THE EFFECT OF DARK CHILLING AND ITS INTERACTION WITH DROUGHT STRESS ON THE PHOTOCHEMICAL, PHYSIOLOGICAL AND ENZYMATIC PROCESSES OF PHOTOSYNTHESIS IN *GLYCINE MAX* (L.) MERRILL

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PROJECT 1:

THE EFFECT OF DARK CHILLING AND ITS INTERACTION WITH DROUGHT STRESS ON THE PHOTOCHEMICAL, PHYSIOLOGICAL AND ENZYMATIC PROCESSES OF PHOTOSYNTHESIS IN SOYBEAN

Due to its chilling sensitivity, minimum night temperature represents one of the main constraints in soybean production in South Africa. With this investigation it was attempted to increase the current understanding of the physiological and biochemical basis of limitation of photosynthesis by dark chilling and drought as well as the interaction between these two stress factors. The investigation was premised on the assumption that by comparing the response of chilling tolerant and highly chilling sensitive soybean genotypes, it would be possible to identify physiological/biochemical traits that convey tolerance.

Three soybean genotypes were selected for direct comparison regarding their physiological and biochemical response to dark chilling and/or drought. Two of them are from temperate origin and exhibit comparable maturity namely 'Maple Arrow' and 'Fiskeby V'. 'Java 29' is of tropical origin and from a different, late maturity group. All experiments were carried out in computer controlled growth rooms at high irradiance levels (1000 µmol photons m^{-2} s^{-1}). Plants of all three genotypes were exposed to dark chilling (8°C) while untreated plants were kept at 20°C. Dark chilled plants were returned to the control growth chamber just before the end of the dark period. During the light period the temperature was kept at 23°C. Dark chilling, as well as dark chilling with superimposed induced drought experiments were conducted on plants during vegetative growth.

At selected intervals during the different treatments, various non-intrusive and intrusive measurements were conducted on both untreated and stressed plants to assess the physiological and biochemical impact of these treatments. Non-intrusive techniques used included assessment of the plastochron index, measurement of direct chlorophyll *a* fluorescence, quantification of the fast phase fluorescence transients (JIP-test) and analysis of photosynthetic gas exchange (CIRAS). Intrusive measurements included determination of pre-dawn leaf water potential, proline content, oxygen evolution rate in isolated thylakoid preparations and enzyme activity (NADP-dependant malate dehydrogenase and chloroplast fructose-1,6-bisphosphatase).

In addition to the data reported during 2001, further investigation of the effect of temperature on the decrease in O_2 evolution was done in 'Java 29'. Isolated chloroplasts were subjected to different temperatures ranging from 5°C to 45°C. At 5°C artificial electron donors (NH_2OH and proline) were able to donate e- to the oxidising side of PS II due to disengagement of the OEC. The data suggest that proline may act as possible alternative e- donor under adverse conditions.
Investigation of the effect of chilling stress on enzyme activity supported these results: Differential changes upon chilling stress occurred in the activation state of NADP-dependent malate dehydrogenase (NADP-MDH) between the genotypes, indicating differences in chilling induced changes in the stromal redox state. In 'Maple Arrow' (chilling tolerant), NADP-MDH activity was down regulated most after one night dark chilling. In addition, dark chilling induced a decrease in chloroplast fructose-1,6-bisphosphatase (FBPase) activity that occurred primarily in 'Maple Arrow' (chilling tolerant) due to a decrease in FBPase protein

content. This effect on FBPase in the chilling tolerant genotype form part of a concerted effort to down regulate photosynthetic activity, thereby limiting damage to the photosynthetic apparatus. For all three genotypes (especially in 'Fiskeby V' and 'Java 29') mesophyll limitation was the main constraint imposed by dark chilling as assessed by photosynthetic gas analysis.

Analysis of the plastochron index, proline content and water potential data suggests that 'Maple Arrow' owes its drought tolerance to its ability to reduce its growth rate to manage plant water status. 'Java 29' also exhibited evidence of drought tolerance, because it could maintain normal growth rates for extended periods. This strategy, however, resulted in a much more negative leaf water potential than in 'Maple Arrow'. Stomatal limitation was followed by mesophyll limitation in both genotypes as assessed by photosynthetic gas exchange analysis. Increases in the internal CO_2 concentration (c_i) proved drought treated and dark chilled plants of 'Java 29' to be severely affected after only one night dark of chilling. 'Java 29' was found to be the most chilling sensitive genotype and did not exhibit the drought regulating abilities evident in 'Maple Arrow' that revealed the latter to also be the most drought tolerant genotype. Significant differences existed in the chilling and drought tolerance of the different soybean genotypes investigated. This information has important practical implications for screening for chilling tolerance in soybean, aimed at directed breeding and genetic transformation.