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

**PHILIPPUS DANIEL RIEKERT VAN HEERDEN M.Sc.**

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Promoter: Prof. Dr. G.H.J. Krüger

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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.

## OPSOMMING

**FISIOLOGIESE- EN BIOCHEMIESE GRONDSLAG VAN LAE NAGTEMPERATUUR- EN GESUPERPONEERDE DROOGTESTRES IN *GLYCINE MAX* (L.) MERRILL.**

Hierdie proefskrif verteenwoordig 'n analise van die effek van lae nagtemperatuur-, asook gesuperponeerde droogtestres, op die fisiologiese- en biochemiese respons van *Glycine max* (L.) Merrill (sojabone). Spesiale aandag is verleen aan die invloed daarvan op fotosintese.

Die doelstellings van hierdie ondersoek was om:

a) die huidige kennis aangaande die fisiologiese- en biochemiese grondslag van die inhibisie van fotosintese deur lae nagtemperatuur te verbreed;
b) moontlike telingsmaatstawwe vir kouetoleransie in sojabone te identifiseer deur middel van 'n ondersoek van die effek van lae nagtemperatuur op verskeie sleutel fisiologiese- en biochemiese prosesse;
c) die impak van gesuperponeerde blootstelling aan lae nagtemperatuur en droogtestres op verskeie fisiologiese prosesse in sojabone te bepaal.

Ten einde direkte vergelykings tussen kultivars t.o.v. hul fisiologiese- en biochemiese respons teenoor lae nagtemperatuur en/of droogtestres moontlik te maak, is twee geneties-verwante sojaboonkultivars van gematigde oorsprong met vergelykbare volwassenheidsgroepering, Maple Arrow (volwassenheidsgroep 00) en Fiskeby V (volwassenheidsgroep 000), vir alle eksperimente geselekteer. Beide hierdie kultivars word as kouetolerant (alhoewel differensieel tolerant) op grond van peulvorming, blomontwikkeling en ander reproduktiewe eienskappe, bestempel.

Plante van beide kultivars is aan lae nagtemperatuur van 8°C in 'n gekontroleerde koelkamer vir een volle donkerperiode blootgestel, terwyl kontroleplante by 23°C gehandhaaf is. Vyftien minute voor die einde van hierdie donkerperiode is die kouebehandelde plante saam met die kontroleplante in 'n gekontroleerde groeikamer vir 'n volle ligperiode by 28°C geplaas. Hierdie temperatuurskedule is vir 1-15 aaneenlopende donker/ligperiodes op dieselfde groep plante herhaal. Lae nagtemperatuur eksperimente is op plante tydens die vegetatiewe- sowel as die laat-reproduktiewe (peulvulling) groeistadia uitgevoer. Benewens

blootstelling aan lae nagtemperatuur, is plante tydens die vegetatiewe groeistadium ook aan droogtestres sowel as gesuperponeerde lae nagtemperatuur en droogtestres blootgestel.

Met geselekteerde tydintervalle gedurende die verskillende behandelings is verskeie nie-destruktiewe bepalings op kontrole- en behandelde plante uitgevoer om die fisiologiese- en biochemiese impak van die behandelings te kwantifiseer. Hierdie bepalings het die meting van pinnaverlenging, chlorofil *a* fluoressensie tesame met analise m.b.v. die JIP-toets en verskeie aspekte van $CO_2$ assimilering ingesluit. In samehang hiermee is verskeie destruktiewe analises ook uitgevoer wat die bepaling van voorsonop blaarwaterpotensiaal, ureïedinhoud van stingel- en wortelknoppieweefsel, prolienakkumulering, aktiwiteite van sleutelensieme betrokke by $CO_2$ assimilering en sukrosesintese, asook aktiwiteite van ensieme betrokke by die beskerming teen oksidatiewe beskadiging ingesluit het.

Dit is die eerste geleentheid waar resultate aangebied word wat die bestaan van prominente en konsekwente genotipiese verskille in die fisiologiese- en biochemiese respons tussen twee kouetolerante sojaboonkultivars in reaksie op lae nagtemperatuur tydens vegetatiewe sowel as laat-reproduktiewe groei demonstreer. In die oorgrote meerderheid van prosesse wat bestudeer is, het Maple Arrow 'n meer geringe of vertraagde respons as Fiskeby V vertoon.

Dit is bevind dat die geringe tot matige inhibisie van $CO_2$ assimilering in Maple Arrow in reaksie op lae nagtemperatuur hoofsaaklik die gevolg was van stomabeperking, terwyl die erge inhibisie in Fiskeby V die gevolg van mesofilbeperking was. 'n Analise van die bevindings aangaande mesofilbeperking het moontlike biochemiese kandidate geidentifiseer wat vir die inhibisie van fotosintese in Fiskeby V verantwoordelik kon gewees het. Na deeglike ondersoek van die onderskeie moontlikhede is dit bevind dat die inhibisie van PS II funksie en ensiemaktiwiteite wat aangetoon is, nie die erge inhibisie van $CO_2$ assimilering volledig kan verklaar nie. Dit is moontlik dat lae nagtemperatuur nie 'n betekenisvolle inhibisie van die sleutelreaksies self teweeg gebring het nie, maar eerder 'n versteuring in die koordinasie tussen die onderskeie reaksies van fotosintese veroorsaak het.

Die ureïedtegniek is gebruik om die invloed van lae nagtemperatuur op simbiotiese stikstoffiksering te kwantifiseer. Daar is bevind dat simbiotiese stikstoffiksering meer sensitief vir lae nagtemperatuur as $CO_2$ assimilering is. Teenstrydighede tussen die ureïedrespons van wortelknoppie- en stingelweefsel in reaksie op lae nagtemperatuur het egter

voorgekom. Die afleiding was dat die bruikbaarheid van die ureïedtegniek, wat gebaseer is op die ureïedinhoud van xileemsap, as indikator van simbiotiese stikstoffiksering tydens lae nagtemperatuurblootstelling twyfelagtig is, veral as moontlike verskille tussen die tempo van ureïedkatabolisme tydens stres geïgnoreer word.

Die hoër kouetoleransie van Maple Arrow teenoor Fiskeby V t.o.v. fotosintese is verder beklemtoon met die gesuperponeerde blootstelling aan lae nagtemperatuur en droogtestres. In teenstelling met Fiskeby V, het gesuperponeerde blootstelling aan lae nagtemperatuur en droogtestres ligversadigde $CO_2$ assimilering by normale en versadigde $CO_2$ konsentrasies in Maple Arrow minder geïnhibeer (tot soveel as 50%) as droogtestres alleen. Dit word gepostuleer dat die meganisme wat verhoogde kouetoleransie van fotosintese in Maple Arrow teweegbring, ook minstens gedeeltelik verantwoordelik is vir die verlening van verhoogde toleransie teenoor gesuperponeerde blootstelling aan lae nagtemperatuur en droogtestres.

In alle behandelings wat droogtestres ingesluit het, het Maple Arrow duidelik 'n groter vermoë tot prolienakkumulering vertoon as Fiskeby V wat 'n belangrike komponent van die strestoleransie van Maple Arrow mag verteenwoordig. Dit wil voorkom of lae nagtemperatuur die aktivering van prolienbiosintese verhoed. Dit is noemenswaardig dat gesuperponeerde blootstelling aan lae nagtemperatuur en droogtestres 'n vertraging in die aanvang van prolienakkumelering veroorsaak het.

Maple Arrow het duidelik 'n hoër kapasiteit besit om die aktiwiteit van askorbaatperoksidase en glutatioonreduktase tydens alle strestoestande te verhoog, wat meestal beskou word as 'n belangrike eienskap van verhoogde strestoleransie.

Die groot hoeveelheid getuienis wat tydens hierdie projek ingewin is, ondersteun ten sterkste 'n meer kouetolerante fisiologiese- en biochemiese samestelling in Maple Arrow as in die geval van Fiskeby V. Verder wil dit voorkom of verhoogde kouetoleransie ook verhoogde toleransie van die fotosintetiese apparaat teenoor droogtestres bewerkstellig, gedurende die ongunstige situasie wanneer hierdie twee streskondisies gesuperponeerd voorkom. Dit is ons opinie dat die sensitiwiteit van ons eksperimentele benadering om genotipiese verskille aantetoon van besonderse waarde behoort te wees in toekomstige teel- en genetiese manupuleringsprogramme met die einddoel om verhoogde strestoleransie in sojabone te bewerkstellig.

SLEUTELTERME: droogte; fotosintetiese gaswisseling; kouetoleransie; lae nagtemperatuur; PS II-funksie; Rubisco; stresinteraksies; sukrosefosfaatsintase; ureïede; *Glycine max* (L.) Merr.