

# Sustainable Production of Photosynthetically Cold Stress Tolerant Soybeans for Northern Canada

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## Abstract

The soybeans’ economic value to the food industry has amplified the need for increased yield and better acclimation response to the harsh norther climates of Canada. This present study aims to identify soy varieties that efficiently acclimate to stress while maintaining photosynthesis and efficient carbon allocation. Nineteen soy varieties were grown in various environments; Control (26°C/24°C); Night Cold Stress (26°C/10°C); Day/Night Cold Stress (15°C/10°C); and Cold Acclimated (26°C/10°C). PM and AM samples were collected prior to light cycle change, 8 PM and 6 AM, respectively, to identify variation in carbon allocation and mobilization. Soluble and insoluble fractions were extracted and analysed via colorimetric Anthrone reaction. Control plants exhibited varying abilities to accumulate/mobilize starch and export sucrose. Cold night conditions show a decreased ability to mobilize accumulated starch, while cold day/night conditions impacted the ability to export sucrose. Cold acclimation identified varieties with the ability to adapt and resume starch mobilization/soluble export. Varieties that successfully acclimated had variation in harvest time; further breeding may help utilize the beneficial aspects of selected germplasms.

## Introduction/Background

There is a great demand to expand production of soy plants in western and northern areas of Manitoba and Saskatchewan. The weather patterns in this area lead to the exposure of crop plants to low day/night temperatures. As a result, photosynthesis and crop productivity is severally affected. Photosynthesis is a process involved in oxygen production and CO<sub>2</sub> fixation, and production of carbohydrate for future growth and development. During the day, newly fixed carbon is allocated to sucrose and starch. Sucrose, during the day, is either used for growth and development of the leaf or is exported from the fully developed (donor) leaf to the growing organs (new leaves flowers and seeds). Starch which accumulated in the chloroplast throughout the day is mobilized back to soluble carbohydrates (glucose, sucrose) during the night, which are exported from the leaf to the growing organs (new leaves flowers and seeds). Low day/night temperatures are suppressing photosynthesis, carbohydrate allocation and abundance and export, possibly affecting starch mobilization. We evaluated soybean germplasm, selected for growth in western and northern areas of Manitoba and Saskatchewan, based on early maturity. However, there is no information existing on whether those varieties are cold stress tolerant. The purpose of the current study was to evaluate if the selected varieties have differential photosynthetic cold stress tolerance, ability for different carbon allocation (sucrose/starch). Differential low temperature starch mobilization and carbohydrate export capacity.

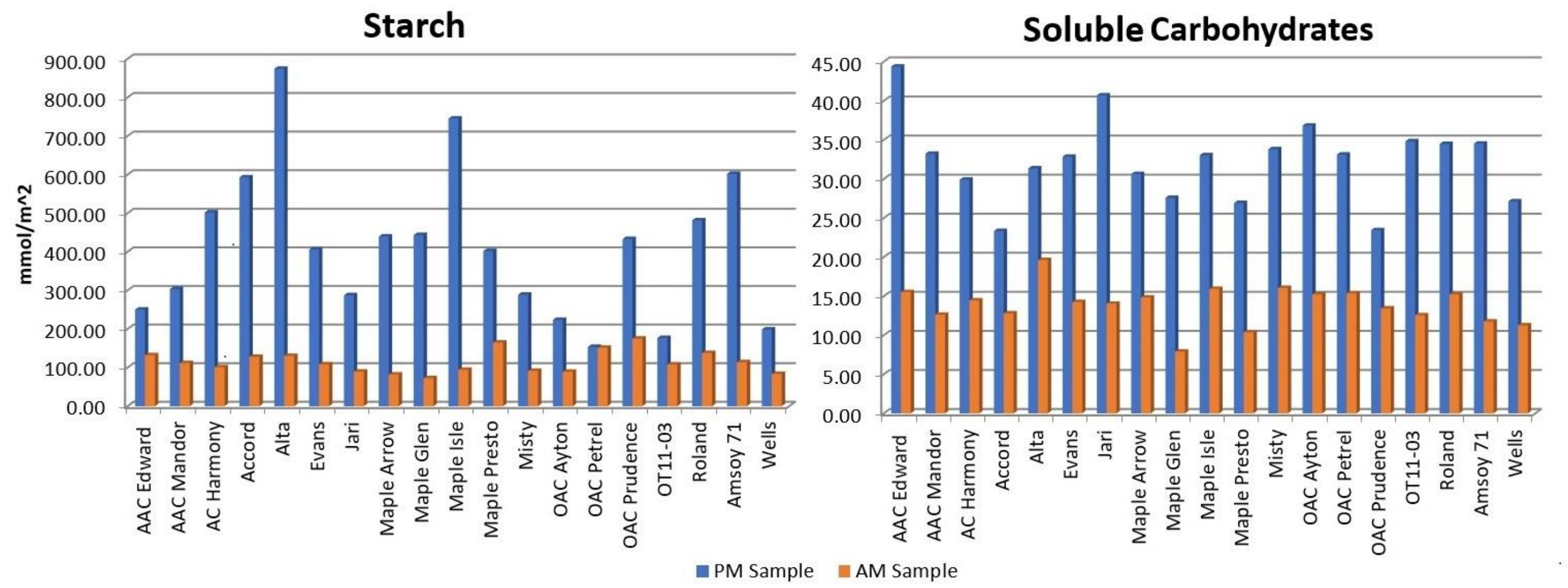
## Method

Phase 1: Soluble Extraction: (1) Mechanical grinding of frozen leaf tissue. (2) Ethanol-water sugar extraction at 95°C. (3) After centrifugation, supernatant was collected for analysis of soluble sugars

Phase 2: Starch Digestion: (1) Pellet washed with D.I. water to remove residual soluble sugars. (2) Samples were dried overnight at 65°C. (3) Chemical predigestion (KOH) of starch at 100°C. (4) Sample neutralization with acetic acid. (5) Enzyme digestion of starch using amyloglucosidase at 55°C. (6) After centrifugation, supernatant was collected for analysis of soluble sugars

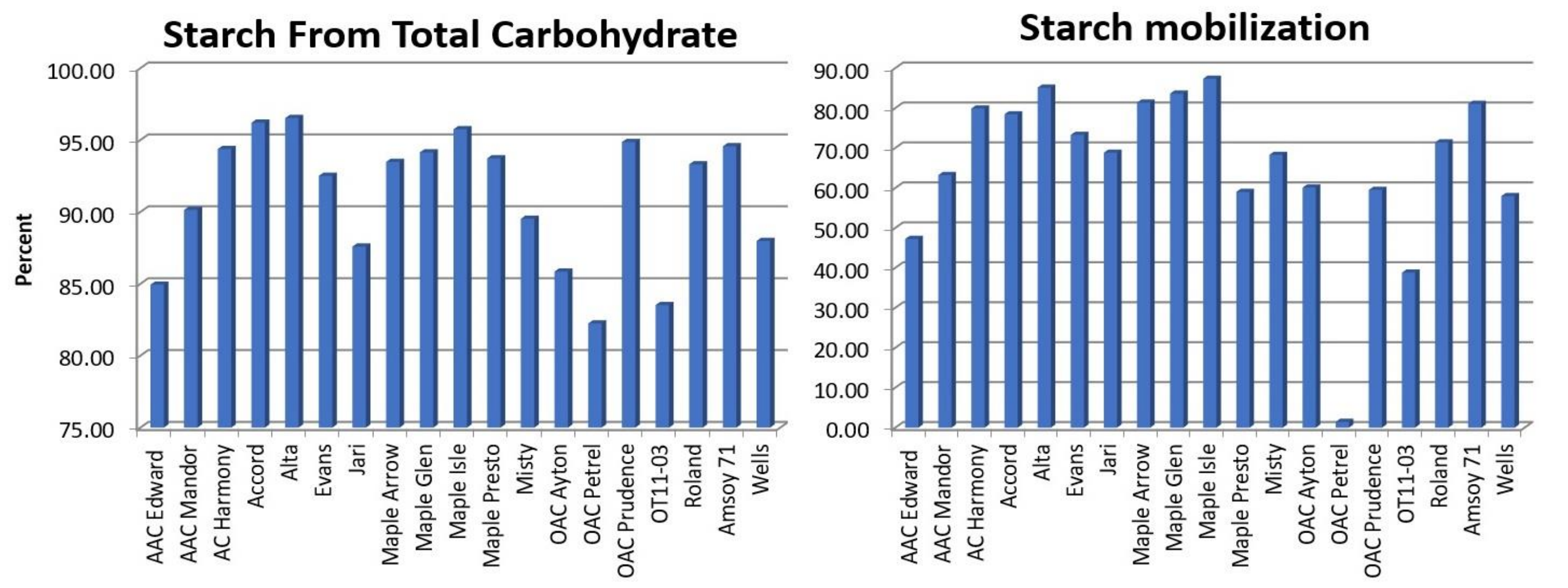
Phase 3: Carbohydrate Analysis: (1) Sample dilution (soluble extract or starch extract). (2) Colorimetric Anthrone reaction was initiated at 60°C. (3) Resulting blue-green complex evaluated using UV/VIS spectrophotometry at 620 nm.

## Results



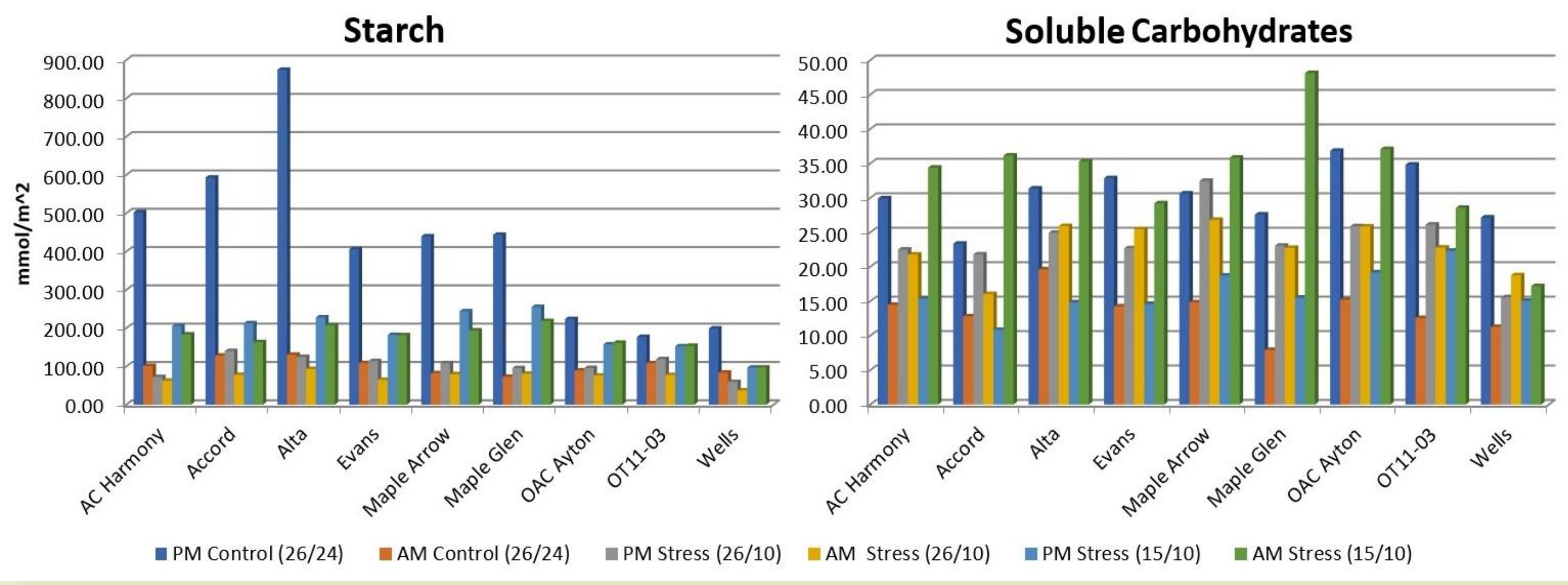
**Figure 1.** Carbon allocation under control conditions (26°C/24°C). (Left) Starch accumulation in leaf tissue after 14 hours photoperiod (PM, blue bars) and after 10 hours of darkness (AM, orange bars). (Right) Soluble sugars accumulated in leaf tissue after 14 hours photoperiod (PM, blue bars) and after 10 hours of darkness (AM, orange bars).

- Germplasms differ by their ability to allocate carbon to starch or soluble carbohydrates. We identified high starch accumulators (Group 1), moderate accumulators (Group 2), and low accumulators (Group 3)
- All tested varieties grown in the control conditions (26°C /24°C) have shown the capacity to mobilize starch and export soluble carbohydrates as seen by the depletion of starch abundance and soluble carbohydrate abundance in the AM samples



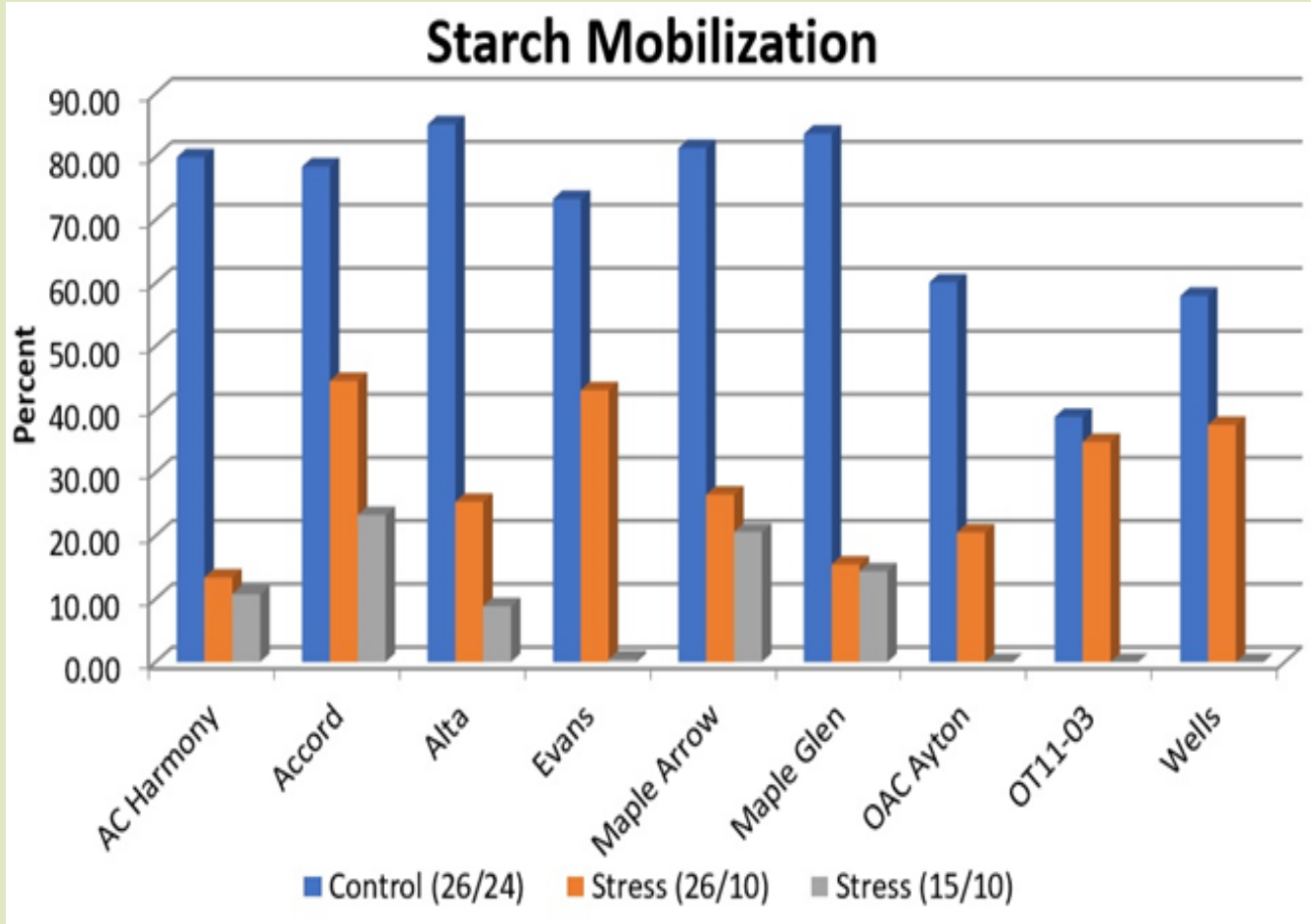
**Figure 2.** (Left) The percentage of starch accumulated from total carbohydrates grown under control conditions (26°C/24°C). (Right) The percent of starch that mobilized in the donor leaves after 10 hours of darkness in plants grown under control conditions (26°C/24°C).

- Tested varieties grown in control conditions are different in the capacity to allocate carbon to sucrose and starch leading to identification of starch accumulating groups (1, 2, and 3)
- Tested varieties grown in control conditions are different in the rate of starch mobilization with highest rates in group 1 and the lowest rates in group 3
- Low starch accumulators, allocate much less carbon to starch, thus do not require efficient starch mobilization



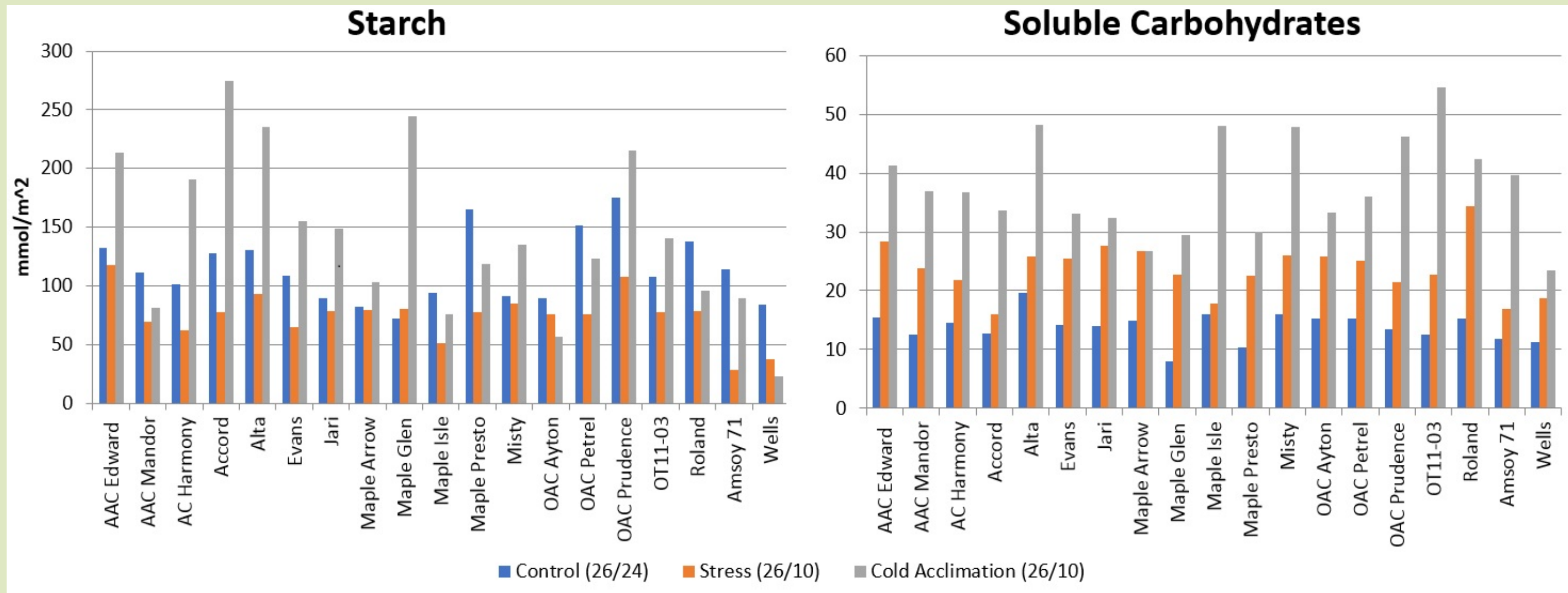
**Figure 3.** The accumulation of starch (Left) and soluble carbohydrates (Right) in control conditions (26°C/24°C, blue and orange bars represent PM and AM samples, respectively), low night temperatures (26°C/10°C, grey and yellow bars represent PM and AM samples, respectively) and low day/night temperatures (15°C/10°C, light blue and green bars represent PM and AM samples, respectively).

- Under all stress conditions, all varieties suppressed photosynthesis and as a result decreased the abundance of starch and soluble carbohydrates at the end of the day (PM)
- All varieties independent of stress conditions suppressed starch mobilization and export of soluble carbohydrates as seen by minor differences in starch abundance (PM vs AM) and soluble carbohydrates abundance (PM vs AM). Those limitations were exacerbated under 15°C/10°C stress conditions compared to 26°C/10°C conditions. Some variability have been observed between varieties with respect to the stress dependent allocation of carbohydrates



**Figure 4.** Starch mobilization under control (blue bars) and after 6 days of stress environments (26°C/10°C orange bars, and 15°C/10°C grey bars).

- Capacity for starch mobilization has been decreased throughout all varieties under all stress conditions, however 15°C/10°C conditions amplified the reduction at the level of starch mobilization
- The effect of stress on starch mobilization in low starch accumulators is less apparent
- Applied stresses differentially affected starch mobilization in tested soybean germplasm



**Figure 5.** Effects of low night temperature stress (6 days, 26°C/10°C, orange bars) and cold acclimation (5 weeks, 26°C/10°C, grey bars) on the abundance of starch (Left) and soluble carbohydrates (Right) in the AM samples.

- Cold acclimation enhanced limitation at the level of starch mobilization and limited export capacity in some varieties (Groups 1 and 2, Table 1) as seen by the increased accumulation of starch and soluble carbohydrates in AM samples
- Cold acclimation enhanced starch mobilization and export in some varieties (Group 3, Table 1) as seen by the limited accumulation of starch and soluble carbohydrates in AM samples

**Table 1.** Days after planting (DAP) required to reach full sexual maturity (R8) of soybean varieties grown in control and cold acclimated conditions. According to the differential ability of varieties to allocate carbon to starch and sucrose, selected germplasm has been ranked as high starch accumulators (Group 1, starch level  $\geq 500$  mmol/m<sup>2</sup> at 26/24 and  $\geq 200$  mmol/m<sup>2</sup> at 26/10), moderate accumulators (Group 2. 300 - 500 mmol/m<sup>2</sup> at 26/24 and 100 - 200 mmol/m<sup>2</sup> at 26/10), and low accumulators (Group 3. < 300 mmol/m<sup>2</sup> at 26/24 and < 100 mmol/m<sup>2</sup> at 26/10).

Variety	26/24C growth R8 DAP (Group)*	26/10C growth R8 DAP (Group)**
AC Harmony	80-95 (1)	92-99 (2)
Accord	77-80 (1)	87-92 (1)
Alta	80-93 (1)	91-95 (1)
AAC Edward	74-83 (3)	87-95 (1)
OAC Prudence	77-83 (2)	85-95 (1)
OAC Petrel	79-83 (3)	91-97 (2)
Maple Isle	79-93 (1)	93-96 (3)
OTII-03	74-78 (3)	90-93 (2)
Roland	74-80 (2)	128-130 (3)
AAC Mandor	77-93 (2)	128-130 (3)
Jari	70-78 (3)	145-152 (2)
Evans	79-80 (2)	139-144 (2)
Maple Arrow	78-80 (2)	130-135 (2)
Maple Presto	77-95 (2)	122-130 (2)
Maple Glen	77-80 (2)	135-150 (1)
OAC Ayton	77-82 (3)	149-152 (3)
Misty	74-79 (3)	145-152 (2)
Wells	79-81 (3)	N/A (3)***
Amsoy 71	79-81 (1)	N/A (3)***

\* PM sampling performed prior to dark cycle (8 PM, Figure 1)

\*\* AM sampling performed prior to light cycle (6 AM, Figure 5)

\*\*\* Full harvest data not available, plants could not reach maturity



**Figure 6.** Effect of low night temperature acclimation on maturity and physiological development in Jari variety. Control grown plants (26 C/24 C, top) 70-78 days to full maturity (R8 stage). Inset: Fully formed pod. Cold acclimated plants (26 C/10 C, bottom) 145-152 days to full maturity (R8 stage). Inset: White flower.

- Figure inset confirms differences in developmental stages

## Conclusion

- Screened soybean varieties differ in their ability to allocate carbon to starch or soluble carbohydrates. As a result we identified high starch accumulators, moderate accumulators, and low accumulators (Groups 1, 2, and 3). Growth in 26°C/24°C conditions does not limit starch mobilization and export of carbohydrates.
- All screened varieties independent of stress conditions suppressed photosynthesis and as a result decreased the abundance of starch and soluble carbohydrates at the end of the photoperiod, suppressed starch mobilization and export of soluble carbohydrates. Some variability have been observed between varieties with respect to the stress dependent allocation of carbohydrates.
- Limited starch mobilization and export capacity in cold acclimation conditions were observed in high and moderate starch accumulator varieties (Groups 1 and 2) and led to the sustained starch mobilization and export in low starch accumulator varieties (Group 3).
- Cold acclimation-induced delay in maturity could not be directly linked to differential abilities of soybean varieties to adjust carbon allocation.

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