Title: Cold Acclimation Potentials of *Glycine max* and *Glycine soja*

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Abstract: Soybean has been considered a cold intolerant species; based largely upon seed germination and soil emergent evaluations. This study reports a distinct acquisition of cold tolerance, in seedlings, following short acclimation periods. Diversity in cold responses was assessed in 8 cultivars of *Glycine max* and 6 accessions of *Glycine. soja*. All varieties of soybean significantly increased in freezing tolerance following acclimation. This study indicates soybean seedlings are indeed capable of sensing cold and acquiring cold tolerance. Germination rates after cold imbibition were negatively correlated with maturity group, but positively correlated with cold acclimation potential in *G. soja*. Seed fatty acid composition was varied between the species, with *G. soja* accessions containing about 2-times more linolenic acid (18:3) than *G. max*. Furthermore, high levels of linoleic acid (18:2) in seeds were positively correlated with germination rates following cold imbibition in *G. soja* only. We suggest that domestication has not impacted the overall ability of soybean to cold acclimate at the seedling stage and that there is some variation within the domesticated species for ability to cold acclimate. Thus, this brief comparative study reduces the enthusiasm for the "wild" species as an additional source of genetic diversity for cold tolerance.

Keywords: cold tolerance, cold acclimation, ion leakage, fatty acid composition, germination, soybean

Running Title: Soybean Cold Acclimation

Introduction

Domesticated soybean, *Glycine max* [L.] Merr., is an important agricultural crop, valued within the United States at $38.7 billion in 2012 (USDA-NASS 2014). *Glycine max* cultivars are grouped into 13 maturity groups, ranging from 000 to X, based primarily on photoperiodism (Zhang, Kyei-Boahen et al. 2007). Higher maturity groups (V to X) generally flower in response to shorter days than lower maturity groups (000 – IV). These maturity group designations are also utilized for *Glycine soja* Sieb. & Zucc., which is considered the closest wild progenitor for *G. max* (Broich and Palmer 1980; Li, Li et al. 2010).

Glycine soja is smaller in both size and yield than *G. max*, possessing hard, long-dormancy seeds and slender vine-like branches (Broich and Palmer 1980; Saitoh, Nishimura et al. 2004). Some cultivars of *G. soja* are less susceptible to salt stress (Luo, Yu et al. 2005) and dehydration stress (Chen, Chen et al. 2006) than their domestic counterparts (*G. max*). Based on sequencing, *G. soja* have a greater genetic diversity and may be a source for expanding the abiotic stress tolerance of *G. max* (Lam, Xu et al. 2010; Li, Li et al. 2010).

In freezing survival experiments *G. max* genotypes were characterized by high death rates following a -3 °C cold treatment with no significant differences between cultivars being observed (Hume and Jackson 1981). The work described here allows for a more quantitative evaluation of cold and freezing tolerance. Though *G. soja*'s cold tolerance has not yet been examined; several soybean cold-accumulated proteins, when transgenically expressed, have been shown to enhance cold tolerance in Arabidopsis (Luo, Bai et al. 2012; Yang, Wu et al. 2014). We have chosen to closely examine the

ability of soybean to cold acclimate and to acquire cold tolerance. Further, we have examined a few selected accessions of *G. soja* to determine whether it is likely the wild varieties are a potential source for variation in cold tolerance. We further examined the variation of cold tolerance (seed germination) in soybeans and whether there is an association of these abilities with seed lipid composition and/or dehydrin abundance and response.

This study aimed to determine the variation in cold tolerance and the relationship to the maturity group of *Glycine* species (*max* and *soja*) at both germinating seed stage and in young seedlings, and the impact of cold acclimation on freezing plant tolerance.

Materials and Methods

Ion Leakage Freezing Assay

To obtain seed, plants were all grown (from May to August) under natural lighting in a greenhouse with temperature ranging from 15 to 38 ^{o}C, except for *G. soja*, several of which were grown with short day periods (10 h days) at 25 ^{o}C in a growth chamber (PI 391587, 483464, and 483071). For experimental work *G. max* or *G. soja* (Table 1) seeds were planted in moist potting soil and grown for 2 weeks at 22 °C under approximately 150 μmol m^{-2} s^{-1} of light on a 16:8 light:dark cycle. At this point the plants were at stage VC – V1, with unifoliate leaves completely unfurled and the first trifoliate set expanding. Cold acclimation was 4 °C at night and 5 °C during the day under the same light cycle as growth conditions. After cold acclimation, ranging from 0 to 10 days, unifoliate leaves from 14 to 16 individual soybean seedlings were removed and 1 cm

diameter leaf discs punched, avoiding the midrib. Each replicate (usually 6 replicates), consisted of 3 to 4 leaf discs in a 16 x 100 mm glass test tube.

Freezing assays were performed in a ethylene glycol bath (Brinkmann Lauda MGW RC 20) with temperatures starting at -1.0 °C and lowered by 0.5 °C every 2 hrs until -2.5 °C was reached (unless otherwise noted) where they were maintained for another 2 hrs prior to removal and recovery overnight at 4 °C. Three ml of distilled water was added to each replicate and vigorous shaking was applied for 6 hrs. Electrical conductivity was measured by a portable conductivity meter (Milwaukee Model MW301 EC meter). One hundred percent electrolyte leakage was determined by rereading conductivity after freezing the same samples at -80 °C.

Germination Assay

Seeds from *G. max* and *G. soja* (Table 1) were covered halfway with water for 24 hrs at 4, 8, or 22 °C in the dark. Seeds were then placed on moistened paper towels at control temperature (22 °C) for 5 days prior to scoring germination. Seeds were considered to have germinated if any visible root extended beyond the seed coat. Each condition consisted of 15 seeds and the experiment was repeated 4 times for a total of 60 seeds for each soybean variety.

Fatty Acid Determination

Seeds from *G. max* and *G. soja* varieties (Table 1) were collected from greenhouse grown plants. For each experiment, 20 seeds of each *G. max* cultivar and 40 to 50 seeds of each *G. soja* variety were ground into a powder in liquid nitrogen. Fatty acid methyl esters were prepared from 2 mg seed powder by transesterification of total lipids (2 % H_2SO_4/methanol) at 80 °C for 1hr. Fatty acid methyl esters (FAMEs) were extracted into

hexanes and analyzed by gas chromatography–mass spectrometry with an Agilent Technologies 7890A GC/5975C MS and DB23 column (J&W Scientific). The fatty acid profile was expressed as the total percent FAMEs.

Protein Analysis

For both leaf and seed samples, proteins were extracted with 2x Laemmli SDS-PAGE sample buffer (Laemmli 1970) containing protease inhibitors (1mM Benzamidine, 1 μg mL-1 aprotinin (Sigma), l μM pepstain A (Sigma), and 1 μg mL-1 leupeptin (Sigma)). Total protein concentration was determined via amido black (Kaplan and Pedersen 1985). Proteins (10 ug) were separated on 12 % SDS polyacrylamide gels (Laemmli 1970). Samples were electrophoretically transferred to nitrocellulose membrane. Membranes were blocked with 5 % (w/v) milk in PBS prior to antibody probing. Seed samples were probed with a dehydrin antibody (1:2000 dilution, K-segment specific (Close, Fenton et al. 1993)), while leaf samples were probed with an antibody that recognizes GmERD14 (1:2000 dilution, (Yamasaki, Koehler et al. 2013)). The secondary antibody was goat anti-rabbit IgG conjugated with horseradish peroxidase (1:4000 dilution, Sigma). Peroxidase was visualized with Super Signal Western Dura (Pierce Biotechnology) and imaged on a BioRad ChemiDoc XRS+ (BioRad Laboratories).

Results

Cold acclimation in soybean seedlings

Exposure to a low, but non-freezing temperature (cold acclimation), resulted in acquisition of freezing tolerance as measured by a decrease in ion leakage in *G. max* cv

Williams 82 (Figure 1A). The acclimation time required for 50 % enhancement of freezing tolerance was 5.2 ± 0.6 days (calculated from the data in Figure 1A and two additional experiments, not shown). Seven days of cold treatment was chosen to compare the *G. max* and *G. soja* genotypes for their ability to cold acclimate. Acclimated and non-acclimated *G. max* cv Fiskeby V (MG 000), Williams 82 (MG III) and *G. soja* PI 391587 (MG II) were examined for ion leakage across a range of freezing temperatures from -1.0 to -3.0 °C (Figure 1B). Following cold acclimation, all 3 cultivars showed significant ($p < 0.05$) improvement in freezing tolerance at all temperatures less than -1.0 °C. However, the cultivars were not significantly different from each other in sensitivity to freezing temperatures regardless of acclimation status. To investigate whether maturity group might impact cold tolerance, we surveyed 8 cultivars of *G. max* and 6 varieties of *G. soja* which encompassed a wide range of maturity groups (Figure 2A). After 7 days of cold acclimation, all soybean varieties showed a significant increase in freezing tolerance (electrolyte leakage after exposure to -2.5 °C). Interestingly, the ability to acclimate was moderately correlated ($R^2 = 0.59$) with maturity group in *G. max, w*hile there was a very weak correlation in *G. soja* (Figure 2B). This suggests that while all soybean genotypes show an ability to cold acclimate there may be inherent (or baseline) differences in cold tolerance across varieties.

The impact of cold on seed germination

Seven cultivars of *G. max* and six varieties of *G. soja* across a range of maturity groups were examined for their ability to germinate after being allowed to imbibe water for 24 hrs at various temperatures (Figure 3A). There was no direct correlation between

maturity group and the ability to germinate under chilling (8 °C) or cold (4 °C) imbibition conditions in *G. max* (Figure 3B and C). Conversely, there was a moderate correlation (R^2 = 0.54, 0.47 at 8 °C and 4 °C; respectively) in *G. soja* with chilling and cold imbibition negatively impacting germination with increasing maturity group (Figure 3B, C). Within the early maturity groups (0 – II), *G. soja* had substantially higher germination rates than *G. max* of the same maturity group (compare 464866A to Shinsei; 101404A & 391587 to Amcor 89). Yet in maturity groups above III, *G. max* exhibited greater than 50 % germination rate, while *G. soja* germination rates were less than 20 %. The 000 and 00 maturity group in *G. max* had very high germination rates following treatments at either 8 or 4 °C.

When examining the cold acclimation potential (as measured by percent difference in electrolyte leakage between acclimated and non-acclimated plants), there was a moderate positive correlation between germination rates when cold imbibed and cold acclimation in *G. soja* (Figure 4A). This trend was absent in *G. max* (Figure 4B).

Fatty acid content and the ability to germinate in the cold

In the Fabaceae family, seeds that were resistant to chilling injury during imbibition generally contained higher unsaturated to saturated fatty acids proportions, particularly linolenic acid (18:3) and linoleic acid (18:2) versus oleic acid (18:1) (Dogras, Dilley et al. 1977). To investigate if this could be used as a physiological marker that relates to the observed cold germination patterns, we examined the total fatty acid content of 14 cultivars of *G. max* and 6 accessions of *G. soja* (Figure 5). Marked differences in *G. max* versus *G. soja* were found in relative amounts of oleic acid (18:1) and linolenic acid (18:3). When comparing the average percentages of all varieties, *G. max* has a higher

percent total fatty acid of oleic acid than *G. soja* (14.8±3.4 vs 7.7±2.1% respectively), while *G. soja* had significantly more linolenic acid than *G. max* (17.1±2.3 vs 8.7±1.1% respectively) which is consistent with other reports (Shibata, Takayama et al. 2008). Within the species, as maturity group increased, the percentage of linoleic acid (18:2) decreased in *G. soja* and increased in *G. max* (Figure 5). In contrast, oleic acid (18:1) and linolenic acid increased in *G. soja* and oleic acid decreased in *G. max* as maturity group increased. No significant differences relative to species or maturity group were observed in the amounts of palmitic acid (16:0) or stearic acid (18:0). Only in the *G. max* cultivar Traff and *G. soja* varieties 447003B and 101404A were 24:0 fatty acids detected at 0.1 % total composition (not shown).

The ability to germinate following cold imbibition was compared with seed lipid content (oleic acid, linoleic acid, and linolenic acid, Figure 6). *Glycine soja* seeds with elevated oleic acid tended to have decreased germination rates after imbibition at 4 and 8 °C (R^2 = 0.41, 0.34 respectively); conversely, for *G. max* higher germination rates at both 4 and 8 °C imbibition were poorly correlated to increased oleic acid (R^2 = 0.01, 0.11 respectively). Interestingly, *G. soja* linoleic acid levels strongly correlated with elevated germination rates after both 4 and 8 °C imbibition (R^2 = 0.81, 0.83 respectively), while there was no significant correlation observed in *G. max* (R^2 = 0.01, 0.20 respectively).

Protein composition and dehydrin content in G. max and G. soja

Proteins, mainly storage proteins, in the seeds of *G. max* and *G. soja* are remarkably similar in quality and quantity (Figure 7A). Additionally, protein quantity and quality in leaves of cold acclimated seedlings were also quite similar both within varieties and between *G. max* and *G. soja* (Figure 7B). Considering the dehydrin family of proteins

are known to be important in cold acclimation and cold tolerance of other plant species (Graether and Boddington 2014; Bannerjee and Roychoudhury 2015), we examined levels of dehydrins in soybean seeds and leaves. Previous examination of *G. max* cv. Young (Yamasaki and Randall, 2013) showed minimal changes of dehydrin family members in response to environment stresses. The examination here shows there was no significant difference in seed dehydrin content regardless of maturity group (Figure 7B); nor was there any increase in the acidic dehydrin GmERD14 content compared to non-cold acclimated seedling leaves in *G. soja* or *G. max* (Figure 7D).

Discussion

Glycine max has been previously characterized as a cold intolerant species with little genetic potential for cold acclimation (Hume and Jackson 1981). In this work we focused on a more detailed analysis of the cold acclimation process and determination of whether there was any correlation with maturity groups. Further, we compared the ability for seedlings to acclimate and seeds to germinate in the cold with lipid content and the expression of the stress tolerance-related dehydrin proteins. In this study, *G. max* cv Williams 82 seedling leaves demonstrated enhanced freezing tolerance with longer cold acclimation periods. After a single day of acclimation there was measurable improvement in freezing tolerance, which continually improved until day 10. All *G. max* and *G. soja* varieties examined had significantly improved freezing tolerance after 7 days of cold acclimation. These data indicate that soybean clearly does have the ability to cold acclimate as previously suggested (Hume and Jackson 1981). The argument can be made that *G. max* cold acclimation is weakly correlated with maturity group, with

northern maturity groups acclimating better than southern cultivars. Previous reports suggested that overall cold tolerance, as measured by seed germination and emergence (Littlejohns and Tanner 1976) and plant damage (Hume and Jackson 1981), did not correlate well with maturity group. This correlation between cold tolerance and maturity group was not apparent in wild genotypes of *G. soja* in the present work; though there were some varieties that performed significantly better than others at low temperatures. Cold germination ability in soybean is a distinctive measure of cold tolerance as imbibition by the seed is a process extremely sensitive to cold. For example; cold imbibition has been shown to clearly decrease the survival rate of *G. max* cv Wayne (MG III), after only 30 min (Bramlage, Leopold et al. 1978). Based on work presented here there is clear genetic potential for enhanced germination rates in the cold, illustrated by the Fiskeby V and McCall *G. max* genotypes and the several *G. soja* (MG 0 to II). Additionally, in *G. soja* the noted correlation between cold imbibition germination rates and cold acclimation suggests that these traits may be linked. The ability to germinate following cold imbibition could be a potential predictor for seedling cold survival.

Lipid (total fatty acid content) analysis of soybean seeds was evaluated to determine whether this parameter might be predictive for the ability of seeds to germinate in the cold and perhaps seedlings to survive freezing damage. Previous studies clearly showed an adaptive change in lipid composition occurs in response to cold acclimation in other members of Fabaceae family (Dogras, Dilley et al. 1977). Additionally, *Arabidopsis thaliana* ecotypes that originate in cooler, mountainous regions of the world have lower levels of very long chain fatty acids compared to ecotypes from warmer, lower altitudes

(Millar and Kunst 1999). Recently, it was shown that soybean seeds maturing in warmer climates generally had higher levels of total fatty acid content with a decreased levels of linolenic acid and increased levels of oleic acid which correlated with mean daily temperatures, but not to maturity group (Song, Yang et al. 2016). In this study, we examined the lipid composition in seeds which had not been acclimated during maturation (all grown under similar constant and normal growth conditions) to identify any potential markers within fatty acid composition that could be used to predict cold/chilling germination success. It was noted that basal levels in linoleic acid correlated with cold and chilling imbibition germination in *G. soja* (higher levels of linoleic associated with higher cold germination rates). Additionally, increases in cold germination ability also correlated with increased freezing tolerance in seedlings of *G. soja*. Neither of these trends were observed in *G. max*. However, as *G. max* consistently had equal or higher linoleic acid percentages as that of *G. soja*, this is unlikely to be an avenue worthy of further pursuit to increase cold imbibition or seedling tolerance. Another physiological potential for the differences observed in freezing acclimation is the presence of dehydrin proteins. Dehydrins are known to be expressed in seeds and vegetative tissue of Arabidopsis and dehydrin expression is highly upregulated in vegetative tissues during periods of cold (Nylander, Svensson et al. 2001) and are important for freezing tolerance (Puhakainen, Hess et al. 2004). The observation that none of the genotypes tested showed cold up-regulated levels of acidic dehydrins, was not unexpected as the lack of cold-induced dehydrin expression was previously reported in the *G. max* cv Young (Yamasaki, Koehler et al. 2013). This is despite the observation that soybean exhibits apparently normal characteristics of functional cold perception and

initial stages in cold responses (Yamasaki and Randall 2016). We conclude that the lack of dehydrin response in soybean is reflective of a partly deficient cold acclimation response system, relative to most cold tolerant plants.

Summary. Overall, there appears to be cold tolerance potential in the *Glycine max* and *Glycine soja* genotypes. This is particularly evident in cold seed germination variability among the various genotypes. For example, maturity groups 000 *G. max* and 0 in *G. soja* genotypes clearly confer cold germination phenotypes.

We present evidence here that young soybean seedlings can cold acclimate, though it should be noted that this ability to adjust molecular and physiological components results in a much less overall cold tolerance than that reported for more cold tolerant species (e.g., Arabidopsis, strawberry). Since all genotypes tested have similar ability to adjust following cold exposure (acclimate); most of the variation in cold tolerance of seedlings is contributed by inherent (under nonacclimating or basal conditions) differences in metabolism and gene expression This suggests that while all soybean genotypes show similar ability to acclimate, there are inherent (or basal) differences in cold tolerance.

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Figure Legends

Figure 1: Cold acclimation potential in *G. max* and *G. soja*. A) Ion leakage in 14 day old *G. max* cv Williams 82 acclimated at 4 °C for 0 to 10 days prior to freezing treatment. All acclimation periods were significantly different than 0 day (T-test $p<0.01$). B) Electrolyte leakage in 14 day old *G. max* cv Williams 82, Fiskeby V and *G. soja* PI 391587 soybean seedlings acclimated for 7 days (solid line, closed symbol) or 0 days (dash lines, open symbol) at 4 °C prior to freezing treatment. Error bars are standard deviations.

Figure 2: Cold Acclimation across maturity groups of *G. max* and *G. soja*. A) Ion leakage in soybean seedlings acclimated for 7 days (open bars) or 0 days (solid bars) at 4 °C prior to freezing treatment (-2.5 °C). B) Correlation of the difference between non-acclimated and acclimated ion leakage versus maturity group in both *G. max* (closed circles) and *G. soja* (open circles). Error bars (standard deviations) not shown are smaller than the symbols.

Figure 3: Cold imbibed germination rates for *G. max* and *G. soja*. A) Germination rates at 8 °C (closed box) or 4 °C (open box) were expressed as a percentage of germination rates at 22 °C. B) Correlation of seed germination rates after cold (4 °C) imbibition with maturity group in *G. max* (closed circles) and *G. soja* (open circles) varieties. C) Correlation of seed germination rates after chilling (8 °C) imbibition with maturity group in *G. max* (closed circles) and *G. soja* (open circles) varieties. Control (22 °C

imbibition) seeds germinated greater than 90% except for *G. max* cv Shinsei (63%) and *G. soja* PI 483464B (53%) and PI 483071B (15%).

Figure 4: Correlation between germination and cold acclimation potential. A) Cold (4 °C, open triangles) and chilling (8 °C, closed triangles) imbibition germination percentages versus cold acclimation (difference between % non-acclimated and % acclimated ion leakage) in *G. soja*. B) Cold (4 °C, open triangles) and chilling (8 °C, closed triangles) imbibition germination percentages versus cold acclimation in *G. max*.

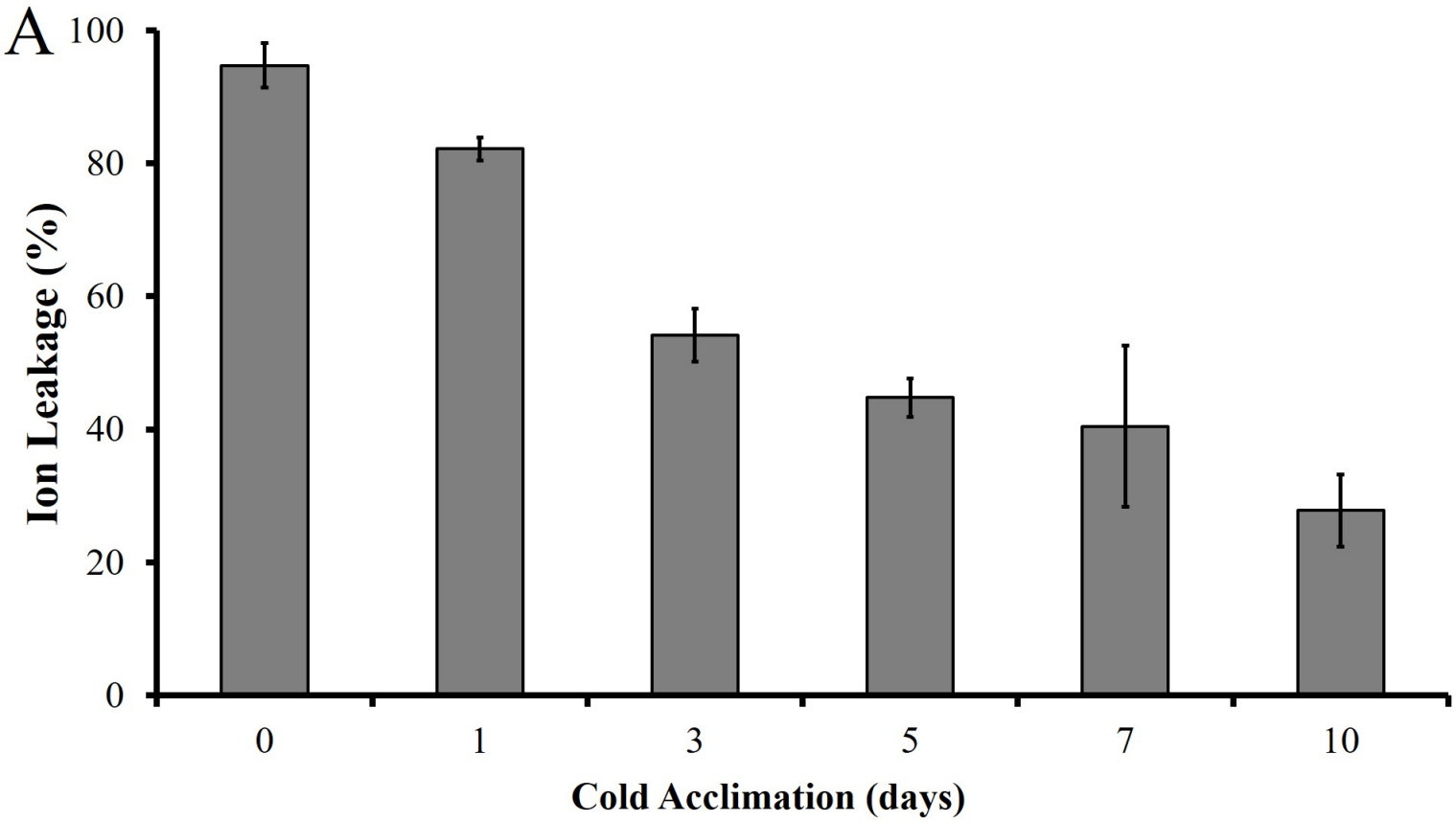
Figure 5: Major fatty acid profile of *G. soja* and *G. max* seeds. Fatty acid composition of *G. soja* and *G. max* seeds expressed as percent of total quantified fatty acids. Fatty acids greater than 20 carbons accounted for less than 1% of the total and are not depicted.

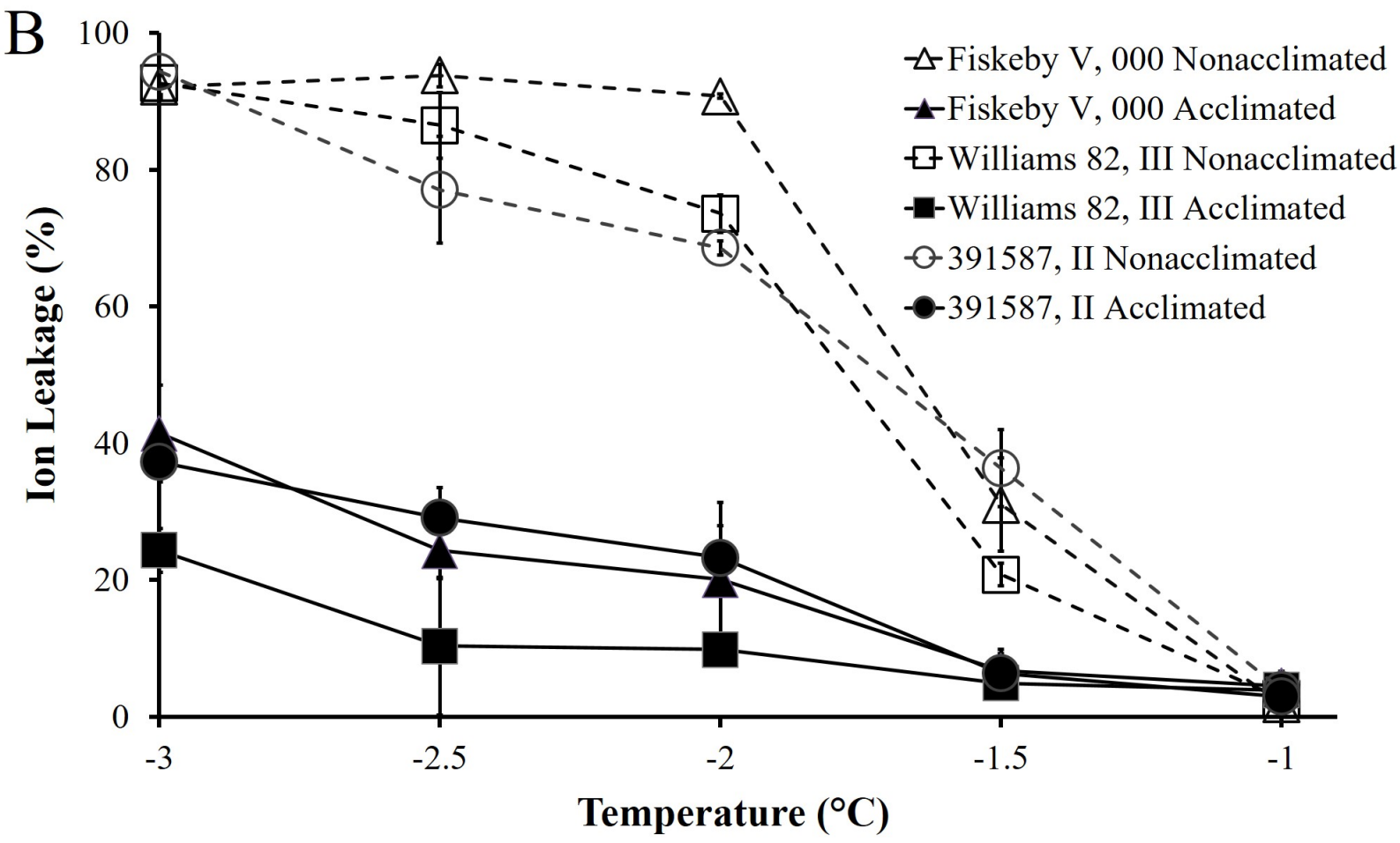
Figure 6: Fatty acid composition and cold germination in *G. max* and *G. soja*. Panels A, C, E; germination following cold inbibition at 4 °C, Panels B, D, F; germination following cold inbibition at 8 °C. Panels A, B; Oleic acid (18:1), Panels C, D; Linoleic acid (18:2), and Panels E, F; Linolenic acid (18:3). Open symbols indicate *G. soja* and closed symbols indicate *G. max.*

Figure 7: Protein content in *G. max* and *G. soja* seeds and leaves. A) Coomassie stained gel from soybean seeds. B) Coomassie stained gel from leaves after either a 7 d cold acclimation (+) or no acclimation (-). C) Western blot for the dehydrin ERD14 protein

levels in soybean seeds. D) Western blot for GmERD14 dehydrin leaves after either a 7 d cold acclimation or no acclimation. Each image is representative of 3 gels/blots.

Species	Maturity Group	Cultivar name	PI #	Country of origin
G. max	000	Fiskeby V	360955A	Sweden
G. max	000	Traff	470930	Sweden
G. max	000	Maple Presto	548593	Canada
G. max	00	Maple Arrow	548593	Canada
G. max	00	McCall	548582	USA
G. max	0	Shinsei	594279	Japan
G. max	I	Koganejiro	317335	Japan
G. max	I		522189	Russia
G. max	II	Amcor 89	546375	USA
G. max	III	Williams 82	518671	USA
G. max	III	Woodworth	548632	USA
G. max	VI	Harbar	561702	Mexico
G. max	VI	Young	508266	USA
G. max	VII	Bragg	548266	USA
G. soja	00		464866A	China
G. soja	I		447003B	China
G. soja	II		101404A	China
G. soja	II	Ye sheng-tou	391587	China
G. soja	III		483464B	China
G. soja	IV		483071B	China


A
100
80
60
40
20
0
Ion Leakage (%)
0
1
3
5
7
10
Cold Acclimation (days)


B
100
80
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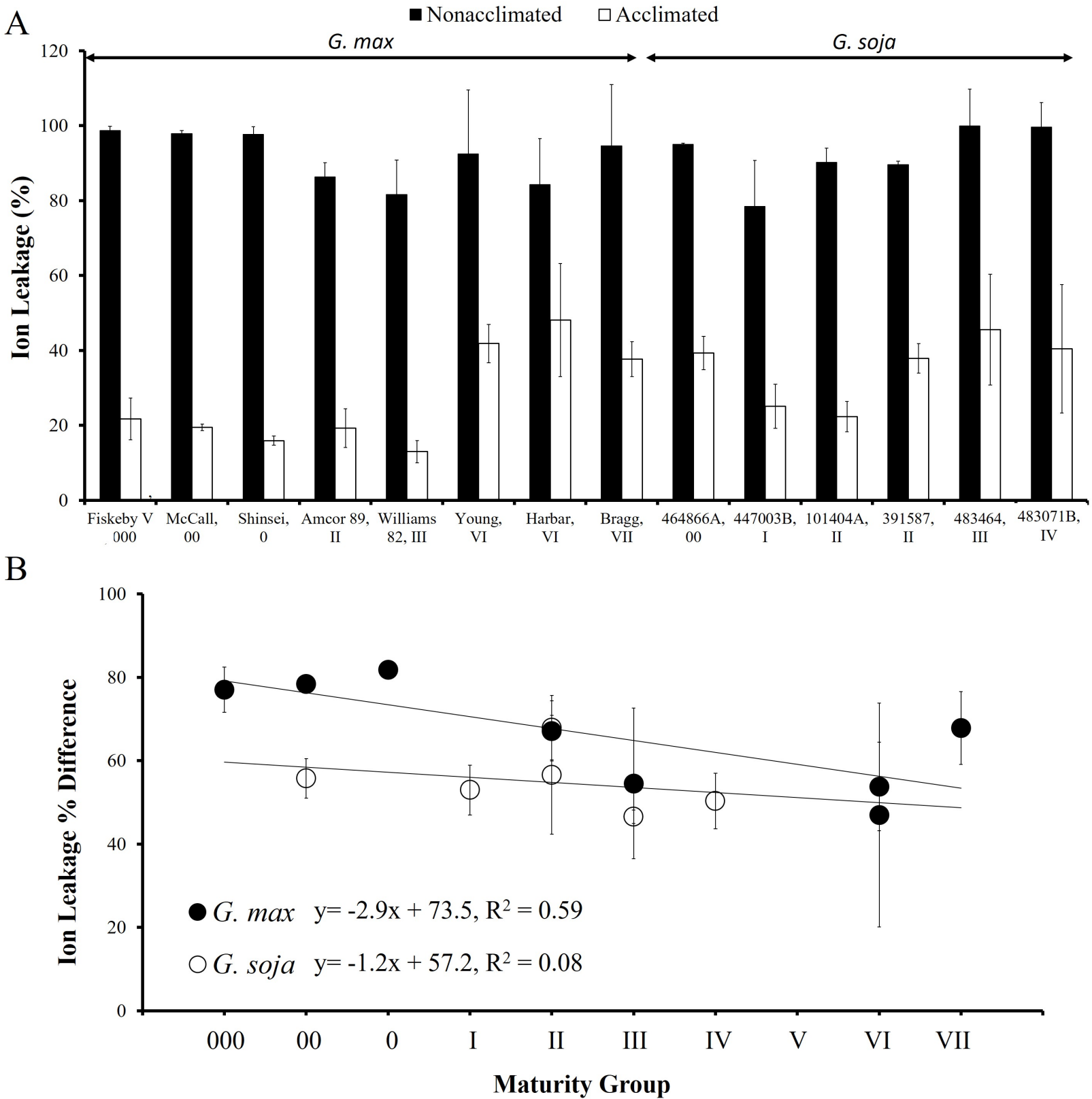
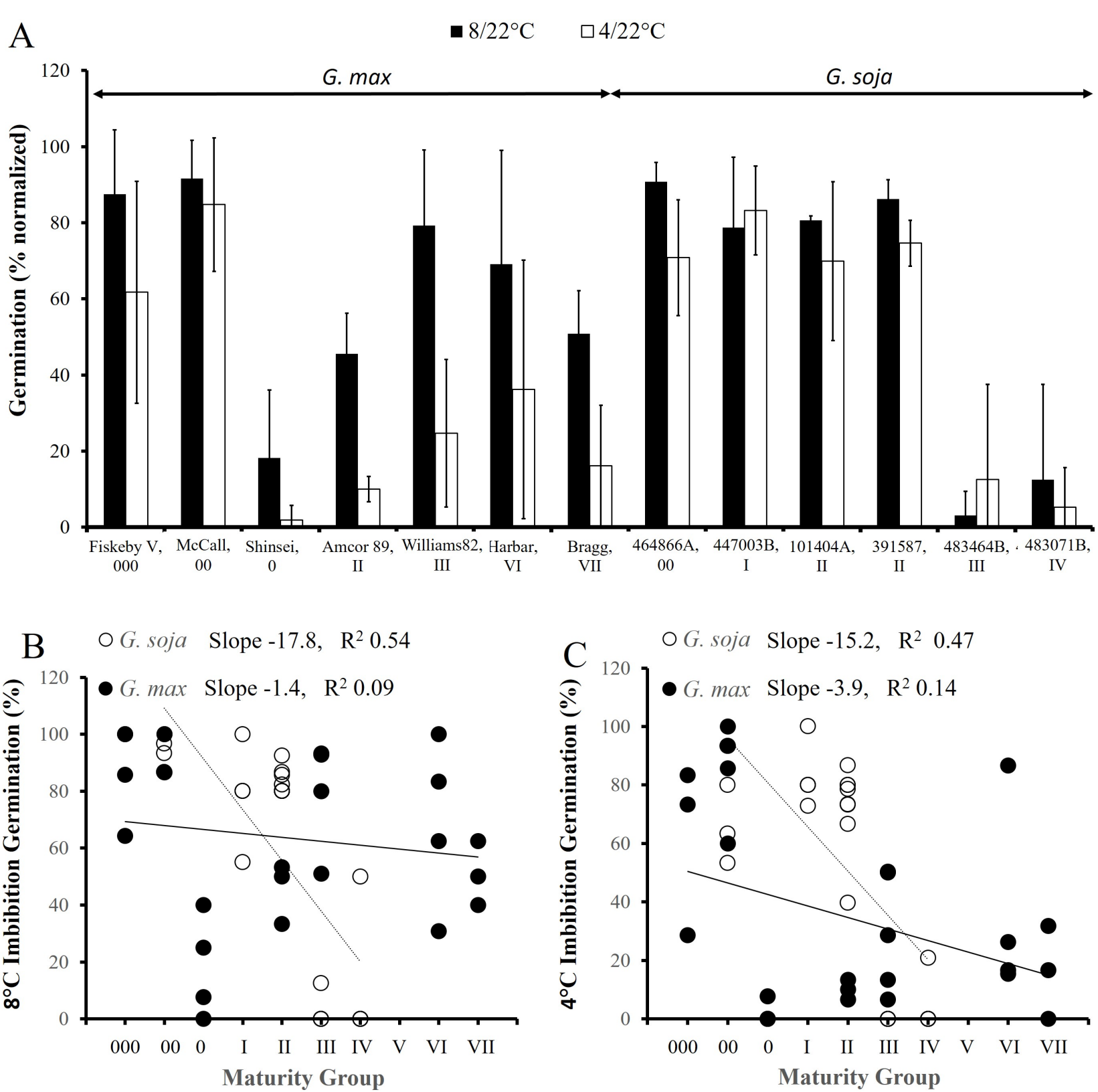
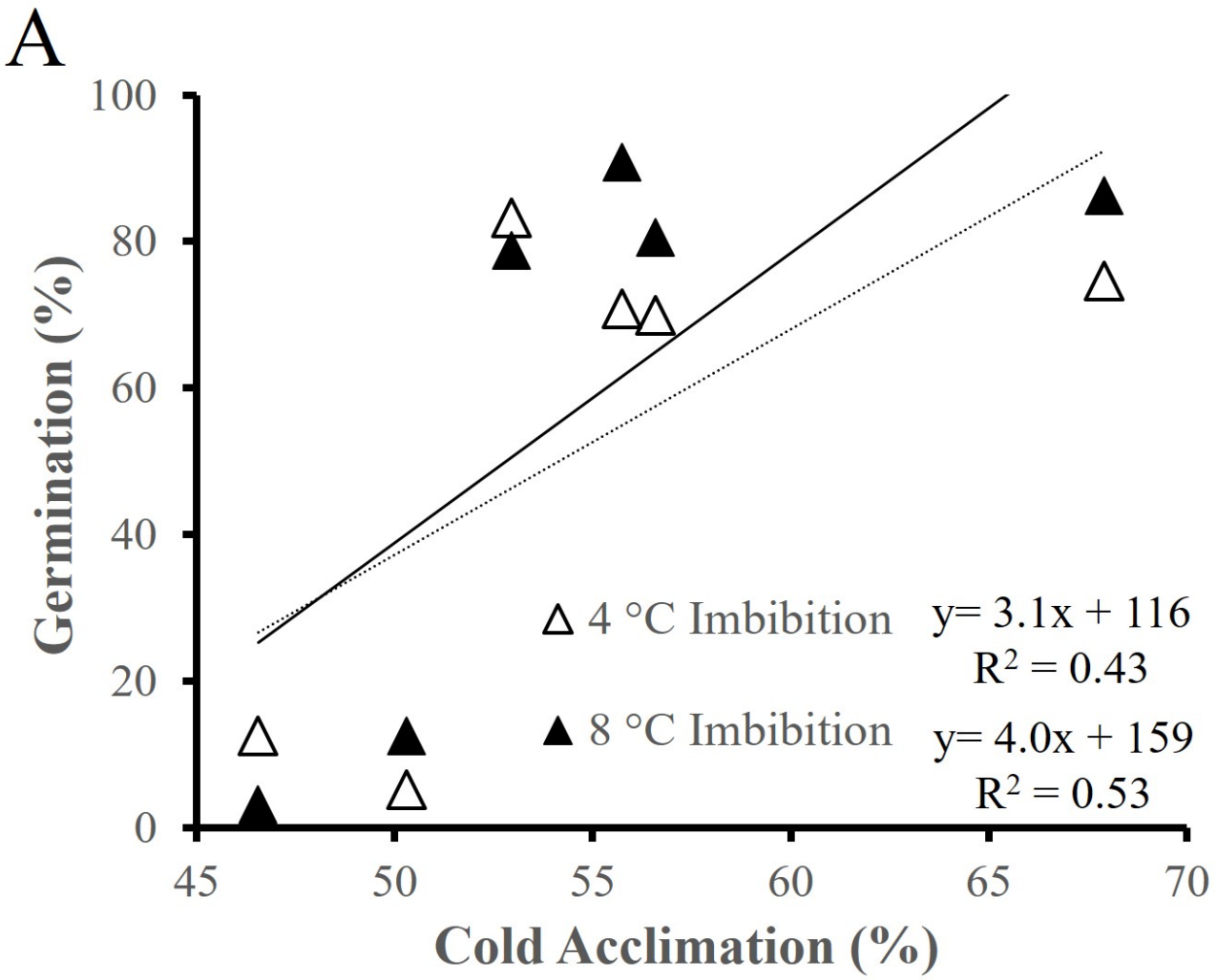
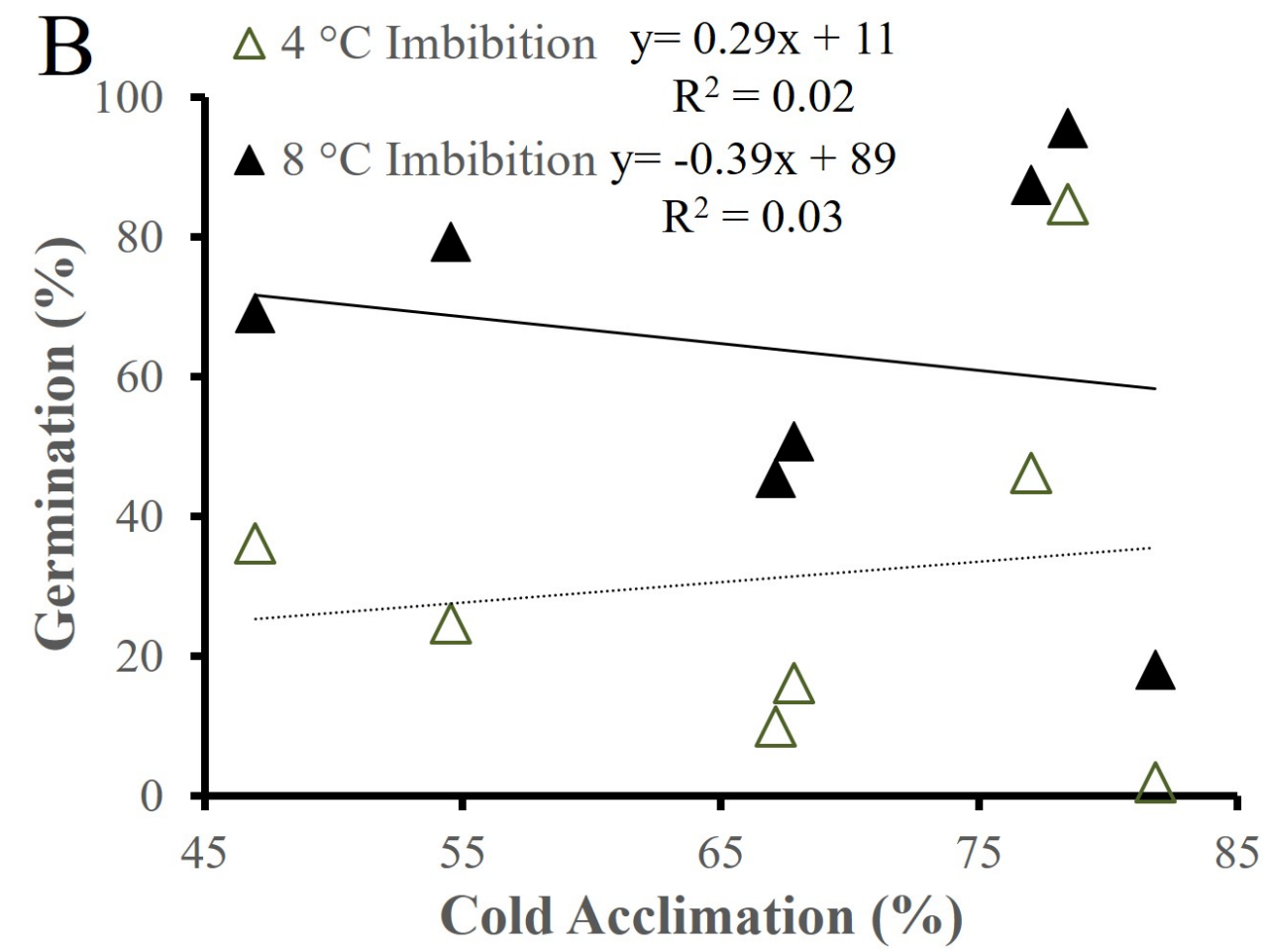
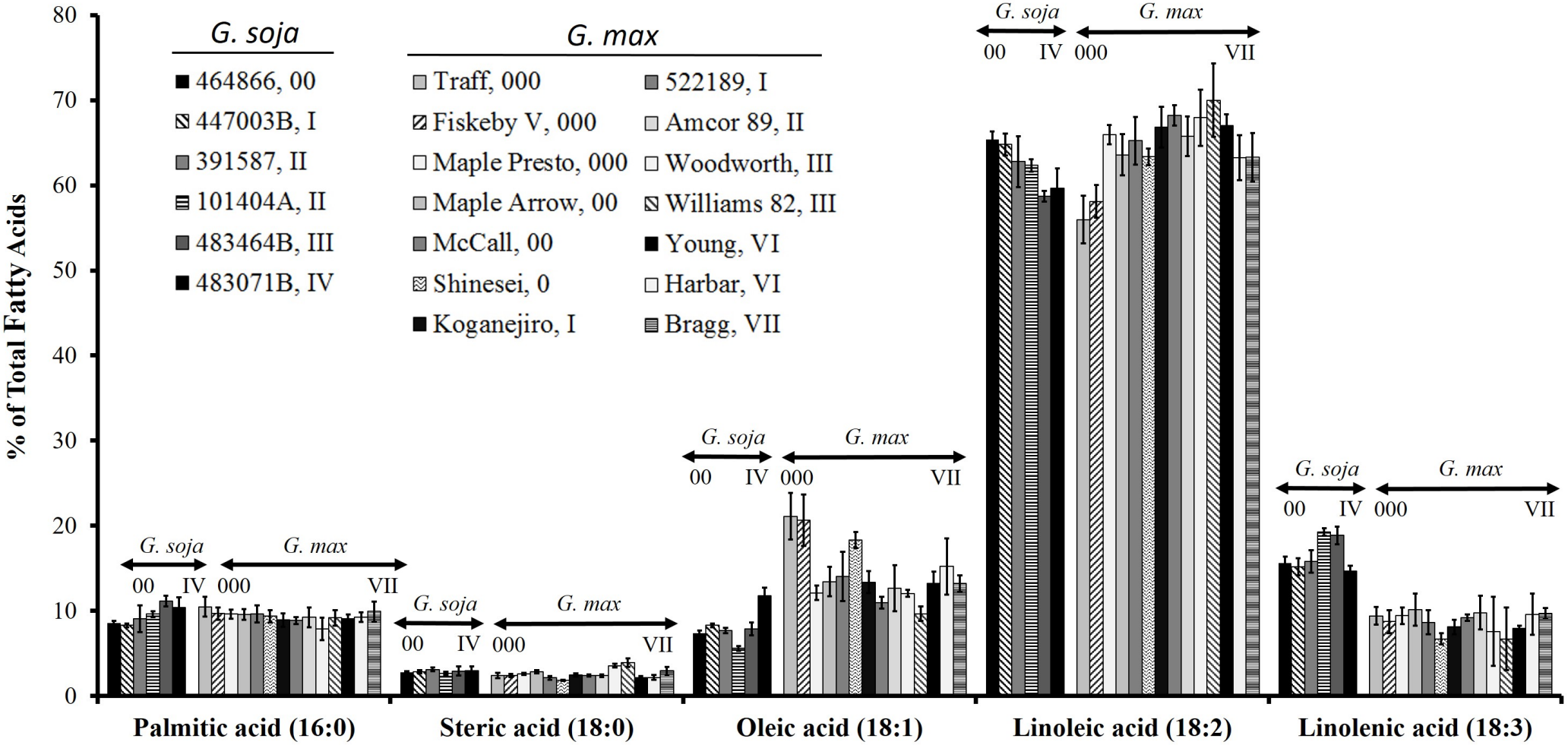
40
20
0
Ion Leakage (%)
-3
-2.5
-2
-1.5
-1
Temperature (°C)
Fiskeby V, 000 Nonacclimated
Fiskeby V, 000 Acclimated
Williams 82, III Nonacclimated
Williams 82, III Acclimated
391587, II Nonacclimated
391587, II Acclimated

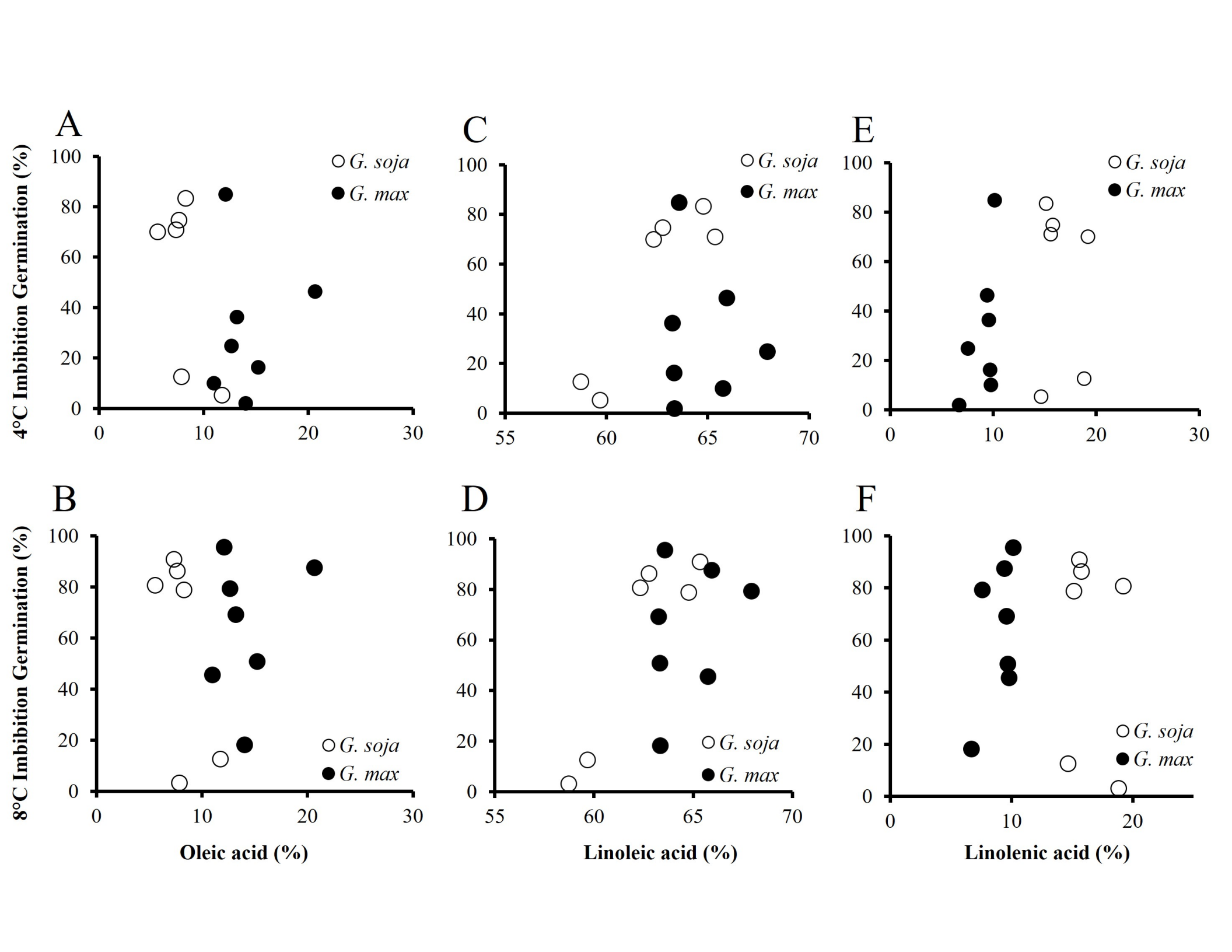

A
Nonacclimated
Acclimated
G. max
G. soja
Ion Leakage (%)
120
100
80
60
40
20
0
Fiskeby V 000
McCall, 00
Shinsei, 0
Amcor 89, II
Williams 82, III
Young, VI
Harbar, VI
Bragg, VII
464866A, 00
447003B, I
101404A, II
391587, II
483464, III
483071B, IV
B
Ion Leakage % Difference
100
80
60
40
20
0
G. max y= -2.9x + 73.5, R2 = 0.59
G. soja y= -1.2x + 57.2, R2 = 0.08
000
00
0
I
II
III
IV
V
VI
VII
Maturity Group

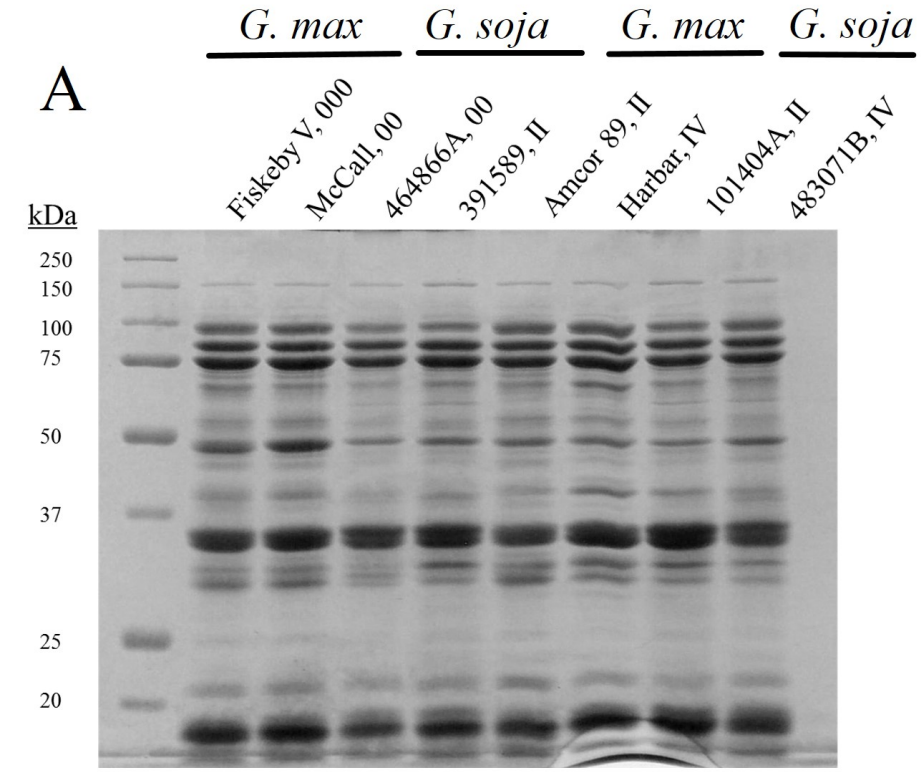

A
8/22°C
4/22°C
G. max
G. soja
Germination (% normalized)
120
100
80
60
40
20
0
Fiskeby V, 000
McCall, 00
Shinsei, 0
Amcor 89, II
Williams82, III
Harbar, VI
Bragg, VII
464866A, 00
447003B, I
101404A, II
391587, II
483464B, III
483071B, IV
B
G. soja Slope -17.8, R2 0.54
G. max Slope -1.4, R2 0.09
8°C Imbibition Germination (%)
000 00 0 I II III IV V VI VII
Maturity Group
C
G. soja Slope -15.2, R2 0.47
G. max Slope -3.9, R2 0.14
4°C Imbibition Germination (%)
000 00 0 I II III IV V VI VII
Maturity Group

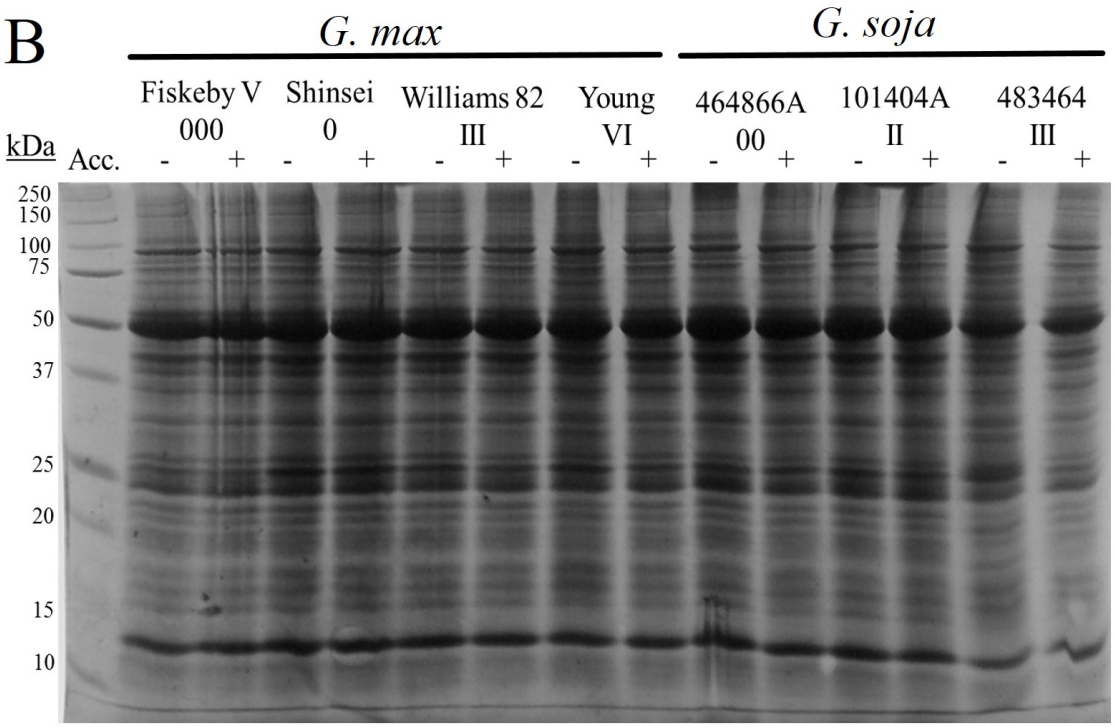

A
100
80
60
40
20
0
Germination (%)
45
50
55
60
65
70
Cold Acclimation (%)
4 °C Imbibition
y= 3.1x + 116
R² = 0.43
8 °C Imbibition
y= 4.0x + 159
R² = 0.53

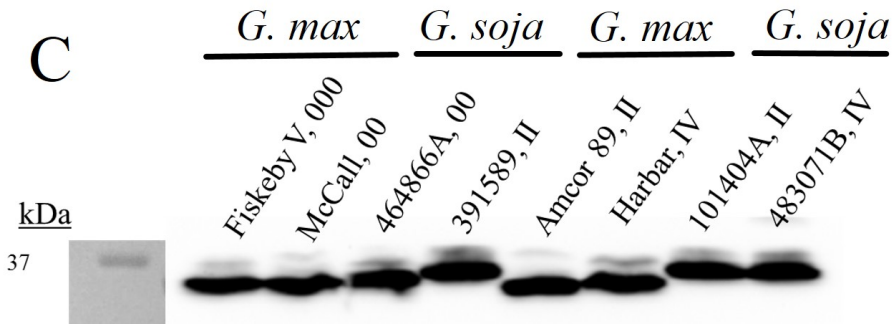

B
4 °C Imbibition
y= 0.29x + 11
R² = 0.02
8 °C Imbibition
y= -0.39x + 89
R² = 0.03
100
80
60
40
20
0
Germination (%)
45
55
65
75
85
Cold Acclimation (%)

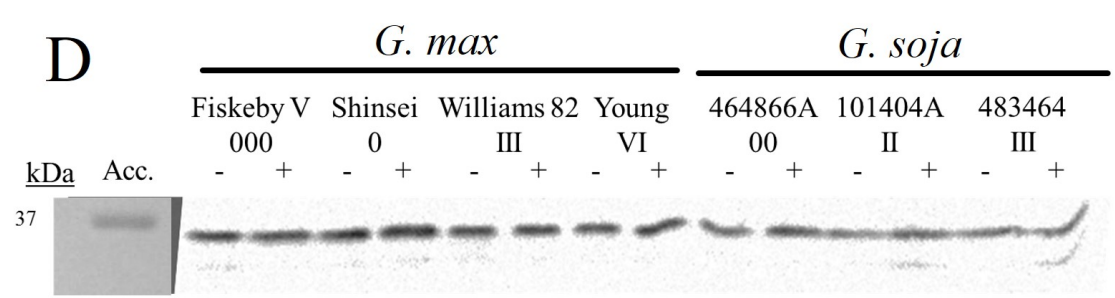

G. soja
464866, 00
447003B, I
391587, II
101404A, II
483464B, III
483071B, IV
G. max
Traff, 000
Fiskeby V, 000
Maple Presto, 000
Maple Arrow, 00
McCall, 00
Shinesei, 0
Koganejiro, I
522189, I
Amcor 89, II
Woodworth, III
Williams 82, III
Young, VI
Harbar, VI
Bragg, VII
% of Total Fatty Acids
0
10
20
30
40
50
60
70
80
G. soja
00
IV
G. max
000
VII
Palmitic acid (16:0)
Steric acid (18:0)
Oleic acid (18:1)
Linoleic acid (18:2)
Linolenic acid (18:3)


A
B
C
D
E
F
4°C Imbibition Germination (%)
8°C Imbibition Germination (%)
Oleic acid (%)
Linoleic acid (%)
Linolenic acid (%)
G. soja
G. max
0
10
20
30
40
50
60
70
80
90
100


A
G. max
G. soja
G. max
G. soja
Fiskeby V, 000
McCall, 00
464866A, 00
391589, II
Amcor 89, II
Harbar, IV
101404A, II
483071B, IV
kDa
250
150
100
75
50
37
25
20


B
G. max
G. soja
Fiskeby V
000
Shinsei
0
Williams 82
III
Young
VI
464866A
00
101404A
II
483464
III
kDa
Acc.
- + - + - + - + - + - + - +
250
150
100
75
50
37
25
20
15
10


C
G. max
G. soja
G. max
G. soja
Fiskeby V, 000
McCall, 00
464866A, 00
391589, II
Amcor 89, II
Harbar, IV
101404A, II
483071B, IV
kDa
37


D
G. max
G. soja
Fiskeby V
000
Shinsei
0
Williams 82
III
Young
VI
464866A
00
101404A
II
483464
III
kDa
Acc.
- + - + - + - + - + - + - +
37