The Effects of Early Season Drought on Stomatal Conductance, Leaf-air Temperature Difference and Proline Accumulation in Sugar Beet Genotypes.

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ABSTRACT

Limited water for irrigation and the coincidence of early growth of sugar beet (Beta vulgaris L.) with the late growth of winter cereals in the Northeast (NE) of Iran, force farmers to allocate their limited irrigation water to the cereal crops and thus their sugar beet crop is subjected to an early water stress. Finding cultivars which are able to withstand early water stress without a significant yield reduction is critical to the farmers’ economy. This study was conducted over a two-year period (1998 and 1999) to evaluate the response of nine sugar beet genotypes to drought stress and to determine the crop traits associated with drought resistance. The results showed that stomatal conductance, leaf-air temperature difference and proline accumulation were associated with levels of water stress in sugar beet genotypes. Among these indices, leaf-air temperature difference was a more precise parameter to measure. A negative correlation between ΔT (leaf-air temperature) and stomatal conductance was found. The correlation coefficients for 1998 and 1999 were -0.87 and -0.58, respectively. There was a positive correlation between ΔT and proline accumulation in sugar beet genotypes. The correlation coefficients for the 1998 and 1999 experiments were 0.61 and 0.49, respectively. The shoot: root ratio (S:R) measured at the end of the stress period showed that genotypes with a lower S:R often had a greater stomatal conductance. In general, genotypes with a lower S:R at the end of the stress period usually had a greater root dry weight. The correlation coefficients of S:R with root dry weight at the end of the stress period were -0.96 and -0.65 for 1998 and 1999, respectively.

Keywords: Drought stress, Leaf conductance, Proline, Shoot: root ratio, Sugar beet, Temperature.

INTRODUCTION

Sugar beet is considered as the main sugar crop in arid to semi-arid climates. In Iran, with its semi-arid climate, sugar beet production is limited by the availability of irrigation water. It is planted in spring at a time when the farmers are allocating their limited irrigation water to their small grain cereals. Thus in the early phenological stages, the sugar beet plants are confronted with drought stress. Although it has been reported the sugar beet is sensitive to drought at the four leaf stage (FAO, 1977). Sadeghian et al. (2000) showed that there are genetic variations for the white sugar yield of sugar beet genotypes subjected to drought stress in the early of growing season.

In several crops, such as spring wheat (Mustafa et al., 1996), faba bean (Link et al.,...
significant relationships between some morphological and physiological characteristics and drought stress have been reported. Thus, morphological and physiological studies of sugar beet genotypes subjected to drought stress may be used in the breeding program.

Brown et al. (1987b) showed that, when confronted with early drought, sugar beet lost the fibrous roots of its undeveloped root system and the plant was thus unable to take up enough water to meet its transpirational demands. Plant transpiration is mainly derived from the difference between plant and atmospheric water potential (Brown et al., 1987a). Groves and Bailey (1994) have studied the effect of different irrigation regimes on sugar beet, ranging from an unirrigated control to applying irrigation water to ensure that the soil moisture deficit never exceeded 35 mm. They have noted that, under these irrigation regimes, sugar beet yield is closely related to irrigation water applied and crop evapotranspiration. Reports indicate that drought could significantly increase sugar beet leaf diffusive resistance and thus decrease leaf photosynthesis (Dressmann et al., 1994; Clover et al., 1999). It was reported that differences in stomatal diffusive resistance might be seen between different genotypes of some crops such as maize and durum wheat (Ray and Sinclair, 1997; Clarke and Clarke, 1996).

Water deficit causes stomatal closure, a reduced transpiration rate, and elevated canopy foliage temperature (Halim et al., 1990). During drought, sugar beet leaves are subjected to both heat and water deficiency stress (Clarke et al., 1993). As a consequence of the reduction in transpiration rates of leaves, leaf temperature increases and the effects of drought and heat stress frequently combine to scorch leaves and subsequently cause leaf death. It has been reported that the transpiring leaves of sugar beet can be up to 10°C cooler than those of non-transpiring plants (Idso, 1982; Sepaskhah et al., 1988). The apparent ease and rapidity of canopy temperature measurements with a handheld infrared thermometer have elicited interest in the technique as a means of screening the germplasm of various crop species for drought resistance in a number of crops such as rice and pearl millet (Garrity and O’Toole, 1995; Singh and Kanemasu, 1983).

Under drought stress conditions, the plant parts should keep a lower water potential ($\psi$) than the soil to maintain turgor and prevent desiccation. Osmotic adjustment is a process by which plants can lower their osmotic and thus their water potential (Heuer, 1993). Proline is an amino-acide known to contribute to the osmotic adjustment and tolerance of plants exposed to unfavorable environmental conditions (Heuer, 1993). Gzik (1996) reported that in sugar beet, osmotic and drought-induced stress resulted in a rapid increase in leaf proline content. He concluded that the accumulation of high levels of proline under stressed conditions indicates the involvement of proline in osmoregulation. Brown et al. (1987a) showed that osmotic adjustment in sugar beet is affected by the rate at which the stress develops. Wallace et al. (1983) suggested that interspecies differences in osmotic potential might reflect different drought tolerances. Genotypic differences in proline accumulation have been reported for various different plants such as barley, sorghum and rice (Blum and Ebercon, 1976; Heuer, 1993). Although Hanson et al. (1977) reported that plant proline accumulating potential should not be utilized as a positive index in screening drought resistance cereals.

Increasing the root:shoot ratio is another mechanism by which plants increase their water-absorbing potential all over the root system while reducing their shoot transpiration capacity (Kramer, 1983). Hang and Miller (1986) showed that shoot:root ratios of sugar beet plants that received less irrigation water than the estimated evapotranspiration demand was generally affected later and much less than the crop growth rate. Abdollahian-Noghabi (1999) reported that, under water deficiency stress in the early growth stages, sugar beet shoot growth was more restricted than that of the roots.
In this research we studied the physiological response of sugar beet genotypes to early season drought stress in order to determine if certain physiological characteristics can be used as a screening tool to select drought resistant genotypes.

**MATERIALS AND METHODS**

Field experiments were conducted over a two-year period (1998 and 1999) at the Khorasan Agricultural Research Station, NE Iran. The soil texture was silty in the first 15-cm layer, and silty loam in the layer below. The field lay fallow and was under a wheat crop before 1998 and 1999, respectively. The soil was fertilised with 100, 200 and 200 kg ha$^{-1}$ of urea, ammonium phosphate and potassium sulfate, respectively. Micronutrients were applied as 20, 40 and 30 kg ha$^{-1}$, zinc sulfate, boric acid and manganese sulfate, respectively. Eighteen tons ha$^{-1}$ of dairy manure and three tons ha$^{-1}$ of chicken manure were also used prior to ploughing in 1998 and 1999, respectively.

The experimental design was a split plot with a randomised complete block arrangement in four replications. The main plots consisted of two irrigation regimes: control (non-stress), where the plants were irrigated about every eleven days, and water stress, where plants were not irrigated for 41 days (9 June.-20 July) and 53 days (1 June.-24 July) after emergence in 1998 and 1999 respectively. Subplots consisted of nine sugar beet genotypes. Only six genotypes were common to both years (Table 2). Plot size was 8 × 5 m$^2$, row distance was 0.61 m and plants were thinned to 0.2 m apart at the 4-leaf stage. During 1998, plants were top-dressed with 150 kg ha$^{-1}$ of ammonium nitrate after thinning in control plots, and just before the end of the stress period in stressed plots. In 1999, 200 kg ha$^{-1}$ ammonium nitrate was drilled into the plots after thinning under both conditions.

Stomatal conductance (cm s$^{-1}$) was determined, using a diffusive porometer (model AP4 manufactured by $\Delta$T Company) according to Clarke and Clarke (1996). The measurements were taken between 12.00-14.00 hours on the adaxial surface of five new fully developed sugar beet leaves four days prior to the termination of stress in 1998. In 1999, the fifth fully developed leaf from three plants in each plot was tagged and all the measurements were made on these leaves. This year, measurements were repeated 16 days after the termination of stress. The analysis of variance in leaf total conductance was carried out using three different methods. For the first method, the Papadakis (Pap) (Clarke and Clarke, 1996) procedure was used to adjust temporal trends in stomatal conductance. This method adjusts observed values with an environmental trend index used as a covariate; the trend index for each plot is the mean of the observed values minus the treatment mean for neighboring plots. In the second method, leaf temperature was used as a covariate. For the third one there was no adjustment of the observed values. Adjustment on the basis of two flanking plots with the Pap method was most consistent in reducing the coefficient of variation compared with the other two methods. Clarke and Clarke (1996) indicated that the Pap method is useful for improving precision in stomatal conductance experiments. We therefore refer only to data that has been adjusted by the Pap method.

Leaf temperatures were measured using an infra-red thermometer (Quick temp 850-1 model, made by Testo Company) between 12.00-14.00 hours on the tenth fully developed leaf prior to the termination of stress in 1998. In 1999, leaf temperatures were recorded on leaves, which were tagged for stomatal conductance measurements.

To measure proline concentration, leaf samples were taken from the new fully developed leaves of five plants and the proline extracted and measured according to Bates et al. (1973). In both years, proline was measured one day before stress termination. In 1998, the measurement was also repeated 16 days after the removal of stress.

Destructive plant samples were taken prior to the termination of stress. The fresh and
dry weight of shoots and roots (five plants in 1998 and three plants in 1999 in each plot) were also measured. Soil moisture was measured in 1999 using TDR (Time Domain Reflectometry) equipment at 0 to 15, 15 to 45 and 45 to 75 cm soil depths. The soil matric potential was calculated using water retention curve.

RESULTS AND DISCUSSION

Matric potential measurements Calculated in the second year of the experiment (1999) showed that the soil matric potentials in stressed plots were lower than in non-stressed plots in all three soil layers (Figure 1). This indicated that the crop was under water stress.

Figure 1. Soil matric potentials (KPa) of three soil layers in stressed and non-stressed conditions in a 0-15 cm b) 15-45 cm and c) 45-75 cm, during 1 Jun. –24 Jul. 1999.

In 1999, the mean leaf conductance values of all genotypes in water stressed plots were significantly lower (72% reduction) than those in non-stressed plots, but these differences were not significant in 1998. However, mean leaf conductance values across all genotypes in stressed plots were 47.5% lower (47.5% reduction) than those in the control plots (Tables 1 and 2). The difference between these two years may be related to the longer duration of the stress period in 1999 which, in turn, caused a more severe stress and thus reduced leaf conductance more than in 1998. The cumulative pan evaporation and temperature data presented in Figure 2 also indicated higher atmospheric demand for transpiration in 1999 and thus a more severe stress condition as compared to 1998.

There were no significant difference between stressed and non-stressed conditions in terms of leaf conductance measurements taken 16 days after removing the stress period in 1999 (Tables 1 and 2). It could be inferred that there was a high correlation between leaf conductance values and soil water potential. This has also been reported for cotton and soybeans by Davies (1977). Dreesmann et al. (1994) also showed that although, stomatal resistance increased 10-fold in 3-month old sugar beet under drought stress conditions, it fully recovered 24 hours after rewatering. The genotypes x stress level interactions were not significant for

Figure 2. Rate of the cumulative evaporation from class A evaporation pan (mm) under stress period in 1998 (9 Jun. –20 Jul.) and 1999 (1 Jun. -24 Jul.) and the cumulative mean of air temperature (°C), at the same period. (data from-Mashhad meteorological station).
leaf conductance. In other words, all genotypes were almost equally affected by stress treatment (Table 1).

In 1998, differences in leaf conductance among the genotypes, just before the termination of stress, were significant under both stressed and non-stressed conditions (P<0.05) (Table 2) while, in 1999, the differences were only significant (P<0.05) in non-stressed plots (Table 2). The more severe stress conditions in 1999 may have caused closure of stomata in genotypes masking the genotypic differences in leaf conductance. In 1998, under water stress conditions, the greatest leaf conductance values were observed for A37.1 and MSTC2 (Table 2). In 1999, after removing the water stress, the leaf conductance of genotypes under control conditions differed significantly (P<0.05) but no significant differences were observed among genotypes which were water stressed (Table2). In fact, continuous irrigation, under non-stressed conditions, may have resulted in the leaching of N below the sugar beet roots in the field, leading to less N than under stress conditions in the soil. So, after the termination of stress there is more N to be taken up by plants. It has been reported that there is a positive correlation between leaf conductance and leaf nitrogen content (Morgan, 1984. Fiscus, et al., 1991). High levels of N and sufficient water for transpiration after the termination of water stress, may have decreased any genotype differences in leaf conductance. Having a higher nitrogen content gives water stressed plants a chance to recover from the stress much sooner. This needs further investigation.

The difference between leaf and air temperature (ΔT) is used as an index of water stress. Sepaskhah et al. (1987) showed that leaf water potential (ψ) can be predicted from ΔT. Water stress significantly increased the ΔT values. The mean ΔT for sugar beet genotypes was -8.7 and -3.9°C in 1998 and -8.0 and -2.1°C in 1999 for control and water stressed conditions, respectively (Table 2), since stress was more severe in 1999 than 1998. Under drought conditions, sugar beet leaves wilt in response to water deficiency and tend to lay flat on the soil and thus increase the effective area exposed to the sun (Clover, 1997). As a consequence of reduction in transpiration rates of such leaves, leaf temperature increases.

Genotypes stress level interactions were significant for ΔT (Table 1). In other words, genotypes responded differently in ΔT to the change from non-stressed to stressed conditions. Similar results have been reported for other crops such as rice, pearl millet (Garrity and O'Toole, 1995; Singh and Kanemasu, 1983). According to Garrity and O'Toole, (1995) ΔT can be used as a screening tool in selecting genotypes for water stress resistance. In 1998, genotypes A37.1, MSTC2 and 7233.P3 and in 1999, 7233.P3 and A37.1 showed lower leaf temperatures than other genotypes (Table 2). As expected, there was a negative correlation between ΔT and leaf conductance (Figure 3). The correlations were significant during 1998 and 1999 (P<0.01 and P<0.1 respectively) which was due to the greater loss of heat through latent heat by higher transpiring genotypes (Wiebelt and Henderson, 1978). It has been reported that the transpiring leaves of sugar beet is up to 10 °C cooler than those of non-transpiring plants (Idso, 1982; Sepaskhah et al., 1988).

Proline accumulation in plant tissues under water stress as a mechanism to maintain cell turgor, water uptake and drought tolerance, has been reported for many crops (Blum and Ebercon, 1976; Heuer, 1993). In our experiments, we observed 30% and 36% increases in leaf proline concentration when under water stress conditions, in 1998 and 1999, respectively (Table 2). Proline accumulation was significantly different between stressed and non-stressed conditions (P<0.01) in 1999 but not in 1998 (Table 1). This may be due to a higher C.V. in 1998. Leaf proline contents of previously stressed plants, assayed 16 days after the removal of stress and a return to their normal (control) levels (2.39 and 2.28 micromole g⁻¹ fresh weight in stressed and non-stressed plots,
This phenomenon has also been reported for other crop plants by Heuer (1993). Although Doney and Theurer (1990) observed no osmotic adjustment in sugar beet under water stress, Brown et al. (1987a) reported osmotic adjustment in sugar beet plants in one of the two years of experimentation and concluded that osmotic adjustment depends on the length of stress duration.

The interaction between genotype x stress level for proline was not significant at P<0.05 in 1999 (Table 1), but the differences between genotypes under stress conditions were significant at P<0.05 (Table 2). The highest proline content was observed in PC9597.P58 and the lowest was in the 7233.P3 genotypes (Table 2). Genotypic differences in proline accumulation have also been reported by Blum and Ebercon (1976) and Heuer (1993) for other crops. There was a positive correlation between proline content and ΔT in 1998 (r=+0.61, P<0.08) and 1999 (r=+0.49, P<0.19) (Figure 4) which indicates that genotypes with lower leaf water content and higher ΔT had higher proline contents.

In these experiments, ΔT had a lower C.V. than leaf conductance and proline content (Table 1), suggesting that ΔT could be a more precise measurement than the other assessments. O’Toole et al. (1984) compared different methods of measuring the degree of stress and concluded that ΔT is the most sensitive and precise method of stress measurement.

Shoot: root ratio (S:R) is considered to be another determinant of the plant transpiration rate (Kramer, 1983). The correlation between S:R and leaf conductance was negative and noticeable (r= -0.58, P<0.1) in 1998 but negligible in 1999 (r=0.23) (Figure 5). If PC9597.P58 is ignored, the correlation would increase to -0.65. A clear relationship between S:R and root yield was observed, demonstrated by a negative correlation between S:R and root yield at the end of the stress period (Figure 5). Genotypes with lower shoot weights and greater root weights used the available water more efficiently and produced higher root yields.

Correlation coefficients for leaf conductance, ΔT and proline content with root dry weight before the removal of stress are given in Table 3. None of the aforementioned correlation coefficients were significant at P<0.05. Although these characteristics could
be used to show the state of plant water, it seems that these criteria are not able to predict yield when the crop is under water stress. Therefore further research is needed to elucidate those characteristics which might have a good correlation with yield when water is limited.

It can be concluded that $\Delta T$ is a more sensitive and easier method to use for measuring the extent of water stress in sugar beet than leaf conductance and proline accumulation. A lower S:R in sugar beet was a good criterion to avoid early season water stress and produce high root yield.

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The Effects of Drought on Sugar Beet Genotypes _________________________________


اءات تشخشکی اواپل فصلرشد بر هدایترزنوهای اختلافدمایبگو و هوا و تجمعپرولین برژنوتیپهای چنددرفند

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جمله

به علت محدودیت منابع آبی و همزمایی آبیاریهای اواپل فصل رشد غلات با آب‌یاریهای اواپل فصل رشد چنددرفند (Beta vulgaris L.) در شمال شرقی ایران، کشاورزان مجبرند از آب‌یاری اواپل فصل رشد چنددرفند خودداری نموده و لذا چنددرفند در این شرایط با تشخشکی مواجه می‌شود. برای افزایش دردکشداری، یافتن ارقامه که بتوانند فصل اواپل فصل رشد بدون کاهش معنی‌داری در عملکرد تجمیع نمایند، با کمیتی می‌باشند. آزمایشی که منتظر ارزیابی قابلیت تجمیع به خشکی 9ژنوتیپ چنددرفند و شناسایی خصوصیاتی که با تجمیع به خشکی ارتباط دارد انجام گرفت. نتایج حاصل از این آزمایشات نشان داد که هدایت روزنوهای اختلاف درجه حرارت هوا - برگ (ΔT) و میزان پرولین می‌توانند برای تشخیص نش در ژنوتیپهای چنددرفند مورد استفاده قرار گیرند. دربنیشاخصیات استفاده شده، ΔT، روش آسان و دقیق تری نسبت به روش‌های دیگری. همین‌گونه مناسبی بین ΔT و هدایت روزنوهای برگ در ژنوتیپهای چنددرفند مشاهده شد (87.85= و 58.19= ΔT و هدایت روزنوهای برگ در ژنوتیپهای چنددرفند میانگین داشت. ΔT و مقدار پرولین همینگی مثبت وجود داشت. ضرایب همینگی این صفات در سالهای 1377 و 1378، به ترتیب 61 و 49 بود. با اندادگیری نسبت‌زن خشکک قسمت‌هایی به رنگ (S:R) در اواپل فصل نش معلوم شده که اکثر ژنوتیپهایی که S:R

دارای کم‌هستند، از هدایت روزنوهای بالایی بندرخوردار می‌باشد. بطورکلی ژنوتیپهای با رنگ

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کم، دارای وزن خشک ریشه بالایی نیز در انتهای دوره تنش بودند. ضریب همبستگی S/R با وزن خشک ریشه در اواخر دوره تنش برای سال‌های ۱۳۷۷ و ۱۳۷۸ به ترتیب، ۰/۹۶ و ۰/۹۵ بود.