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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ABSTRACT

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

Due to its chilling sensitivity, minimum night temperature represents the main constraint 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 namely 'Maple Arrow' and 'Fiskeby V' are from temperate origin and exhibit comparable maturity. 'Java 29' is of tropical origin and from a different, late maturity group. All experiments were carried out in computer controlled growth rooms under 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 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 measurements 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, and 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 vivo quantification of PS II function by direct chlorophyll fluorescence (JIP-test, http://come.to/bionrj), revealed that dark chilling (8°C) inhibited PS II function by deactivation of reaction centres and the conversion of excitation energy to electron transport, especially in the chilling sensitive genotype 'Java 29'. The inhibitory effect increased with time of exposure to light following dark chilling. Computing the difference of the relative variable fluorescence (ΔV) plots revealed that in 'Java 29' (chilling sensitive genotype) uncoupling of the oxygen evolving complex (OEC) occurred, indicated by an increase in fluorescence emission after about 250µs (ΔK peak) which increased with number of dark chilling treatments. In 'Maple Arrow' (chilling tolerant), one night dark chilling lead to a decrease in fluorescence emission at 2ms ($-\Delta J$ peak) indicating a decrease in the level of the Q_A^- pool, which may be due to chilling-induced slow down of e^--donation from H_2O. 'Maple Arrow' showed signs of recovery after the second and third dark chilling periods as the $-\Delta J$ peak tended to decrease, indicating an increased availability of electrons from the OEC. The genotype 'Fiskeby V' responded in a fashion intermediate to above-mentioned extremes. In the latter case only after the third dark chilling treatment did a ΔK peak, indicating damage to the OEC, appear. The JIP-test proved to be a quick, practical, non-destructive screening method for chilling tolerance in soybean. These findings were corroborated by the concomitant changes in the *in vitro* O_2 evolution rate measured polarographically on chloroplast level. The effect of temperature on the decrease in O_2 evolution was further investigated 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 which cause uncoupling of the OEC. 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 decrease in chloroplast fructose-1,6-bisphosphatase (FBPase) activity 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 is the main constraint imposed by dark chilling as assessed by photosynthetic gas analysis.

Our data revealed that differences exist in the response upon dark chilling when whole plants were chilled opposed to when only above ground parts were chilled. Furthermore an interaction between inorganic N-fertilisation of plants and the effect of dark chilling was revealed. It was

evident that addition of inorganic N ameliorated the effect of dark chilling. Chilling of above ground parts only had a less severe inhibitory effect on PS II activity, even in the sensitive genotypes ('Fiskeby V' and 'Java 29'). Recovery was also better.

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, as 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 chilling. Six days drought seemed to cause inhibition of the CO_2 draw-down mechanism of drought treated plants. Increased PI_{ABS} values were evident in 'Java 29' and 'Maple Arrow' which could have been the result of concentration of reaction centres (RCs) due to shrinking of the leaves, or alterations in the normal reversible regulation of PS II activity. No disengagement of the OEC occurred. An unambiguous conclusion regarding the mechanism involved, could however not be made from this information alone. 'Java 29' was found to be the most chilling sensitive genotype and did not exhibit the drought regulating abilities exhibited by the most drought tolerant genotype, 'Maple Arrow'.

Significant differences existed in the cold and drought tolerance of the different soybean genotypes investigated. Our data has important practical implications for screening of soybean genotypes for chilling tolerance, aimed at directed breeding and genetic transformation.

KEY WORDS: chilling tolerance; dark chilling; drought; photosynthetic gas exchange; PS II function; stress interactions; NADP-dependant malate dehydrogenase; chloroplast fructose-1,6-bisphosphatase; photosynthetic oxygen evolution; proline accumulation; *Glycine max* (L.) Merr.

OPSOMMING

DIE INVLOED VAN LAE NAGTEMPERATUUR EN DIE WISSELWERKING DAARVAN MET DROOGTE OP DIE FOTOCHEMIESE, FISIOLOGIESE EN ENSIEMATIESE PROSESSE VAN FOTOSINTESE IN *GLYCINE MAX* (L.) MERRILL

Weens kouegevoeligheid is minimum nagtemperatuur die hoof beperking vir sojaboonproduksie in Suid-Afrika. Met hierdie ondersoek is gepoog om die begrip van die fisiologiese/biochemiese grondslag van die remming van fotosintese deur lae nagtemperatuur en droogte asook die wisselwerking van hierdie stressors, te verbeter. Daar is van die veronderstelling uitgegaan dat deur die reaksie van koue-tolerante en koue-sensitiewe sojagenotipes met mekaar te vergelyk, dit moontlik sal wees om fisiologiese/biochemiese eienskappe te identifiseer wat toleransie verleen.

Vir hierdie doel is drie genotipes geselekteer. Twee van hulle, naamlik 'Maple Arrow' en 'Fiskeby V' is van gematigde oorsprong en besit dus 'n vergelykbare volwassenheidsperiode. 'Java 29' is van tropiese oorsprong en val in 'n laat volwassenheidsgroep. Alle eksperimente is in rekenaarbeheerde groeikamers by hoë ligintensiteit (1000 µmol fotone m^{-2} s^{-1}) uitgevoer.

Die proefplante is onderwerp aan lae nagtemperatuur (8°C) terwyl die kontroleplante by 20°C gehou is. Die koue behandelde plante is net voor die einde van die nagperiode teruggeplaas in die kontrole groeikamer. Tydens die ligperiode was die temperatuur deurgans 23°C. Die koue- sowel as die koue met gesuperponeerde droogte eksperimente, is uitgevoer tydens die vegetatiewe groeistadium.

Op gekose tussenposes is verskeie nie-destruktiewe en destruktiewe metings op proef- en kontroleplante uitgevoer om die fisiologiese en biochemiese invloed van die behandeling te bepaal. Nie-destruktiewe metings het ingesluit: (1) bepaling van die plastochronindeks, (2) meting van direkte chlorofil *a* fluoressensie en kwantifisering van die vinnigefase fluoressensiekurwe (JIP-toets), (3) analise van fotosintetiese gaswisseling (IRGA). Die destruktiewe tegnieke het ingesluit (1) bepaling van voordagbreek-waterpotensiaal, (2) bepaling van prolieninhoud, (3) bepaling van die suurstofvrystellingstempo in geïsoleerde tilakoïed preparate, (4) *in vitro* bepaling van NADP-afhanklike malaat dehidrogenase en chloroplast fruktose-1,6-bisfosfatase.

Met *in vivo* kwantifisering van PS II funksie deur chlorofilfluoressensie (JIP-toets) is aangetoon dat 'n nagtemperatuur van 8°C PS II funksie benadeel deur deaktivering van reaksiesentrums

asook remming van die omsetting van eksiteringsenergie na elektrontransport, veral in die koue-sensitiewe 'Java 29'. Hierdie inhibering het toegeneem met toenemende tyd van blootstelling aan lig, tydens die daaropvolgende ligperiode. Berekening van die verskil tussen die fluoressensiekurwes van die kontrole en behandeling (ΔV), het aan die lig gebring dat ontkoppeling van die OEC in 'Java 29' plaasgevind het, soos aangetoon deur 'n toename in fluoressensie na ongeveer 250µs (ΔK piek) wat toegeneem het met aantal kouebehandelings. In 'Maple Arrow' (kouebestand) het een nag koue gelei tot 'n afname in fluoressensie by 2ms ($-\Delta J$ piek) wat 'n afname in die vlak van die Q_A^- poel aandui en wat te wyte kan wees aan 'n vertraging in e^- -vloei vanaf H_2O. 'Maple Arrow' het egter tekens van herstel getoon na die tweede en derde koue nagte, aangesien die $-\Delta J$ piek algaande verminder het wat 'n toename in beskikbaarheid van elektrone vanaf die OEC aangedui het. Die genotipe 'Fiskeby V' se gedrag het tussen hierdie twee uiterstes geval. In hierdie geval het 'n ΔK piek wat beskadiging van die OEC aandui, eers na die derde koue nag verskyn. Die JIP-toets het geblyk 'n vinnige, praktiese, nie-destruktiewe keuringsmaatstaf vir kouetoleransie in sojabone te wees. Genoemde bevindings is bevestig deur gelyktydige polarografiese meting van die suurstofvrystellingstempo op chloroplastvlak wat aangedui het dat O_2 vrystelling inderdaad deur die kouebehandeling gerem word. Verdere ondersoek van die invloed van temperatuur op die afname in O_2-vrystelling is uitgevoer op 'Java 29'. Geïsoleerde chloroplaste is blootgestel aan verskillende temperature tussen 5°C tot 45°C. By 5°C was kunsmatige e^- skenkers soos NH_2OH en prolien instaat om elektrone te skenk aan die oksiderende kant van PS II as gevolg van die ontkoppeling van die OEC. Die resultate suggereer dat prolien mag optree as moontlike alternatiewe e^- skenker aan die oksiderende kant van PS II onder ongunstige omstandighede. Data ten opsigte van die invloed van koue op ensiemaktiwiteit het hierdie data ondersteun: Verskille het voorgekom tussen genotipes ten opsigte van die invloed van nagkoue op die aktiveringstaat van NADP-afhanklike malaatdehidrogenase (NAPD-MDH), wat daarop wys dat verskille voorkom ten opsigte van koue-geinduseerde veranderinge in die stroma redokstoestand. In 'Maple Arrow' (koue bestand) was die NADP-MDH aktiwiteit die meeste verminder na een nag koue. Hierbenewens het koue-geïnduseerde verlaging van fruktose-1,6-bisfosfaat aktiwiteit, weens die verlaging in proteïeninhoud hoofsaaklik in 'Maple Arrow' voorgekom. Hierdie invloed op FBPase in die kouebestande genotipe vorm deel van 'n georkestreerde poging om die fotosintesetempo te verlaag om skade aan die fotosintetiese-apparaat te beperk. Met behulp van fotosintetiese gasanalise kon aangetoon word dat in al drie genotipes (maar hoofsaaklik 'Fiskeby V' en 'Java 29') mesofilbeperking die belangrikste gevolg van kouebehandeling was.

Die data het verder aangetoon dat wanneer die hele plant verkoel word die invloed verskil van wanneer slegs die bogrondse gedeelte verkoel word. Daar het ook 'n wisselwerking bestaan tussen N-voeding en die reaksie van die plant op koue. By al drie genotipes het toegevoegde

anorganiese N die invloed van die koue versag aangesien dit die inhibisie van PS II verminder het en so ook herstel van die plante bevorder het na die tweede en derde koue behandelings, selfs in 'Java 29' en 'Fiskeby V'.

Analise van die data ten opsigte van plastochronindeks, prolieninhoud en waterpotensiaal, suggereer dat 'Maple Arrow' se droogtebestandheid toe te skryf is aan sy vermoë om sy groeitempo te verminder ten einde sy waterstatus te beheer. 'Java 29' het ook tekens van droogtebestandheid getoon aangesien dit 'n normale groeitempo vir langer periodes kon volhou. Dit het egter gelei tot laer waterpotensiaalwaardes as in die geval van 'Maple Arrow'. Fotosintetiese gaswisseling data het daarop gedui dat geïnduseerde droogte hoofsaaklik mesofilprosesse nadelig beïnvloed het: In 'Java 29' is die assimilering van CO_2 deur die Calvinsiklus verminder (verhoging in c_i). 'n Toename in die intersellulêre CO_2 konsentrasie (c_i) het aangetoon dat 'Java 29'plante blootgestel aan droogte en kouestres, ernstig beïnvloed is deur een nag kouestres. Dit het voorgekom asof 6 dae droogte inhibering van die CO_2 assimilerings meganisme veroorsaak het in die plante blootgestel aan droogtestres. 'Java 29' en 'Maple Arrow' het 'n toename in PI_{ABS} waardes getoon wat moontlik die gevolg kon wees van die konsentrering van die reaksiesentrums (RCs) of veranderinge in die normale omkeerbare regulering van PS II aktiwiteit. Geen onkoppeling van die OEC het plaasgevind nie. Dit was moeilik om alleenlik uit hierdie inligting 'n afdoende gevolgtrekking oor die meganisme betrokke te maak. Daar is bevind dat 'Java 29' nie alleen die koue sensitiefste genotipe is nie, maar dat dit ook nie droogte regulerende vermoëns besit van 'Maple Arrow' wat in ons ondersoek as die mees droogte verdraagsame genotipe geklassifiseer is nie.

Betekenisvoll verskille kom voor in die koue- en droogtetoleransie van die verskillende sojaboon genotipes wat in hierdie studie ondersoek is. Hierdie inligting hou belangrike implikasies in vir die keuring van verskillende genotipes vir koue verdraagsaamheid met die oog op genetiese transformasie.

SLEUTELTERME: droogte; fotosintetiese gaswisseling; kouetoleransie; lae nagtemperatuur; NADP-afhanklike malaat dehidrogenase; chloroplast fruktose-1,6-bisfosfatase; prolienakkumulering; PS II funksie; stresinteraksies; fotosintetiese suurstofvrystelling; *Glycine max* (L.) Merr.