Chlorophyll fluorescence as a method to detect moisture-limiting stress in canola

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ABSTRACT

Chlorophyll fluorescence (CF) is a measure of photosynthetic performance and is widely used by plant physiologists and ecophysiologists. The basic principle of CF analysis is relatively straightforward. When sunlight is absorbed by the leaf one of three processes occur: the light is used for photosynthesis, excess energy is converted and dissipates as heat, or it is reflected back as light – that is, as chlorophyll fluorescence. The three processes are linked and changes in one will be reflected by changes in the other two. The reaction sites of Photo-system II are very sensitive to stress, particularly heat and moisture stress. These changes are not obvious to the naked eye, making measuring changes in chlorophyll fluorescence a potentially useful tool to assess drought tolerance.

Nine canola genotypes were grown in a rain-out shelter experiment in 2010, where three water treatments were imposed: wet, dry and very dry. Chlorophyll fluorescence was measured on three occasions, starting on 2 September, and then 4 weeks and 8 weeks later. Significant differences were detected between genotypes and between moisture treatments. There was significant genotype x water-treatment interaction, with one genotype's CF being particularly sensitive to drought stress.

Key words: *Brassica napus* – drought susceptibility index – photosynthesis

INTRODUCTION

Chlorophyll fluorescence has been used as a measure of photosynthetic performance of plants (Krause and Weis 1991), particularly in relation to physiology and ecophysiology studies. It has been touted as a potential means to screen for tolerance to moisture stress to improve crop production strategies and assess drought survival (Baker and Rosenqvist 2004; Woo *et al.* 2008). There is very little evidence to suggest that tolerance of the photosystem biochemistry to limiting moisture could serve as a direct target for plant breeding (Blum 2011). Rather, chlorophyll fluorescence is a potential tool to provide a quantitative measure of a plant's performance under stress (Maxwell and Johnson 2000) and may serve to identify genotypes that have other dehydration avoidance or drought resistance traits, rather than improved photosynthetic performance *per se*, that may serve as targets for plant breeding.

Inexpensive and accurate hand held devices are available to measure chlorophyll fluorescence, the mode of operation in each is similar. There are two basic types of fluorometers – imaging fluorometers that produce whole leaf data, or leaf clip models that can be used in the field. Using a leaf clip, a rapid pulse of high intensity light is absorbed by the leaf inducing fluorescence which is then measured by the sensor. Numerous parameters are recorded by the sensor and the whole process is very rapid. The most meaningful measurement, in terms of detecting stress tolerance is F_v/F_m (Maxwell and Johnson 2000). The F_v/F_m ratio is defined as the "Maximum efficiency at which light absorbed by light-harvesting antennae of PSII is converted to chemical energy" (Baker and Rosenqvist 2004). In other words, it is a measure of the photochemical efficiency of the light harvesting apparatus within leaf tissue. The site of electron transfer within PSII is sensitive to environmental stress, particularly heat and moisture stress (Sayed 2003).

In this study, chlorophyll fluorescence was used to assess the effect of varying moisture treatments on the photosynthetic performance of nine canola genotypes grown in a field-soil rain-out shelter. Yield data, converted to a Drought Susceptibility Index (DSI), was compared with chlorophyll fluorescence data to determine if relationships exist.

MATERIALS AND METHODS

Nine genotypes were sown in small plots in a rain-out shelter trial in 2010. The rain-out shelter consisted of 63 plots in a 7 x 9 array (range x rows). Genotypes were spatially optimised using DiGGer design software. Three water treatments were imposed – "wet", "dry", "very dry", aligned with ranges with unequal replication (2 reps wet treatment, 3 reps dry, and 2 reps very dry). The trial was sown 18 May 2010 with the rain-out shelter roof closed for the duration of the growing season. Neutron probe access tubes are located throughout the trial in rows 1, 3, 5, 7 and 9, and soil moisture was monitored weekly. Irrigation was applied using a calibrated dripper system. Total moisture equal to 470 mm in-season rainfall in the wet treatment, 300 mm in the dry treatment, and 240 mm in the very dry treatment was available to the plants. Subsoil moisture equal to 134 mm was present at the time of sowing and is included in the totals given above.

Chlorophyll fluorescence measurements were taken using a hand held fluorometer - FluorPen FP100 (Photon Systems Instruments, Czech Republic). Chlorophyll fluorescence data was collected on 3 occasions. Three plants in each plot were tagged so the successive readings could be taken on the same plant. The most recent fully-expanded leaves were selected for measurement. Due to natural leaf senescence it was not possible to measure fluorescence on the same leaf on each sampling occasion, particularly with the final reading in the dry and very dry treatments when the plants were experiencing significant moisture stress.

Three chlorophyll fluorescence parameters were assessed: Ft (instantaneous chlorophyll fluorescence), QY (quantum yield) and the OJIP protocol (chlorophyll fluorescence induction kinetic, which is used to calculate the F_v/F_m ratio). Only the F_v/F_m ratio data is presented here. The first reading was taken on 2 September on the wet and very dry plots only. Measurements were taken at 3 positions on each leaf, 3 leaves were assessed on each plant and 3 plants were assessed in each plot, giving a total of 27 measurements for each character in each plot. In the second and third readings (27 September and 28 October), the number of assessments was reduced to two positions on two leaves on two plants per plot, after analysis of the first reading indicated that plant, leaf, position and their interaction were not significant. Sampling positions were chosen to be equally spaced along one side of each leaf. The sampling time corresponded approximately to commencement of flowering, peak flowering, and end of flowering for the genotypes in the experiment. Chlorophyll fluorescence data was analysed as repeated measures using ASREML in R.

Plots were hand harvested and the plot yield was converted to t/ha for analysis. The following yield components were recorded: harvest index, biomass, pod and branch number, 1000 seed weight, and seeds per pod. Analysed grain yield data was used to calculate the Drought Susceptibility Index (DSI):

$$\text{DSI} = \frac{1-\left(Y_{di}/Y_{pi}\right)}{D_m} \qquad (1)$$

where Y_{di} is the grain yield (t/ha) of genotype *i* under moisture stress, Y_{pi} is the grain yield of genotype *i* under irrigation, and D_m is the ratio of (site mean yield under moisture stress) / (site mean yield under irrigation) (Fischer and Maurer 1978).

RESULTS and DISCUSSION

The effect of genotype and sampling date were significant ($P < 0.001$) for chlorophyll fluorescence. The interaction between genotype and water treatment was also significant ($P < 0.05$), and the three way interaction between genotype, water and sampling date ($P < 0.001$).

Genotypes RIVETTE and 46C76 maintained photosynthetic performance on each of the sampling times for all water treatments (Figure 1). AG-OUTBACK and TARCOOLA also maintained photosynthesis for the wet treatment, but differed in the response in the very dry treatment. In contrast, BLN3343-CO0401 and SARDI607 had significantly ($P>0.05$) reduced performance on the final measurement. In the very dry treatment, SARDI607 experienced

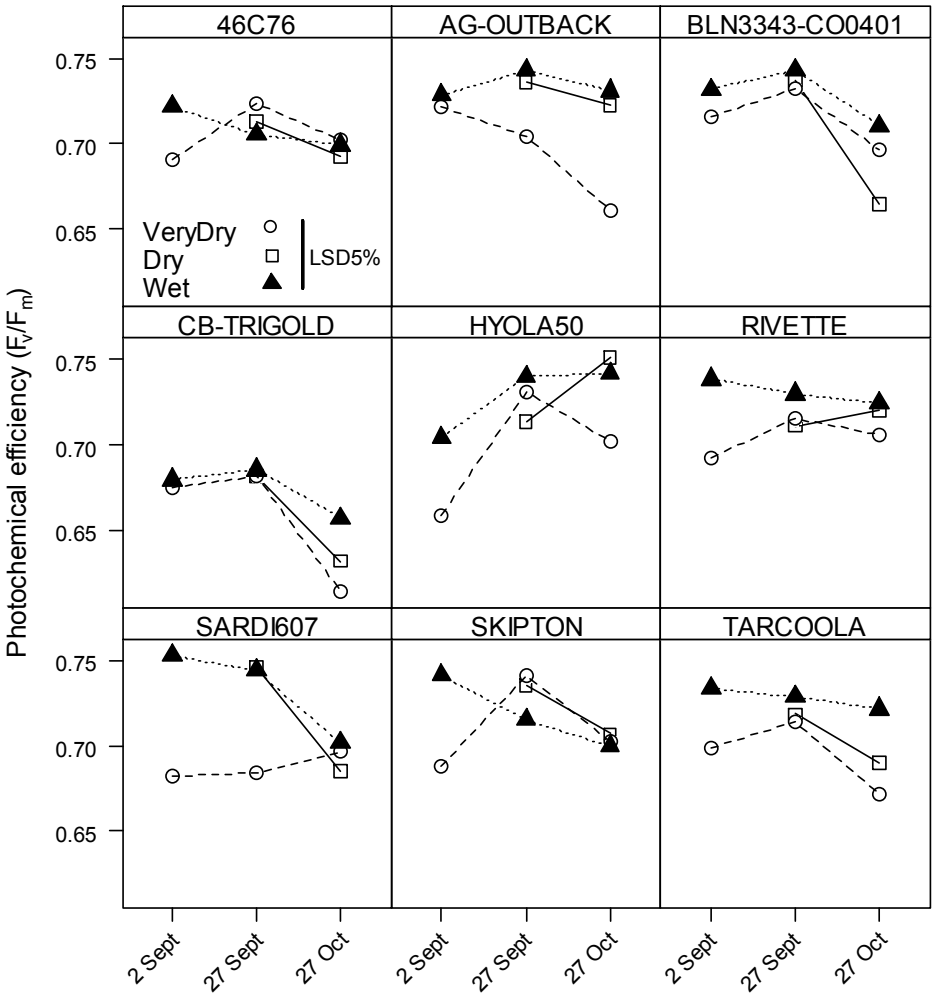

Fig. 1. Photochemical efficiency (F_v/F_m) measured with a chlorophyll fluorometer on three occasions for each water treatment in a rain-out shelter experiment in 2010. Data was not collected for the dry treatment on 2 September.

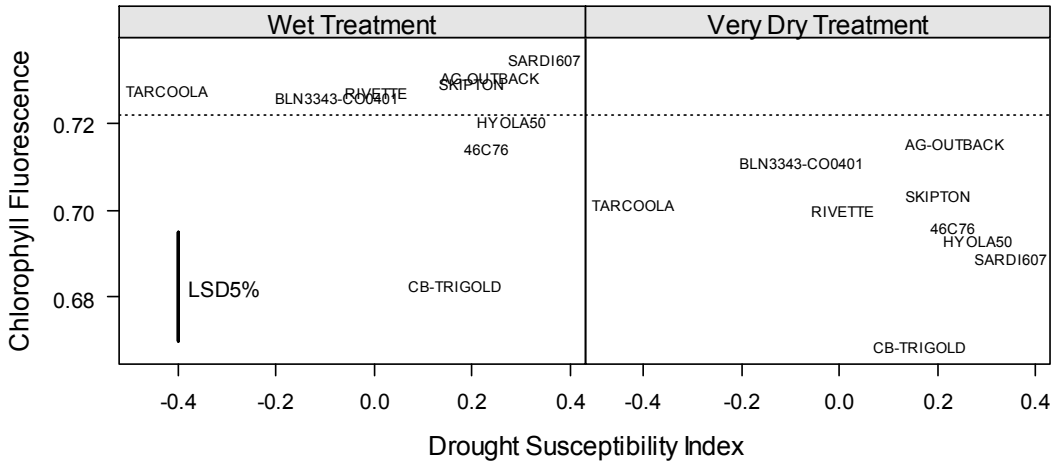

Fig. 2. Relationship between Drought Susceptibility Index and chlorophyll fluorescence for the wet and very dry treatments. The dashed vertical line indicates the mean F_v/F_m value for the wet treatments.

stress throughout the flowering period (Fig.1). Similarly the photosynthetic performance of AG-OUTBACK in the very dry treatment declined as the stress period progressed. CB-TRIGOLD, the only triazine-tolerant (TT) variety in the experiment, had the lowest photosynthetic performance of the genotypes tested, reflecting the well-documented photosynthetic deficiency in TT canola (e.g. McGuire and Thurling 1992). Considering the first two sampling times, there was no difference between the wet and very dry treatments for genotype CB-Trigold, suggesting that is optimised for dry environments.

The effect of genotype, water treatment and their interaction on grain yield was significant ($P < 0.001$). Genotypes TARCOOLA, BLN3433-CO0401 and RIVETTE had very low or negative DSI (-0.42, -0.07, 0.00, respectively) indicating they performed better under moisture stress conditions compared with the wet treatment. SARDI607 and HYOLA50 had the highest DSI (0.35, 0.28 respectively).

In durum wheat significant correlation was found between DSI and chlorophyll fluorescence (Flagella *et al*. 1995). In this study the correlation was not significant.

All of the genotypes had a decreased chlorophyll fluorescence due to the water treatment applied (Fig. 2). SARDI607 had the greatest reduction in chlorophyll fluorescence due to moisture stress (0.76 in the wet treatment and 0.68 in the very dry treatment).

The results of this study suggest that chlorophyll fluorescence, as a measure of photosynthetic performance, is a potential tool to assess stress in canola due to limiting soil moisture.

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