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CHLOROPHYLL FLUORESCENCE: A POTENTIAL INDICATOR FOR RAPID ASSESSMENT OF WATER STRESS TOLERANCE IN CANOLA (*BRASSICA NAPUS* L.)

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Abstract

An experiment was conducted to assess the differential morpho-physiological responses to simulated water stress of two canola (*Brassica napus* L.) cultivars in hydroponic culture. Seeds of two canola cultivars (Cyclone and Dunkeld) were germinated and allowed to grow for three weeks. Three week-old canola plants were grown at 0 MPa (control) or -0.6 MPa (PEG 18.2%) in nutrient solution for further three weeks. Water stress strongly reduced the growth of both canola cultivars. However, cultivar Dunkeld proved to be more drought tolerant as it was higher almost in all growth attributes under water stress conditions, particularly in shoot and root biomass and leaf area. Furthermore, growth of Dunkeld was also higher than that in Cyclone. Leaf chlorophyll 'a', carotenoids and quantum yield of PSII was also reduced due to water deficit conditions, but all these were less affected in drought tolerant Dunkeld. Although leaf osmotic potential was lower in water stressed plants of both cultivars, water stress induced reduction in leaf osmotic potential was less in cv. Dunkeld. However, there was no relationship between growth and osmotic adjustment in canola cultivars examined in the present study. Water deficit caused a substantial decrease in photosynthetic rate in both canola cultivars but canola cultivars did not differ significantly in net CO_2 assimilation rate under water stress conditions. Furthermore, parallels between A, and leaf chlorophyll 'a' or F_v/F_m of both cultivars can not be drawn under water stress conditions. Thus, the differential drought tolerance in canola cultivars was related to leaf area and root growth. There was no relationship between growth and osmotic adjustment in canola cultivars examined here. Overall, quantum yield of PSII and leaf chlorophyll 'a' can be referred as a potential selection criterion for drought tolerance in canola cultivars examined in the present study.

Keywords: *Brassica*, chlorophyll fluorescence, F_v/F_m, chlorophyll, selection criterion

Introduction

Water stress reduces plant growth and crop yields on a world wide scale (Boyer, 1982). However, plant responses to water deficit depend upon the intensity, rate and duration of exposure and the stage of crop growth (Brar *et al.*, 1990). Of various plant organs, leaf growth is generally more sensitive. However, reduced leaf growth or senescence and abscission of older leaves under water stress conditions are one of adaptive mechanisms in plants resulting in reduced transpiration (Athar & Ashraf, 2005). However, leaf growth is mainly restricted due to non-availability of water and nutrients that are taken up from soil through roots. In addition, water stress induced reduction in growth mainly occurs due to its adverse effects on various physiological and biochemical processes including activity of enzymes, cell membrane integrity, plant water status, and photosynthetic rate (Ashraf *et al.*, 1992a; 1998). As different crop species have varying

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responses to water stress (Ashraf & Mehmood, 1990) one finds a spectrum of responses within same species and may be classified as tolerant or sensitive to water stress. However, water stress tolerant plants have ability to maintain water and ion homeostatic conditions by lowering water loss through transpiration, and/or accumulation of organic compatible solutes (Ashraf & Foolad, 2007). In view of water conservation strategy in plants to acclimatize water stress, leaf stomatal conductance has long been considered a promising selection criterion for drought resistance although some contrasting reports have been found in the literature. For instance, Blum (1974) suggested that higher stomatal frequency might lead to drought avoidance. By contrast, Teare *et al*. (1973) suggested that rapid stomatal response might be a drought resistance mechanism to conserve soil water for later use and to maintain high leaf water potential. In another study, leaf stomatal response was found not to be a good screening criterion for drought resistance (Winter *et al*., 1988). Recently it has been reviewed that a decreased photosynthesis under deficit soil moisture may not necessarily be related to stomatal opening; rather non-stomatal control of photosynthesis might have greater influence (Athar & Ashraf, 2005). Long ago, Sobrado & Turner (1983) suggested that differences in water relation characteristics (leaf water potential, osmotic adjustment) which show the differences between the cultivars can be considered as an indicator of drought resistance or adaptation to drought (Sobrado & Turner, 1983). Similarly while working with various sunflower line, Ashraf & O'Leary (1996) found that water stress tolerant genotypes maintained their leaf turgor through the process of osmotic adjustment. In view of all these reports, it is suggested that plant characteristics related with photosynthetic capacity or maintenance of plant water status could be used as a potential selection criteria for breeding for drought tolerance in crops.

Thus the primary objective of the present study was to determine the effects of polyethylene glycol induced water deficit stress on the growth, and photosynthetic capacity of the two canola cultivars, on the basis of which relative tolerance of each cultivar to drought stress could be assessed. Furthermore, relationships between growth, photosynthetic capacity, osmotic adjustment or ion accumulation were drawn to find out potential selection criterion for drought tolerance in canola at early growth stages.

Materials and Methods

The experiment was conducted in the growth room of Department of Botany, University of Agriculture, Faisalabad. Seeds of two cultivars of canola (*Brassica napus* L.), Dunkeld and Cyclone, were sown in glass Petri plates. Then the 7-dayold seedlings were transplanted in the plastic dishes containing Hoagland nutrient solution (half strength) and allowed to grow for two weeks. Three week old plants were grown for further three weeks in half strength Hoaglands nutrient solution with or without PEG $_{(8000)}$ (-0.6 MPa, 18%) in plastic dishes. The nutrient solution was continuously aerated with aquarium pumps.

Osmotic potential: The 2nd leaf from each plant was excised at 7.00 a.m. and was frozen into 2 cm^3 polypropylene tubes at –40°C in an ultra-low freezer for two weeks, after which time plant material was thawed and the frozen sap was extracted by crushing the material with a glass rod. After centrifugation (8000 x *g*) for four minutes, the sap was directly used for osmotic potential determination using a vapor pressure osmometer (Wescor 5500).

Chlorophyll contents: The chlorophyll ‘a’ contents and carotenoids were extracted and determined according to the method of Arnon (1949). Fresh leaves (0.2 g) were ground in 80% acetone and centrifuged at 10,000 x *g* for 5 minutes. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Chlorophyll fluorescence: The polyphasic rise of fluorescence transients was measured by a plant Efficiency Analyzer (PEA, Handsatech Instruments Ltd., King’s Lynn, UK) according to Strasser *et al*. (1995). The transients were induced by red light of 3000 µmol m^{-2} s^{-1} provided by an array of six light emitting diodes (peak 650 nm), which focused on the sample surface to give homogenous illumination over exposed area of sample surface. All the samples were dark adapted for 30 minutes prior to fluorescence measurements.

Gas exchange parameters: Measurements of gas exchange attributes were made on the 2nd intact leaf from top of each plant using an ADC LCA-4 portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). These measurements were made from 10.30 a.m. to 12.30 p.m. with the following specifications/adjustments: leaf surface area, 11.25 cm^2; water vapor pressure into chamber ranged from 0.0006.0 to 0.00089 MPa, ambient CO_2 concentration, 352 µmol mol^{-1}; temperature of leaf chamber varied from 28.4 to 32.4 °C; leaf chamber gas flow rate (U), 251 µmol s^{-1}; molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}; RH of the chamber ranged from 25.4 to 41.2 %; PAR (Q_{leaf}) at leaf surface during noon was maximum up to 918 µmol m^{-2} s^{-1}; and ambient pressure 98.8 kPa.

Statistical analysis of data: The data were subjected to analysis of variance using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor & Cochran (1980).

Results

All growth attributes (shoot and root fresh and dry weights, shoot length, leaf area) were significantly reduced in both canola cultivars due to water stress (Fig 1). However, Dunkeld excelled in both shoot and root fresh and dry weights under water stress conditions, whereas under non-stress conditions cultivars did not differ significantly only in root fresh and dry weight (Fig 1). Similarly, both cultivars did not differ significantly in shoot length when grown under normal conditions, whereas under water stress conditions cv. Dunkeld was higher in shoot length than that in cv. Cyclone. In contrast, Dunkeld was significantly higher in leaf area than that in Cyclone under both water stress and non-stressed conditions.

Water stress significantly reduced (more negative values) the leaf osmotic potential in both canola cultivars (Fig 2). However, this reducing effect on leaf osmotic potential was less in cv. Dunkeld compared with that of cv. Cyclone. Similarly, leaf chlorophyll ‘a’ was also significantly reduced in both cultivars when plants were exposed to PEG induced water deficit for 18-days. However, cv. Dunkeld had higher leaf chlorophyll a than that in Cyclone under water stress conditions. In contrast, under non-stress conditions, both cultivars did not differ significantly in leaf chlorophyll a. Imposition of water deficit conditions significantly increased total carotenoids in both cultivars, particularly in Dunkeld. Both the cultivars did not differ significantly in total carotenoids when grown under non-stressed conditions (Fig. 2). Similarly, imposition of water stress reduced photochemical efficiency (PS II measured as F_v/F_m). However, adverse of water stress was less on photochemical efficiency of cv. Dunkeld.

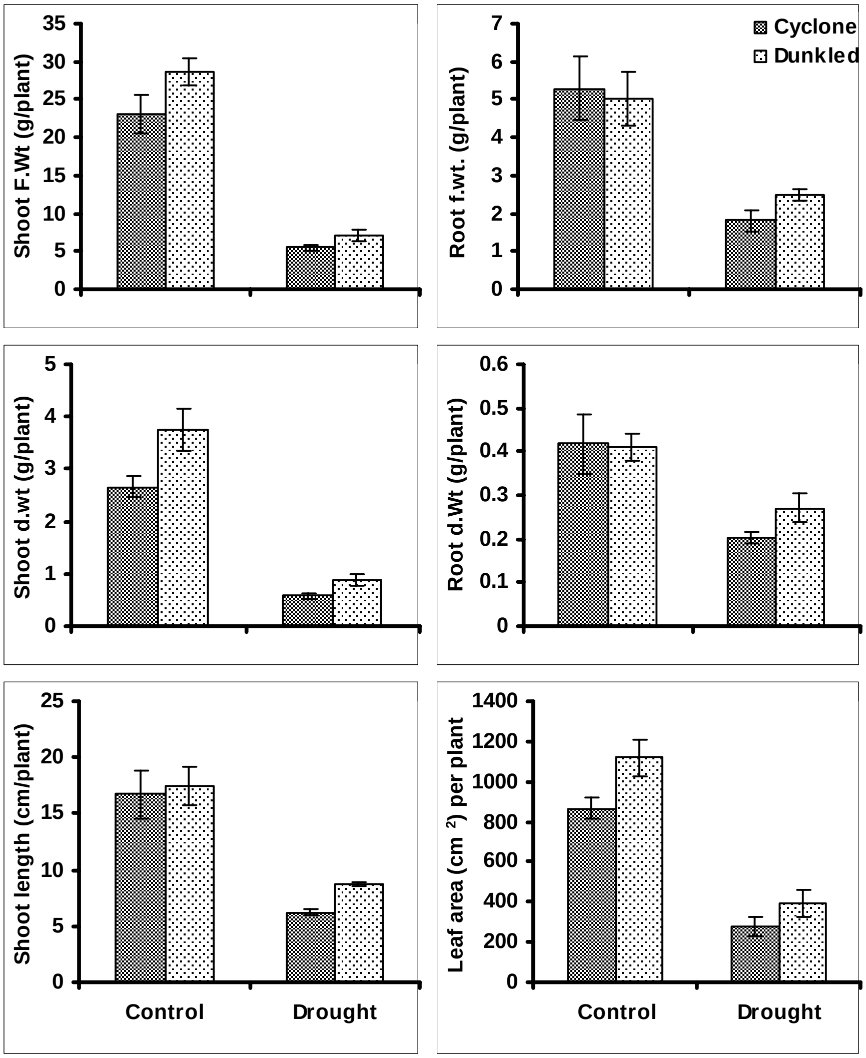

Fig. 1. Growth attributes of two canola (*Brassica napus* L.) cultivars when 27 day-old plants were grown under normal and PEG induced drought stress for three weeks.

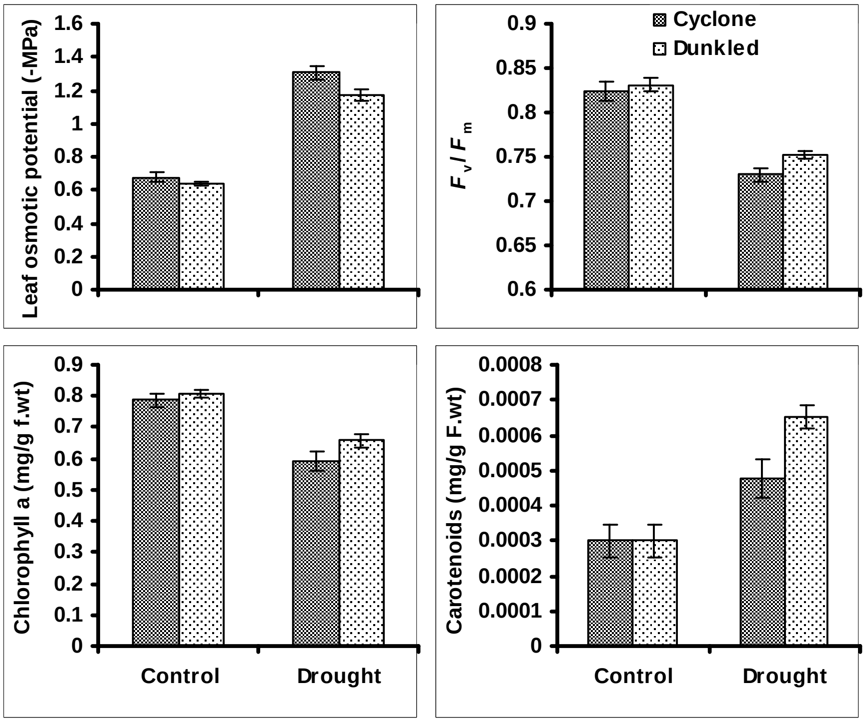

Fig. 2. Leaf osmotic potential, chlorophyll a, quantum yield of PSII, and leaf carotenoids of two canola (*Brassica napus* L.) cultivars when 27 day-old plants were grown under normal and PEG induced drought stress for three weeks.

Net CO_2 assimilation rate (*A*) reduced in both canola cultivars due to water deficit conditions. Furthermore, cultivars did not differ significantly under both water stress and non-stress conditions. Similarly, all other gas exchange characteristics (stomatal conductance g_s, sub-stomatal CO_2 C_i, transpiration rate *E*, water use efficiency WUE) of both cultivars were reduced due to PEG-induced water stress. Furthermore, cultivars did not differ in all these gas exchange characteristics significantly under both water stress and non-stress conditions (Fig 3). Positive relationships have been found among *A* and g_s, *E*, C_i and WUE (r = 0.844***, 0.837***, 0.75***, 0.658***). Although growth is positively associated with *A*, cultivars variation for growth under both water stress or non-stress conditions can not be related to changes in *A*.

Discussion

Polyethylene glycol (PEG) induced water stress imposed for a period of three weeks had a significant detrimental effect on growth of both canola cultivars. However, of the two canola cultivars examined in the present study, Dunkeld was found to be more tolerant to water deficit conditions as compared to Cyclone in view of its higher biomass production (Fig. 1). This reduction in growth of canola cultivars under simulated drought water stress conditions may be due to decrease in photosynthetically active leaf area or

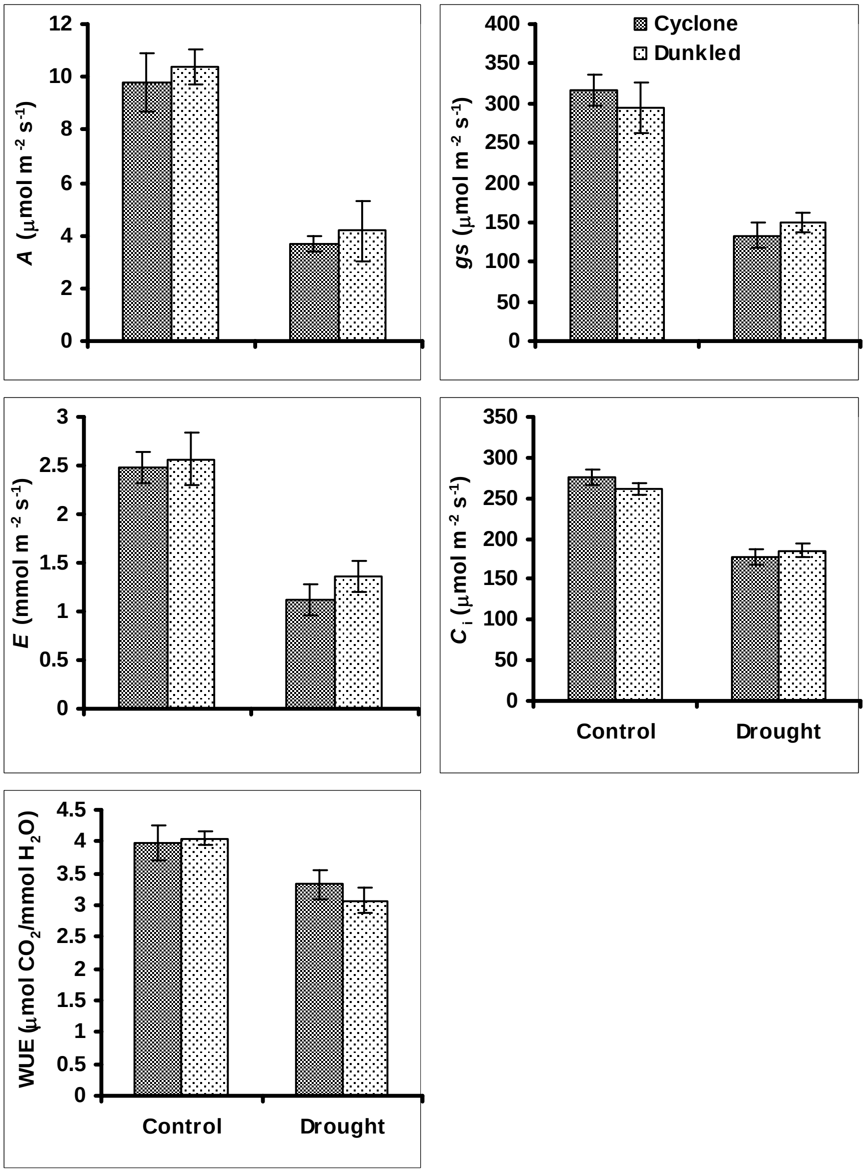

Fig. 3. Gas exchange characteristics of two canola (*Brassica napus* L.) cultivars when 27 day-old plants were grown under normal and PEG induced drought stress for three weeks

reduced root growth (Lawlor, 2002). Reduction in leaf growth rate under water deficit conditions is an early phenomenon, which occurs before decrease in leaf water potential or leaf turgor potential. It has been suggested that a signal produced by roots under drying conditions probably ABA, is transmitted via the xylem to the leaves, thus affecting their growth rate (Davies & Zhang, 1991). However, higher growth of Dunkeld under water stress conditions was possibly due to its higher root growth which helps in water uptake and nutrients thus causing increased growth under water deficit conditions (Kage *et al*., 2004). Similar differential inter-cultivar variation for water stress tolerance has already been observed in barley (Blum, 1989), black gram (Ashraf & Karim, 1991), lentil (Ashraf *et al*., 1992b), and *Brassica carinata* (Ashraf & Sharif, 1998). Thus, higher root growth in Dunkeld under water stress conditions might have increased water and nutrient uptake resulting in increased photosynthesizing tissue or growth.

In some previous studies, Dunkeld was found to be salt tolerant (Qasim *et al*., 2003). It is now well established that water stress tolerance and salinity tolerance in most plant species share the mechanism of osmotic adjustment (Blum, 1985, 1989; Ashraf & O'leary, 1996). Thus, an assay was undertaken to determine whether the observed variability in growth inhibition due to PEG induced water stress might be due to the differences in osmotic adjustment of canola cultivars. Osmotic adjustment was calculated by subtracting the osmotic potential values at water deficit conditions from that at well-watered conditions. Water stress tolerant Dunkeld had lower capability of osmotic adjustment (0.53 MPa) compared with water stress sensitive Cycolne (0.63 MPa). Such a lack of positive relationship of drought tolerance with osmotic adjustment in the two canola cultivars has been found in *Brassica carinata* (Ashraf & Sharif, 1998), *Brassica napus* and *B. juncea* (Niknam & Turner, 1999), Okra (*Hibiscus esculentus*) (Ashraf *et al*., 2002). Thus, the overall differential growth response of canola cultivars to water stress was not due to osmotic adjustment (OA), but due to some other biological processes controlling growth.

Water deficit significantly decreased chlorophyll a in both canola cultivars and cultivars did not differ significantly in both these variables under water stress conditions. Decline in photosynthetic pigments such as chlorophyll 'a' may be the consequence of reduced synthesis of the main chlorophyll pigment complexes encoded by the *cab* gene family (Allakhverdiev *et al*., 2002) or destruction of chiral macro-aggregates of light harvesting chlorophyll 'a' or 'b' pigment protein complexes (CHCIIs) which protect the photosynthetic apparatus (Gussakovsky, *et al.*, 2002) or due to oxidative damage of chloroplast lipids, pigments and proteins (Tambussi *et al*., 2000). Chloroplasts are the major source of production of reactive oxygen species (ROS) in plants (Ormaetxe *et al*., 1998). Fortunately, chloroplasts are the organelles that have the highest antioxidative protection due to presence of carotenoids, tocopherols, and antioxidative enzymes that scavenge ROS and help maintain the integrity of photosynthetic membranes thereby minimizing oxidative damage (Foyer & Noctor, 2000; Athar & Ashraf, 2005). In the present study, total carotenoid content were increased in both cultivars due to water stress. However, carotenoids were far higher in Dunkeld than that in Cyclone, indicating that Dunkeld had relatively higher antioxidant capacity and protection of its photosynthetic apparatus. Thus higher leaf chlorophyll 'a' in Dunkeld was possibly due to its better antioxidant capacity. A positive association has been found between quantum yield of PSII as calculated by F_v/F_m and leaf chlorophyll 'a' and carotenoids. Furthermore, from the results of the present study it is clear that water stress induced reduction in quantum yield of PSII was less in drought tolerant Dunkeld which is similar to some of earlier findings in *Eragrostis curvula* (Colom & Vazzana, 2003). Since photosystem II (PSII) plays a key role in the response of leaf photosynthesis to environmental perturbation (Baker, 1991), changes in photosynthesis under water stress conditions are expected. In the present study, water deficit caused a substantial decrease in photosynthetic rate in both canola cultivars but canola cultivars did not differ significantly in net CO_2 assimilation rate under water stress conditions. Furthermore, parallels between A, and leaf chlorophyll 'a' or F_v/F_m of both cultivars can not be drawn under water stress conditions, which suggested that leaf chlorophyll 'a' did not affect *A*. This in contrast to some earlier findings in which high correlation between the chlorophyll content of leaves and CO_2 uptake has been found in maize (Ashraf & Rehman, 1999) and wheat (Waseem *et al*., 2006). Furthermore, parallels between differential growth responses of canola cultivars and *A* also cannot be drawn. From all these results of the present study, the differential drought tolerance of the two canola cultivars can be related to higher photosynthetic pigments and cannot be related to their net CO_2 assimilation rate. However, water stress induced reduction in photosynthesis may be due to stomatal limitations, metabolic limitations or altered chlorophyll fluorescence or combination of these factors (Athar & Ashraf, 2005).

In conclusion, the differential drought tolerance in canola cultivars was related to leaf area and root growth. Although water stress induced reduction in growth of canola cultivars was strongly associated with photosynthetic capacity, cultivar variation under water stress conditions can be related with their photosynthetic pigments as well as a mechanism which protect photosynthetic pigments. There was no relationship between growth and osmotic adjustment in canola cultivars examined in the present study. Overall, quantum yield of PSII and leaf chlorophyll 'a' can be referred as a potential selection criterion for drought tolerance in canola cultivars examined in the present study.

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