THE IMPACT OF DARK CHILLING FOLLOWED BY EXPOSURE TO HIGH LIGHT INTENSITIES ON ULTRASTRUCTURE AND SELECTED BIOCHEMICAL REACTIONS OF PHOTOSYNTHESIS IN *GLYCINE MAX* (L.) MERRILL.

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SUMMARY

THE IMPACT OF DARK CHILLING FOLLOWED BY EXPOSURE TO HIGH LIGHT INTENSITIES ON ULTRASTRUCTURE AND SELECTED BIOCHEMICAL REACTIONS OF PHOTOSYNTHESIS IN *GLYCINE MAX* (L.) MERR.

The impact of dark chilling on ultrastructure and key reactions of photosynthesis was assessed in two soybean genotypes, 'Maple Arrow' and 'Fiskeby V'. Both these genotypes are regarded as chilling tolerant (albeit differentially tolerant). These genotypes were subjected to three nights of dark chilling followed by exposure to high light intensities. Half of the plants were subjected to whole plant chilling, while the other half were only shoot-chilled. The shoot-chilled plants were grown in the presence/absence of nitrate supplementation. The aim of this study was to increase the current understanding of the physiological, biochemical and ultrastructural basis for the limitation of photosynthesis by dark chilling in soybean genotypes. The photosynthetic response to dark chilling in the presence or absence of root chilling was also compared and the ameliorating effect of nitrate treatment on photosynthetic limitation during dark chilling was evaluated.

Non-intrusive analysis conducted to assess the physiological and biochemical impact of dark chilling included photosynthetic gas exchange measurements. Intrusive analysis included ultrastructural and anatomical studies, measurement of the activities of key enzymes involved in photosynthesis and Western Blot analysis.

The results demonstrated the existence of genotypic differences in the response of photosynthesis in two chilling tolerant soybean genotypes to dark chilling. Photosynthesis was inhibited to a lesser extent in 'Maple Arrow' than in 'Fiskeby V'. It appeared that not only mesophyll limitation, but also stomatal limitation, was much more evident in 'Fiskeby V'.

Novel evidence is provided for the existence of an inverse relationship between loss of FBPase activity and loss of photosynthetic capacity in the two chilling tolerant soybean

genotypes. The results presented suggest that 'Maple Arrow' is capable of "sensing" dark chilling stress much better than 'Fiskeby V'.

No severe ultrastructural disruption was visible in the plants investigated. The changes observed in ultrastructure corroborate the changes in photosynthetic capacity. No genotypic differences in leaf anatomy could be seen in untreated plants.

The comparative investigation regarding the effects of dark chilling, in the presence or absence of root chilling, on the photosynthetic response in 'Maple Arrow' and 'Fiskeby V' confirmed that whole-plant dark chilling caused a greater decrease in photosynthetic capacity than shoot chilling alone. In the absence of root chilling it was not possible to evaluate the ameliorating effect of nitrogen on the inhibition of photosynthesis by dark chilling because photosynthesis was not inhibited in the two genotypes under these conditions. Although dark chilling of only the shoots did not induce a significant decrease in photosynthesis, the trend did suggest that 'Maple Arrow' was more responsive to the change in temperature.

In conclusion, the results presented strongly suggest a more dark chilling tolerant physiological and biochemical make-up in 'Maple Arrow' compared to 'Fiskeby V'. However, this effect was only visible during whole plant chilling. Genotypic differences in the effectiveness of the host – Bradyrhizobium interaction could have contributed towards the chilling sensitivity of 'Fiskeby V'. When roots remained at higher temperatures during chilling, the differential chilling sensitivity between the genotypes were not revealed, suggesting that 'Fiskeby V' is more sensitive to root chilling than 'Maple Arrow'.

KEY WORDS: Chilling stress; chilling tolerance; dark chilling; Fructose-1,6-bisphosphatase; *Glycine max* (L.) Merr.; photosynthetic gas exchange; NADP-dependent malate dehydrogenase; Ribulose-1,5-bisphosphate carboxylase/oxygenase; Sedoheptulose-1,7-bisphosphatase; ultrastructure.

OPSOMMING

DIE EFFEK VAN LAE NAGTEMPERATUUR EN DAAROPVOLGENDE BLOOTSTELLING AAN HOË LIGINTENSITEIT OP ULTRASTRUKTUUR EN GESELEKTEERDE BIOCHEMIESE REAKSIES VAN FOTOSINTESE IN *GLYCINE MAX* (L.) MERR.

Die invloed van lae nagtemperatuur op ultrastruktuur en sleutelreaksies van fotosintese is in twee sojaboongenotipes, 'Maple Arrow' en 'Fiskeby V', ondersoek. Beide die genotipes word as kouetolerant (alhoewel differensieel tolerant), beskou. Die genotipes is aan drie nagte van kouebehandeling, gevolg deur hoë ligintensiteit, blootgestel. Helfte van die plante is aan heelplant-verkoeling blootgestel, terwyl die ander helfte slegs bogronds verkoel is. Genoduleerde, bogrond-verkoelde sojaboonplante is in die teenwoordigheid of afwesigheid van nitraattoevoeging gegroei.

Die doel van die studie was om die huidige begrip van die fisiologiese, biochemiese en ultrastrukturele basis van die beperking van fotosintese deur lae nagtemperatuur te verbeter. Die reaksie van fotosintese op lae nagtemperatuur in die teenwoordigheid of afwesigheid van wortelverkoeling is vergelyk. Verder is die moontlike vermindering van die negatiewe effekte van lae nagtemperatuur deur nitraattoevoeging geëvalueer.

Nie-destruktiewe metings, wat fotosintetiese gaswisselingsanalise ingesluit het, is uitgevoer om the fisiologiese en biochemiese impak van lae nagtemperatuur te bepaal. Destruktiewe analises het ultrastrukturele en anatomiese studies, bepaling van die aktiwiteit van sleutelensieme betrokke by fotosintese en 'Western Blot' analise ingesluit.

Resultate het aangetoon dat daar genotipiese verskille in die reaksie van fotosintese na kouebehandeling in die twee genotipes voorgekom het. Fotosintese was minder geaffekteer in 'Maple Arrow' as in die geval van 'Fiskeby V', en dit het verder geblyk dat nie net mesofilbeperking nie, maar ook stomatale beperking in 'Fiskeby V' oorheers het.

Die studie het nuwe bewys gelewer vir die bestaan van 'n omgekeerde verband tussen die afname in FBPase aktiwiteit en die afname in fotosintetiese kapasiteit in die twee kouetolerante genotipes. Die resultate dui daarop dat 'Maple Arrow' 'n beter vermoë as 'Fiskeby V' openbaar om kouestres waar te neem.

Geen ernstige ultrastrukturele skade is in die genotipes waargeneem nie. Die ultrastrukturele veranderinge wat wel voorgekom het, ondersteun die veranderinge in fotosintetiese kapasiteit. Verder is geen genotipiese verskille in blaaranatomie in kontroleplante van die twee genotipes waargeneem nie.

Die vergelykende ondersoek rakende die invloed van lae nagtemperatuur, in die teenwoordigheid of afwesigheid van wortelverkoeling, het bevestig dat heelplant-verkoeling 'n groter afname in die fotosintetiese kapasiteit veroorsaak het as slegs bogrond-verkoeling. Dit was nie moontlik om die effek van stikstof op die inhibisie van fotosintese gedurende kouestres in die afwesigheid van wortelverkoeling te evalueer nie, omdat fotosintese nie in die twee genotypes onder hierdie omstandighede geïnhibeer was nie. Geen gevolgtrekking kon gemaak word t.o.v. die verwantskap tussen kouetoleransie en nitraattoevoeging nie. Alhoewel bogrond-verkoeling nie 'n afname in fotosintesetempo veroorsaak het nie, is bevind dat 'Maple Arrow' beter gereageer het op veranderinge in temperatuur.

'n Meer kouetolerante fisiologiese en biochemiese samestelling is in 'Maple Arrow', in vergelyking met 'Fiskeby V', aangetoon Die effek was egter net sigbaar tydens heelplant-verkoeling. Tydens bogrond-verkoeling is die verskil in sensitiwiteit tussen die genotipes opgehef. Dit dui daarop dat 'Fiskeby V' meer sensitief vir wortelverkoeling is as 'Maple Arrow'. Genotipiese verskille in simbiotiese assosiasie tussen die twee genotipes en die entstofras kon bygedra het tot 'Fiskeby V' se groter kouesensitiwiteit.

SLEUTELTERME: fotosintetiese gaswisseling; Fruktose-1,6-bisfosfatase; *Glycine max* (L.) Merr.; kouestres; kouetoleransie; lae nagtemperatuur; NADP-afhanklike malaat

dehidrogenase; Ribulose-1,5-bisfosfaat karboksilase/oksigenase; Sedoheptulose-1,7-bisfosfatase; ultrastruktuur.