THE INFLUENCE OF DARK CHILLING ON THE ENDOGENOUS RHYTHMS OF PHOTOSYNTHESIS AND SUCROSE SYNTHESIS IN *Glycine max* [L.] MERRILL

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ABSTRACT

Low night temperatures (dark chilling) can inhibit photosynthesis in chilling sensitive plant species, such as soybean [*Glycine max* (L.) Merrill], by as much as 50% during the day following the chilling event. In the case of soybean, the underlying cause of this severe inhibition is largely unknown. The inhibition of individual biochemical processes is often not large enough to explain the severe decrease of photosynthesis in full. Recently, Jones *et al.* (1998) provided evidence that suggests that dark chilling interfered with the regulation of enzymes and the co-ordination between them, rather than direct effects on enzymes *per se*. Jones *et al.* (1998) demonstrated that the endogenous rhythms in the activity of sucrose-phosphate-synthase (SPS) and nitrate reductase (NR) were severely disrupted by dark chilling in chilling sensitive tomato. Kerr *et al.* (1985) also reported the existence of an endogenous rhythm in SPS activity in soybean, but the effect of dark chilling on the rhythm is unknown.

In this investigation, a thorough characterisation of the effects of dark chilling on photosynthesis was conducted in two soybean genotypes of contrasting chilling tolerance. The main objectives of this study were to improve the understanding of the physiological and biochemical basis of chilling tolerance and that this information could be used for future directed breeding and genetic transformation programmes for increased chilling tolerance in soybean.

'Maple Arrow' (chilling tolerant) and 'Java 29' (chilling sensitive) were used throughout the study. The above mentioned genotypes were subjected to three consecutive nights of chilling. A number of key parameters of photosynthesis were determined using a portable photosynthesis system.

We demonstrated that dark chilling resulted in a very similar inhibition of CO_2 assimilation rate and stomatal conductance in the two genotypes, but that the underlying cause of the inhibition was very different. In 'Java 29', the chilling sensitive genotype,

gas exchange analysis revealed that mesophyll limitation was a major factor contributing towards the inhibition of photosynthesis, while in 'Maple Arrow', the chilling tolerant genotype, only minor mesophyll limitation occurred.

To further quantify the cause of the large inhibition of photosynthesis, plants were subjected to dark chilling and then kept in the growth camber at saturated atmospheric CO_2 concentration (1500 μmol mol^{-1}). This caused the photosynthetic rate to increase, but even though the rates were higher, the dark chilled plants never totally recovered to control values, especially in the case of 'Java 29'. This observation supported the assumption that the reduction in photosynthesis was of biochemical origin.

By keeping the plants under constant light, it was possible to visualise the endogenous rhythms of CO_2 assimilation and stomatal conductance. Although dark chilling caused a decrease in both processes, it did not disturb these endogenous rhythms.

Dark chilling caused a disruption in the regulation of SPS that prevented the normal diurnal increase in SPS activity during the light period following dark chilling. Inhibition of SPS activity was identified as a distinguishing characteristic between the two genotypes and could have been a major factor contributing towards the observed mesophyll limitation of photosynthesis in 'Java 29'. In both genotypes, a very similar endogenous rhythm in SPS activity was identified. Dark chilling disrupted this endogenous rhythm, but only in 'Java 29'. Disruption of the rhythm was not due to a perturbation of the actual biochemical mechanism (change in steady state SPS protein content) that controls the rhythm. Rather, dark chilling introduced some unknown factor that caused SPS to be "locked" in an inactive state.

Disruption of the internal time-keeping mechanism of SPS, which co-ordinates its activity with diurnal changes in the rate of photosynthesis, could be only one of many simultaneous disruptions that occur in chilling sensitive soybean genotypes. It is concluded that this may still prove to be one of the main reasons for the difference in chilling tolerance between soybean genotypes of the early and late maturity groups.

Key words: Dark chilling, endogenous rhythm, photosynthesis, soybean, sucrose-phosphate-synthase, sucrose-synthase.

OPSOMMING

Lae nagtemperatuur kan fotosintese in koue sensitiewe plantspesies soos sojabone [*Glycine max* (L.) Merrill], met soveel as 50% inhibeer gedurende die dag na die kouebehandeling. In die geval van sojabone is die oorsaak van hierdie verskynsel grootliks onbekend. Die skade aan individuele biochemiese prosesse is skynbaar nie groot genoeg om die oorsaak te kan wees vir hierdie erge inhibisie van fotosintese nie. Jones *et al.* (1998) het voorgestel dat lae nagtemperatuur die regulering van ensieme en die koördinering tussen ensieme beïnvloed, eerder as deur 'n direkte effek op die ensieme self. Jones *et al.* (1998) het aangetoon dat die endogene ritme in die aktiwiteit van sukrose-fosfaat-sintase (SPS) en nitraatreduktase (NR) ernstig versteur is deur lae nagtemperatuur in koue sensitiewe tamatieplante. Kerr *et al.* (1985) het ook 'n endogene ritme in SPS aktiwiteit in sojabone ontdek, maar die effek wat koue op hierdie ritme uitoefen, is egter onbekend.

Tydens hierdie studie is 'n deeglike ondersoek gedoen aangaande die effek van lae nagtemperatuur op fotosintese in twee sojaboongenotipes van kontrasterende koue toleransie. Die hoofdoel van hierdie studie was om die fisiologiese- en biochemiese basis van koue toleransie beter te kan begryp en om hierdie inligting te gebruik in toekomstige teling- en genetiese transformeringsprogramme om sodoende koue toleransie in sojabone te verhoog.

'Maple Arrow' (koue tolerant) en 'Java 29' (koue sensitief) is deurgaans in die studie gebruik. Die twee bogenoemde genotipes is vir drie opeenvolgende nagte aan lae temperatuur blootgestel. 'n Paar belangrike parameters van fotosintese is bepaal deur gebruik te maak van 'n draagbare fotosintese sisteem.

Die lae nagtemperatuur het 'n soortgelyke inhibisie in CO_2 assimileringstempo en stomageleiding veroorsaak, maar die oorsaak van die inhibisie het tussen die twee genotipes verskil. In 'Java 29', die koue sensitiewe genotipe, het gaswisselingsanalise

aangetoon dat mesofilbeperking die hoof oorsaak was vir die inhibisie van fotosintese, terwyl in 'Maple Arrow', die koue tolerante genotipe, slegs minimale mesofilbeperking voorgekom het.

Die effek van lae nagtemperatuur op fotosintese is verder gekwantifiseer deur die plante vir 'n verdere nag aan koue bloot te stel en daarna in die groeikamer by versadigde atmosferiese CO_2 konsentrasie (1500 µmol mol^{-1}) te hou. Dit het veroorsaak dat die fotosintesetempo toegeneem het, maar selfs by hierdie hoë CO_2 konsentrasie, is die inhibisie van fotosintese nie opgehef tot kontrole waardes nie, veral in die geval van 'Java 29'. Hierdie verskynsel het die aanname, dat die afname in fotosintese van biochemiese oorsprong is, verder ondersteun.

Om die endogene ritmes in CO_2 assimileringstempo en stomageleiding waar te neem, is die plante onder konstante ligkondisies gehou. Die lae nagtemperatuur het 'n afname in beide prosesse veroorsaak, maar nie die endogene ritmes self versteur nie.

Lae nagtemperatuur het 'n versteuring in die regulering van SPS veroorsaak, wat skynbaar tot gevolg gehad het dat die normale daaglikse toename in SPS aktiwiteit gedurende die daaropvolgende ligperiode nie plaasgevind het nie. Inhibisie van SPS aktiwiteit is geïdentifiseer as 'n kenmerkende eienskap tussen die twee genotipes en kan 'n belangrike rol speel tydens die waargenome mesofilbeperking van fotosintese in 'Java 29'. In beide genotipes is 'n soortgelyke endogene ritme in SPS aktiwiteit geïdentifiseer. Lae nagtemperatuur het hierdie endogene ritme in SPS aktiwiteit versteur, maar alleenlik in 'Java 29'. Die versteuring in die ritme was nie as gevolg van 'n effek op die biochemiese meganisme (verandering in SPS proteïeninhoud) wat die ritme beheer nie. Lae nagtemperatuur het eerder 'n onbekende faktor teweeggebring wat veroorsaak het dat SPS in 'n onaktiewe status "vasgevang" is.

Versteuring van die interne tydmetingsmeganisme, wat daaglikse SPS aktiwiteit met die tempo van fotosintese koördineer, kan een van baie gelyktydige versteurings wees wat in koue sensitiewe sojaboongenotipes voorkom. Sulke versteurings kan heel moontlik een

van die hoofoorsake wees wat die verskil in kouetoleransie tussen sojaboongenotipes van vroeë en laat volwassenheidsgroeperings verklaar.

Sleutelterme: Endogene ritme, fotosintese, kouestres, sojabone, sukrose-fosfaat-sintase, sukrose-sintase.