus it appeared that both percentage and time of germination were inhibited in Pioneer by cold.

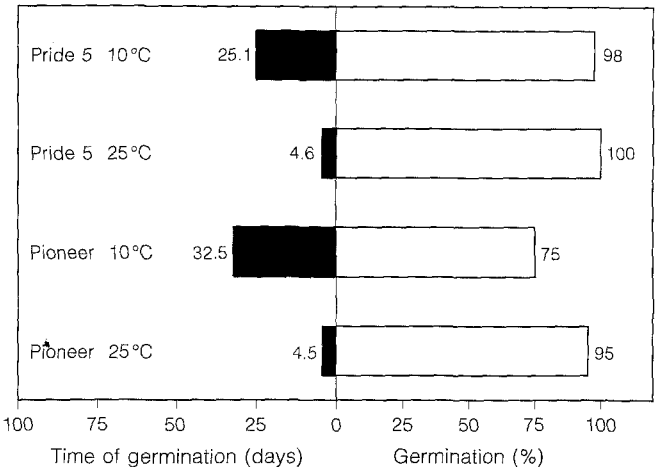

Fig. 1. Time of germination (*black boxes*) and percentage of germination (*white boxes*) of Pride 5 and Pioneer 3902 at 10° C and 25° C

The level of root colonization was independent of both temperature and plant genotype. Overall, vesicular colonization was higher (≈22%) than arbuscular (≈7%).

Shoot mass, chlorophyll and metabolites

Under nonmycorrhizal conditions at 25° C and 10° C, the shoot mass of Pride was comparable to that of Pioneer (Fig. 2a). However, mycorrhizal colonization of Pride plants resulted in greater biomass than that of Pioneer at both temperatures. This was of particular importance at 10° C, where the shoot mass of Pride was more than twofold greater than Pioneer (Fig. 2a; Table 1).

For both mycorrhizal and nonmycorrhizal plants, chlorophyll concentration was higher ($P<0.05$) at 25° C than at 10° C, and higher in Pioneer than in Pride at 25° C (Fig. 2b; Table 1). Overall, mycorrhizal plants tended to have lower chlorophyll concentrations than nonmycorrhizal plants.

For both hybrids, the concentration of proteins was higher ($P<0.05$) at 10° C than at 25° C (Fig. 2c; Table 1). There was a significantly higher ($P<0.001$) protein content in the mycorrhizal plants than in the nonmycorrhizal plants except for Pioneer at 10° C (Fig. 2c, Table 1).

The sugar content, like the protein content, was significantly higher ($P<0.001$) in the mycorrhizal plants than in the nonmycorrhizal plants for both hybrids at 10° C (Fig. 2d; Table 1). The sugar content of Pioneer was considerably higher at 10° C than 25° C, especially in the mycorrhizal plants. The converse was observed for Pride plants.

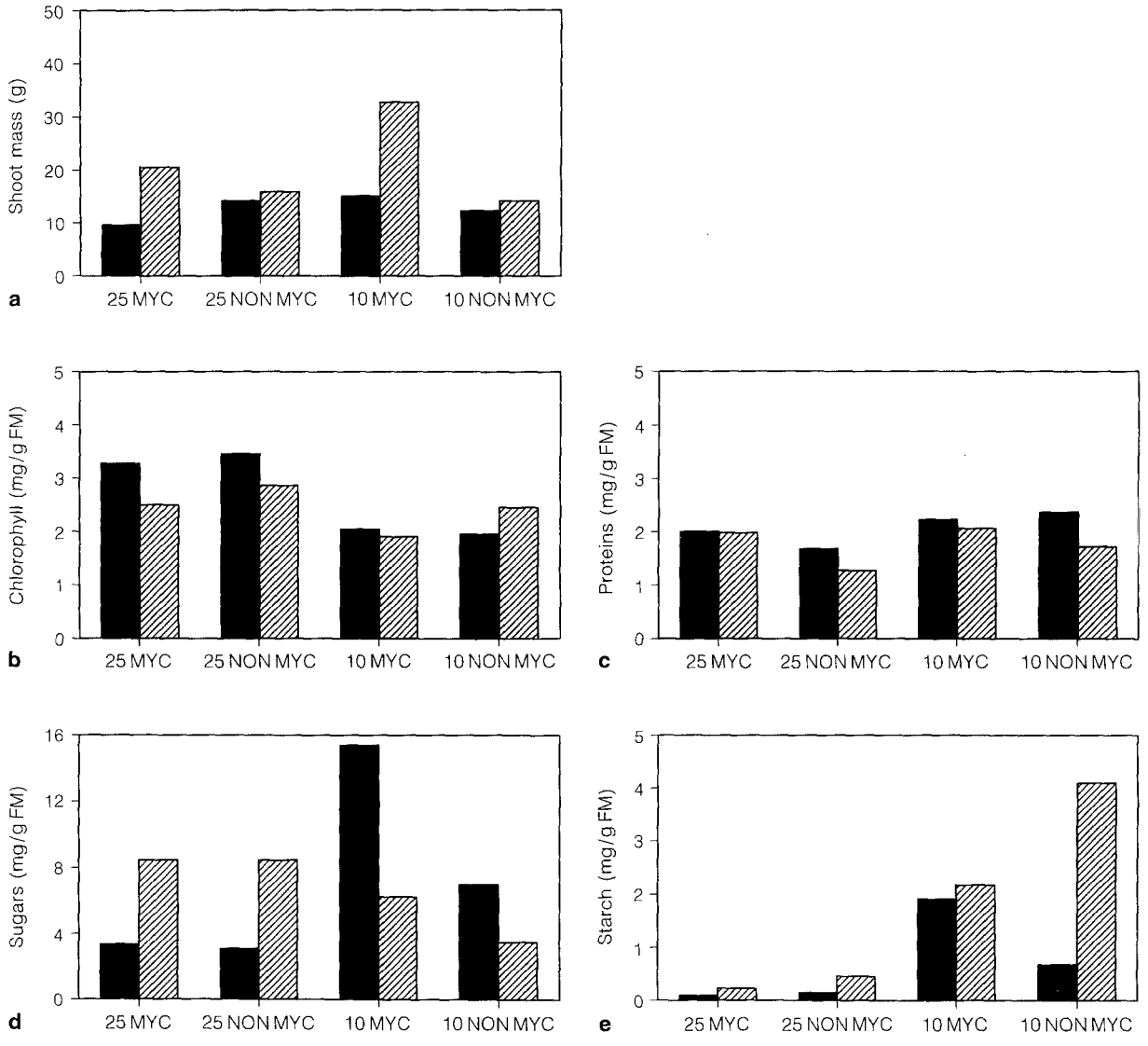

Fig. 2. **a** Shoot mass, **b** total chlorophyll concentration, **c** protein, **d** sugar, **e** starch contents in Pioneer 3902 (*black bars*) and Pride 5 (*hatched bars*) mycorrhizal (*MYC*) and nonmycorrhizal (*NON MYC*) plants at 10° C and 25° C. *FM* Fresh mass

The starch content was significantly higher ($P<0.001$) at 10° C than at 25° C in both hybrids (Fig. 2e; Table 1). Overall, the starch levels were significantly higher ($P<0.001$) in Pride than in Pioneer. There was no clear mycorrhizal effect; in general starch content was lower in mycorrhizal than nonmycorrhizal plants, but was exceptionally high in Pioneer at 10° C. The highest starch content was found in Pride nonmycorrhizal plants at 10° C (Fig. 2e; Table 1).

Table 1. Levels of significance for ANOVA for shoot mass (Smass), chlorophyll (Chl), protein, sugar, and starch contents in leaves of Pride and Pioneer hybrids at 10° C and 25° C for mycorrhizal and nonmycorrhizal plants

Variables	Smass	Chl	Protein	Sugar	Starch
Mycorrhizae (M)	—***	—*	—***	—***	NS
Temperature (T)	NS	—***	—***	NS	—***
Hybrids (H)	—***	—***	—***	—***	—***
M×T	—***	NS	—***	—***	NS
M×H	—***	NS	—***	—***	—***
T×H	—*	—***	—*	—***	—***
M×T×H	NS	—*	NS	—***	—***

* $P<0.05$; *** $P<0.001$; NS, not significant

Discussion

At the germination stage, Pride 5 appears to be more resistant to chilling than Pioneer 3902, even though Pride was initially considered to be most sensitive to chilling (Dwyer and Tollenaar 1989). Chilling resistance is dependent on the physiological age of the plant, and may change as the plant matures (Lyons 1973; Tollenaar and Dwyer 1990).

In the absence of mycorrhizae, the shoot mass of the two hybrids decreased at low temperature. Chilling-sensitive plants such as maize usually exhibit physiological dysfunction when exposed to low temperature

(Lyons 1973; Stamp 1988). When colonized with *G. mosseae*, only the Pride hybrid showed an increased shoot mass in cold conditions. Our results agree with those of Volkmar and Woodbury (1988) with barley, and with the study of Stahl and Christensen (1991) on *Melilotus officinalis* L., and suggest the potential of using mycorrhizae as a chilling protector.

With regard to chlorophyll content, Pioneer was more affected than Pride at low temperature. Chlorosis is a current phenomenon in chilling-sensitive plants (Hodgins and Van Huystee 1985; Potvin and Charest 1991), while in more chilling-resistant plants chlorophyll synthesis capacity may be increased (Kröl and Huner 1985).

Protein content increased in both hybrids in response to both chilling and VAM fungi treatments. An increase in protein content during chilling appears to be an indicator of plant tolerance (Charest and Phan 1990; Guy 1990). An enhancement of soluble proteins was also revealed by Dumas et al. (1990) in the roots of *Nicotiana tabacum* L. colonized by *G. mosseae*.

The increase in sugar content, often observed at low temperature with chilling-resistant plant (Potvin and Charest 1991), was obtained only in the Pioneer hybrid. This conforms to the original assumption of greater chilling resistance for Pioneer (Dwyer and Tollenaar 1990). Although sugar content did not increase at low temperature in Pride, several other analyzed parameters (germination test, shoot mass, protein and starch contents) suggest there is potential chilling resistance in the Pride hybrid.

Overall, mycorrhizal formation, with or without cold temperature treatment, had a beneficial effect by increasing plant growth at the level of shoot mass, protein, and carbohydrate contents. This significant growth promotion suggests that the use of VAM fungi in maize cultivation may counteract chilling injury and could be of economic value.

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