PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF DARK CHILLING AND SUPERIMPOSED DROUGHT STRESS IN *GLYCINE MAX* (L.) MERRILL

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ABSTRACT

PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF DARK CHILLING AND SUPERIMPOSED DROUGHT STRESS IN *GLYCINE MAX* (L.) MERRILL.

This thesis constitute an analysis of the impact of dark chilling and superimposed drought stress on the physiological and biochemical response of *Glycine max* (L.) Merrill (soybean). Special consideration was given to the impact of these stress factors on photosynthesis.

The objectives of this investigation were to:

a) increase the current understanding of the physiological and biochemical basis for the limitation of photosynthesis by dark chilling;
b) identify possible selection criteria for dark chilling tolerance in soybean, through investigation of the effects of dark chilling on several key physiological and biochemical processes;
c) assess the impact of superimposed exposure to dark chilling and drought stress on several physiological processes in soybean.

In order to facilitate direct comparison between cultivars regarding their physiological and biochemical response to dark chilling and/or drought, two genetically related soybean cultivars of temperate origin that exhibit comparable maturity, Maple Arrow (maturity group 00) and Fiskeby V (maturity group 000), were chosen for all experiments. Both these cultivars are regarded as chilling tolerant (albeit differentially tolerant) on the basis of pod formation, flower development and other reproductive traits.

Plants of both cultivars were exposed to dark chilling in a refrigerated chamber controlled at 8°C for one entire dark period, while untreated plants were kept at 23°C. Fifteen minutes before the end of this dark period, the chilled plants were returned to the control growth chamber for the entire light period at 28°C. This temperature regime was repeated for between 1-15 consecutive dark/light periods on the same set of plants. Dark chilling experiments were conducted on plants during vegetative growth and also during late reproductive growth (pod filling). In addition to separately induced dark chilling treatments, plants during vegetative growth were also exposed to separately induced drought stress as well as simultaneously induced dark chilling and drought stress.

At selected intervals during the different treatments, various non-intrusive measurements were conducted on both untreated and stressed plants to assess the physiological and biochemical impact of these treatments. These included measurement of leaflet elongation, chlorophyll *a* fluorescence accompanied by analysis with the JIP-test and various aspects of CO_2 assimilation. In addition, various intrusive analysis were conducted, which included measurement of pre-dawn leaf water potential, ureide content of stem and nodule tissue, proline accumulation, activities of key enzymes involved in CO_2 assimilation and sucrose synthesis and activities of enzymes responsible for providing protection against oxidative damage.

For the first time, results were presented which demonstrated the existence of pronounced and consistent intra-specific differences in the physiological and biochemical response of two chilling tolerant soybean cultivars to dark chilling during both vegetative and late reproductive growth. In most of the processes studied, Maple Arrow was characterised by a less severe or more delayed response than Fiskeby V.

It was demonstrated that the slight to moderate inhibition of CO_2 assimilation in response to dark chilling in Maple Arrow was predominantly the result of stomatal limitation, whilst the often severe inhibition in Fiskeby V, was the result of mesophyll limitation. Dissection of the findings regarding mesophyll limitation identified possible biochemical candidates that could have been responsible for the inhibition of photosynthesis in Fiskeby V. After investigation of these possibilities, it was concluded that the inhibition of PS II function and enzyme activities observed, was not large enough to account for the large inhibition of CO_2 assimilation in Fiskeby V. It would seem that dark chilling did not result in any significant direct inhibition of the key reactions themselves, but rather resulted in disruption of the co-ordination between individual reactions of photosynthesis.

The ureide technique was used to quantify the effect of dark chilling on symbiotic nitrogen fixation. Symbiotic nitrogen fixation was found to be more sensitive to dark chilling than CO_2 assimilation. However, discrepancies in the ureide response between nodule and stem tissue during dark chilling was observed. It was concluded that the suitability of the ureide technique, based on xylem ureide concentration, as an indicator of symbiotic nitrogen fixation during dark chilling is questionable, especially if possible differences in the rate of ureide catabolism during stress is ignored.

The superior dark chilling tolerance regarding photosynthesis of Maple Arrow compared to Fiskeby V was even further emphasised during exposure to simultaneously induced dark chilling and drought stress. In contrast to Fiskeby V, simultaneously induced dark chilling and drought stress inhibited light saturated CO_2 assimilation at ambient and saturating CO_2 concentrations in Maple Arrow to a lesser extent (as much as 50%) than separately induced drought stress alone. It is hypothesised that the mechanism conveying superior dark chilling tolerance regarding photosynthesis in Maple Arrow, are also, at least in part, responsible for conveying superior tolerance against simultaneously induced dark chilling and drought stress.

In all treatments involving drought stress, Maple Arrow clearly had a much larger capacity for proline accumulation than Fiskeby V, which could be an important factor of stress tolerance in Maple Arrow. Dark chilling appeared to prevent the activation of proline biosynthesis. It is noteworthy that simultaneously induced dark chilling and drought stress caused a delay in the onset of proline accumulation.

Maple Arrow clearly had a superior capacity to increase the activity of ascorbate peroxidase and gluthatione reductase during all stress treatments, which is generally regarded as an important trait of enhanced stress tolerance.

The large volume of evidence acquired during this project strongly implicates a more dark chilling tolerant physiological and biochemical make-up in Maple Arrow compared to Fiskeby V. In addition, enhanced dark chilling tolerance also appears to convey enhanced tolerance of the photosynthetic apparatus to drought stress, in the unfortunate event when these two stress factors occur simultaneously. We are of the opinion that the sensitivity of our experimental approach in revealing intra-specific differences should be of great value in future screening and genetic transformation programs aimed at increasing stress tolerance in soybean.

KEY WORDS: chilling tolerance; dark chilling; drought; photosynthetic gas exchange; PS II function; Rubisco; stress interactions; sucrose-phosphate synthase; ureides; *Glycine max* (L.) Merr.