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Inhibition of photosystem II activities in soybean (*Glycine max*) genotypes differing in chilling sensitivity

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ABSTRACT

Due to chilling sensitivity, minimum night temperatures represent the main constraint in soybean production in South Africa. In vivo quantification of photosystem II (PSII) function by direct chlorophyll fluorescence revealed that dark chilling (8°) inhibited PSII function in the extreme chill sensitive genotype, Java 29 (JAs) mainly by deactivating reaction centers and inhibiting the conversion of excitation energy to electron transport and electron transfer from reduced plastoquinone to the PSI end electron acceptors. Further analysis of the normalized fast fluorescence transients, revealed that in JAs, upon dark chilling, disengagement of the oxygen evolution complex (ΔV_K band) occurred which coincided with a concomitant decrease in O_2 evolution measured in vitro. The chilling resistant Maple Arrow (MAr), though one night cold stress lead to a decrease in fluorescence emission at 2 ms ($-\Delta V_J$ band) indicating a decrease in the Q_A^- concentration due to cold-induced slow-down of electron donation from P_{680}, however showed clear signs of recovery after the second and third cold nights. The moderate chill sensitive genotype, Fiskeby V (FBm) responded in a fashion intermediate to above-mentioned extremes. A second experiment showed that in JAs the inhibitory effect increased with increasing time of exposure to light following dark chilling. Our data demonstrated that significant differences exist in the cold tolerance of different soybean genotypes: (a) In respect to activity criteria, expressed by the quantum yields for primary photochemistry $\varphi_{Po} = TR_o/ABS$, for electron transport from photosystem II to photosystem I as $\varphi_{Eo} = ET_o/ABS$ and the efficiency, $\varphi_{Ro} = RE_o/ABS$, to reduce the end electron acceptors of photosystem I up to NADP; (b) In respect to stability criteria, dependent on structure and conformation, such as the capability of energetic cooperativity (grouping) among photosynthetic units quantified by the grouping probability for exciton movements within the energetically connected group of entire photosynthetic units. Therefore analyzing the O-J-I-P fluorescence transient according to the JIP-test offers a practical and sensitive in vivo screening test for dark chilling tolerance in soybean.

1. Introduction

Soybean (*Glycine max* L Merr.) is a crucial source of food for both humans and animals. In South Africa, the demand for soybean oil cake exceeded the supply recently, creating the incentive for increased production (Smit, 1998). However, due to high altitude, in soybean producing areas of South Africa the daily minimum temperature is critically low. Since soybean is chilling sensitive, with growth, development and yield being affected negatively at temperatures below 15 °C (Gass et al., 1996), chilling stress proves to be the most important constraint in soybean production in South Africa. McKersie and Leshem (1994) showed that even a brief chilling event could lead to symptoms gradually appearing after a plant has been returned to optimal growing conditions.

Chilling stress is known to limit a wide range of physiological processes in soybean, including photosynthesis (Allen and Ort, 2001; Caulfield and Bunce, 1988; Strauss et al., 2007; Van Heerden and Krüger, 2002). The temperature sensitivity of photosynthesis is dependent on both plant species and time of exposure to the stress temperature regime. Lundmark et al. (1988) and Nie et al. (1992) pointed out that inhibition of photosynthesis by low temperature cannot fully be accounted for by stomatal limitations under light saturating conditions.

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This phenomenon is also true for soybean (Van Heerden et al., 2003a), although the relative contribution of stomatal and mesophyll limitations at the chloroplast level upon dark chilling is found to be genotype specific and dependent on the chilling sensitivity of the genotype (Van Heerden and Krüger, 2002; Van Heerden et al., 2003b; Strauss and Van Heerden, 2011). Van Heerden et al., 2003b pointed out that chilling-induced mesophyll limitation at the chloroplast level is an important cause of the inhibitory effect. Membranes have been considered a prime candidate as primary causative agent of chill sensitivity and therefore photosynthetic parameters associated with the thylakoid membranes have been intensively studied in relation to chilling injury to photosynthesis. Although it is known that the photochemical events of light absorption, energy transfer and charge separation associated with PSII and PSI, are insensitive to temperature in the biologically relevant range of 0 °C to 50 °C (Mathis and Rutherford, 1987), the combined effect of light and the differential sensitivity to temperature exhibited by the photochemical and thermochemical processes of photosynthesis can lead to a significant impairment of photosynthesis as a result of photoinhibition (Powells, 1984; Bertamini et al., 2006). The rate of non-cyclic electron transport measured at room temperature in chloroplasts isolated from pre-chilled leaves of chilling sensitive plants was found to be inhibited (Kislyuk and Vas'kovskii, 1972; Smillie and Nott, 1979). Fork et al. (1981), working with tomato, corn and pepper, pointed out that, though chilling both in the dark and the light impairs electron flow on the water oxidizing side of PSII, the main damage caused in the light is closer to the reaction center of PSII than is the damage caused in the dark.

Studies using chlorophyll *a* fluorescence supported the notion that the primary target of low-temperature limitation of the photosynthetic apparatus in chilling sensitive species is the photosystem II (PS II) reaction center (Fryer et al., 1998). By simultaneous measurements of chlorophyll fluorescence and absorbance changes at 820 nm, Ren and Gao (2007) showed that both PSII and PSI activities are inhibited by chilling stress under weak light in susceptible soybean. Kee et al. (1986) showed that chilling tomato leaves in darkness inhibited the light saturated whole chain of PSII electron transport, whereas PSI-mediated activity appeared to be stable. PSII inhibition appeared to be localized to the O_2 evolving complex. Similarly, using the ratio of variable to maximum fluorescence, F_v/F_M (representing the maximum quantum yield of PSII primary photochemistry), (measured as trapping flux per light absorption flux TR/ABS) to assess chilling stress in soybean, Tambussi et al. (2004) found no change in this parameter when subjecting the plants to a chilling temperature (7 °C) in darkness, whereas a marked decrease occurred when the stress was applied under illumination. The drop in F_v/F_M was exacerbated by treatment of leaves with the chloroplast protein synthesis inhibitor lincomycin, suggesting that concurrent repair ameliorated chilling-induced damage to PSII.

By means of direct chlorophyll fluorescence measurements, Strauss et al. (2006) demonstrated considerable intra-genotypical differences in the inhibition of PSII function by dark chilling in a large selection of soybean genotypes. These genotypes were ranked according to chilling tolerance, but the physiological mechanisms explaining the intra-genotypical variation in the inhibition of PSII function were not explored in detail (Strauss et al., 2006). The current investigation was based on the belief that intra-genotypical differences in the response of PSII to dark chilling would contribute to an understanding of the physiological basis of tolerance. Such information is needed to increase production by directed breeding and genetic transformation for cold tolerance. As PSII is known to be highly sensitive to temperature changes (Krause and Weis, 1991), our aim was to investigate the biochemical basis of the inhibition of PSII function after dark chilling and at selected time intervals during the subsequent light period, using three soybean genotypes of differing cold tolerance. Dark chilling-induced changes in PSII function and plant vitality was quantified in vivo by chlorophyll *a* fluorescence kinetics (JIP test), and O_2 evolution capacity of chloroplasts was assessed in vitro. The endeavor was also to identify biochemical traits associated with chilling tolerance/sensitivity which could serve as tool for directed breeding and genetic transformation.

2. Material and methods

2.1. Plant material and treatments

Glycine max plants of the cultivars Maple Arrow, Java 29 and Fiskeby V were used in this investigation. The genotypes were chosen based on the fact that they originate from temperate (Maple Arrow and Fiskeby V) and tropical (Java 29) climates. Maple Arrow and Fiskeby V exhibit comparable, early maturity (maturity groups 00 and 000, respectively) (Seddigh et al., 1988), while the third, Java 29, belongs to a late maturity group. Maple Arrow is regarded as chilling tolerant (therefore denoted as MAr), Fiskeby V is regarded as moderately chilling tolerant (therefore denoted as FBm) (Hume and Jackson, 1981) while Java 29 is regarded as extremely chilling sensitive (therefore denoted as JAs) (Lawn and Hume, 1985). Plants were grown in computerized growth chambers under optimal controlled conditions: 15 h/9 h and 23 °C/20 °C light/dark cycle at a light intensity of 1000 $\mu Em^{-2}\ s^{-1}$. After 27 days (trifoliate stage) the experimental plants were subjected to low night temperature (8 °C) for 3 consecutive nights, while the controls were maintained at normal conditions.

2.2. Chlorophyll fluorescence

Chlorophyll fluorescence (JIP test, (Strasser et al., 2000)) was measured on the second trifoliate leaf just before onset of the light period and for the second experiment also at different time intervals during the day period. Chlorophyll fluorescence measurements were conducted pre-dawn (6:00) and for the second experiment also at specific points in time during the subsequent light period, namely at 8:00, 9:00, 10:00, 12:00 and 14:00. Plants measured during the light period (second experiment) were darkened one hour before measurement using leaf clips. For every hourly data point a separate set of plants was used, i.e. a particular set of plants was used only once and for the particular light exposure period. The instrument used was a Plant Efficiency Analyser, Hansatech Ltd., King's Lynn, Norfolk, UK. Each fluorescence induction transient was induced by red light (peak 650 nm) at 3000 $\mu Em^{-2}\ s^{-1}$ (sufficient excitation intensity to ensure complete closure of PSII reaction centers to obtain true fluorescence intensity of F_M in vivo) and recorded for 1 s on a 4 mm diameter area of a dark-adapted, attached leaf sample. Quantification of the fast phase fluorescence transients was done according to Strasser et al. (2000) using the 'Biolyzer' computer program (Maldonado Rodriguez, Biolyzer@ fluoromatics). The JIP-test represents a translation of the original data to biophysical parameters and calculation of the performance indexes (PI_{ABS} and $PI_{ABS,total}$). The average value of each of these parameters for all measurements was calculated. The parameters which all refer to time zero (start of fluorescence induction) are: (a) the specific energy fluxes (per reaction center, RC) for absorption [ABS/RC = antenna size of PSII = $(M_o/V_J)\cdot F_M/(F_M - F_o)$], trapping [$TR_o/RC = M_o/V_J$], electron transport [$ET_o/RC = (M_o/V_J)\cdot(1 - V_J)$] and dissipation at the level of the antenna chlorophylls [DI_o/RC] and electron transport from PQH_2 to the reduction of PSI end electron acceptors [$RE_o/RC = (M_o/V_J)\cdot(1 - V_I)$]; (b) the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry [$\varphi_{Po} = TR_o/ABS = F_V/F_M$], the efficiency with which a trapped exciton, having triggered the reduction of Q_A to Q^-_A can move an electron further than Q^-_A into the electron transport chain [$\psi_{Eo} = ET_o/TR_o$], the quantum yield of electron transport [$\varphi_{Eo} = ET_0/ABS = \varphi_{Po}\cdot\psi_0$], the quantum yield of dissipation [$\varphi_{Do} = DI_o/ABS = 1 - \varphi_{Po}$] and the efficiency of electron transfer from reduced plastoquinone to the PSI end electron acceptors [$RE_o/ET_o = \delta_{Ro} = (1 - V_I)/(1 - V_J)$]; (c) the phenomenological energy fluxes the (estimated per excited cross section, CS or leaf area) for absorption [ABS/CS] which is proportional to F_o for a given physiological state,