

# Physiological Basis for Differences in Zoysiagrass Freeze Tolerance

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## Objective

Determine the role of carbohydrates, proline and proteins in the freeze tolerance of zoysiagrass cultivars.

## Rationale

Zoysiagrass (*Zoysia* spp.) is a warm-season grass well-adapted for lawns and golf turfs in the transitional and warm climatic regions of the USA and requires minimal maintenance inputs. Thus, expanded use of zoysiagrass could play a key role in making transition zone golf courses and lawns more environmentally friendly and sustainable. However, key barriers to widespread zoysiagrass use are slow establishment rate and lack of cold hardiness. This report summarizes the physiological basis for differing freeze tolerance among zoysiagrass cultivars.

To examine the physiological basis for differences in cold-hardiness, freeze tolerance of several cultivars was first determined. A positive relationship ( $r^2 = 0.48$ ,  $p = 0.009$ ) between freeze tolerance ( $LT_{50}$ , determined with growth/freeze chambers) and winter injury in the field indicates growth chamber based procedures produced can be used to predict field survivability. The physiological basis for differences in freeze tolerance was examined using SDS-PAGE, immunoblotting, carbohydrate, and proline analysis (Figs 1 and 2).

## Results

Certain carbohydrates and proline were found to play a role in improving freezing tolerance. High concentrations of glucose, total reducing sugars, total soluble sugar:starch ratio, and proline were also found to play a role in improving freezing tolerance, whereas low levels of starch were associated with improved freeze tolerance (Figs. 1 and 2). Concentrations of glucose, total soluble sugar:starch ratio, sucrose:total reducing sugars ratio, a 23 kDa dehydrin-like polypeptide, total reducing sugars, proline, and starch in cold-acclimated plants were correlated ( $r^2 = 0.55, 0.45, 0.43, 0.41, 0.41, 0.39$ , and  $0.37$  respectively) with  $LT_{50}$  and could be used as predictors of zoysiagrass freeze tolerance. Additionally, the change  $[\Delta\text{content} = (100 * ((1 + \text{CA content}) - (1 + \text{NA content})) / (1 + \text{NA content}))]$  in glucose, total soluble sugar:starch ratio, sucrose, and total soluble sugars during cold-acclimation were also correlated ( $r^2 = 0.55, 0.44, 0.51$ , and  $0.31$ , respectively) with  $LT_{50}$  and could be used as predictors of zoysiagrass freeze tolerance.

Several prominent polypeptides were found in zoysiagrass stolons and rhizomes, but the abundance of relatively few polypeptides changed in response to cold acclimation and their levels were not related to zoysiagrass freeze tolerance. However, immunoblotting revealed that dehydrin-like polypeptides are associated with zoysiagrass cold-acclimation, and that a 23 kDa dehydrin-like polypeptide plays a role in improving freezing tolerance (Fig. 2E).

During cold-acclimation in a growth chamber, proline, dehydrins, total reducing sugars (glucose + fructose), total soluble sugars, and sucrose (listed in order with largest percentage increase during cold-acclimation first) increased in concentration and could be used as indicators that zoysiagrass has been cold-

acclimated. Future researchers could measure these solutes as good indicators of cold acclimation in zoysiagrass.

The concentrations of proteins, proline and carbohydrates were measured from plants cold-acclimated in a growth chamber, but it is likely that plants sampled in the field contain similar concentrations. The cold-acclimation induced in the growth chamber increased zoysiagrass freeze tolerance and dramatically changed concentrations of sugars, proline and dehydrin. Thus, researchers could choose to sample zoysiagrass plant material from the field during winter or to use a growth chamber to cold acclimate plants.

## **Summary**

This research establishes that high levels of dehydrin-like polypeptides, glucose, total reducing sugars, total soluble sugar:starch ratio, and proline play a role in improving freezing tolerance, whereas high levels of starch are detrimental to freeze tolerance. Prior to the initiation of these studies, previous knowledge about cold-hardiness of zoysiagrass promoted a hypothesis that high levels of starch were advantageous to zoysiagrass cold-hardiness and that soluble sugars were likely not important. Proper understanding of the role of carbohydrates, dehydrin, and proline in zoysiagrass freeze tolerance will be a useful tool for breeders to select freeze tolerant phenotypes and improve the winter performance of future releases.

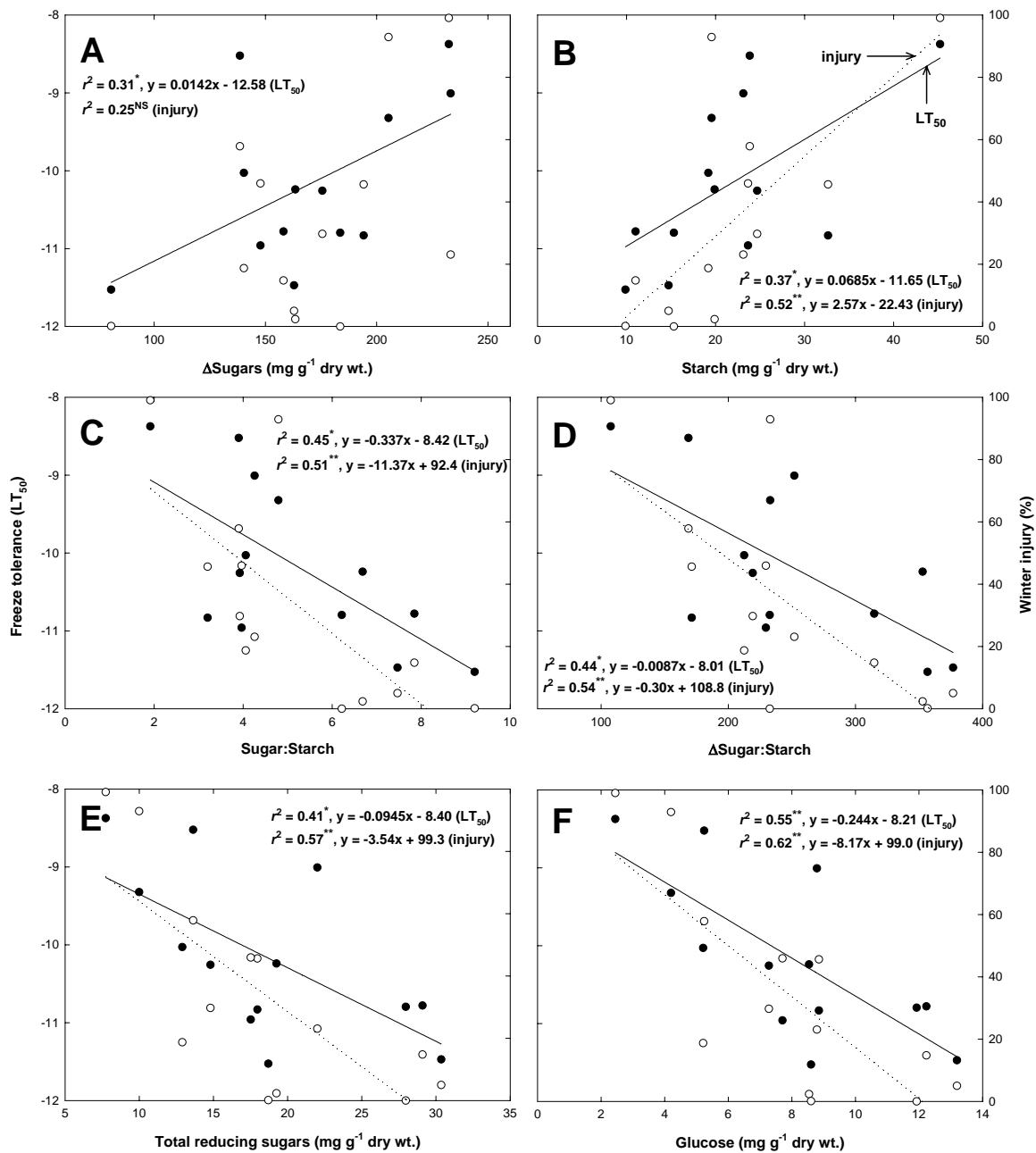


Figure 1. There is a relationship between zoysiagrass freeze tolerance ( $LT_{50}$ ) (●), winter injury in the field (○) and the percent change in sugars during acclimation ( $\Delta$ total soluble sugars) (A), starch concentration in cold-acclimated plants (B), sugar:starch ratio in cold-acclimated plants (C), the percent change in sugar:starch ( $\Delta$ sugar:starch) during acclimation (D), total reducing sugars in cold-acclimated plants (E), and glucose in cold-acclimated plants (F). Each point represents the mean of six replications for  $LT_{50}$  and tissue contents (carbohydrates and proline) and the mean of 8 values (2 years of 4 replications each) for winter injury. \*, \*\* Significant at the 0.05, and 0.01 probability levels, respectively. NS, not significant.

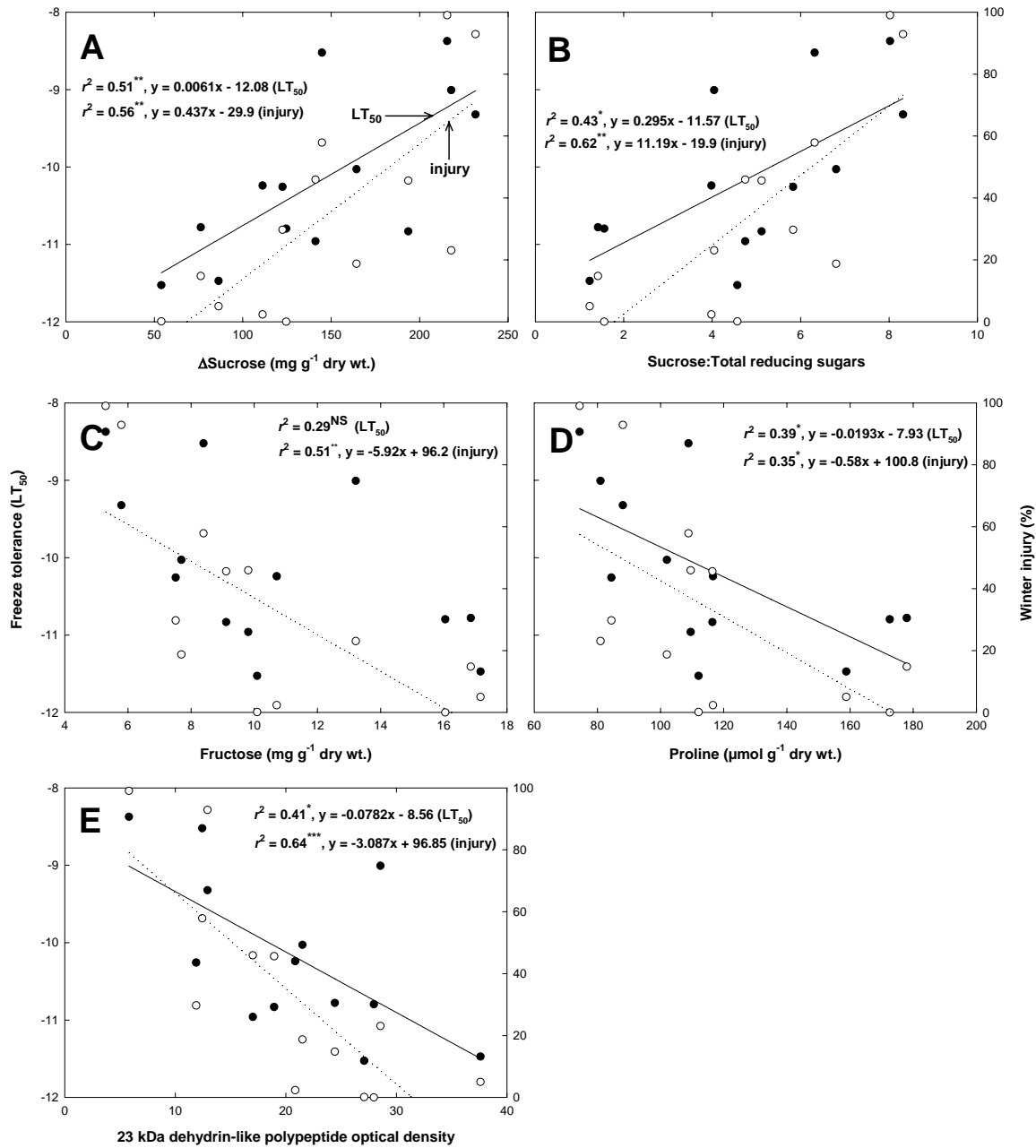


Figure 2. There is a relationship between zoysiagrass freeze tolerance ( $LT_{50}$ )(●), winter injury in the field (○) and the percent change in sucrose during acclimation ( $\Delta$ sucrose) (A), sucrose:total reducing sugars in cold-acclimated plants (B), fructose in cold-acclimated plants (C), proline in cold cold acclimated plants (D), and a 23 kDa dehydrin-like polypeptide in cold acclimated plants (E). Each point represents the mean of six replications for  $LT_{50}$  and tissue contents (carbohydrates and proline), 1 replication for dehydrin abundance and the mean of 8 values (2 years of 4 replications each) for winter injury. \*, \*\*, \*\*\* Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. NS, not significant.