Effect of Drought Stress on Carbohydrate Composition and Concentration in White Clover

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ABSTRACT : To investigate the changes in the composition and pool size of carbohydrates under drought stress, white clover (*Triforium repens* L.) were exposed to -0.04 Mpa (well-watered, control) or to -0.12 Mpa (drought-stressed) of soil water potential during 28 days. Dry weight of leaves in drought-stressed plants was remarkably decreased by 45% within 14 days and 74% within 28 days compared to those of the control. Glucose concentration in drought-stressed plants was increased, while that of control was slightly decreased or remained at same level throughout experimental period. Fructose and sucrose concentrations in leaves were not significantly changed for drought-stressed plants, but those of the control were significantly decreased on plant after 14 days. Fructose and sucrose concentrations in stolon of control plants were sharply decreased, while that of drought-stressed plants was less varied. Those concentrations in roots were generally increased in drought-stressed plants. The concentration of total soluble sugars at 28 day was 438.0 and 632.6 mg g^{-1} dwt. in control and drought stressed plants, respectively. Starch concentration of stolon and roots of control plants was significantly increased to 2.0 and 1.4 times of initial level, respectively, whereas those of drought stressed plants was nearly same level or slightly decreased compared to initial level.

Keywords : Glucose, Fructose, Sucrose, Starch, Drought stress, White clover

W ater deficit is a major component of environmental stresses such as drought, salinity and low temperature and 40-60% of the agricultural land around the world suffers from drought (Nabors, 1990; Bray, 1997).

Drought is the major climatic factor limiting annual production of forages, cereals and other crops in temperate regions (Boyer, 1982). It is well documented that nutrient uptake of plants is inhibited in dry soil. Soil drought decreases mineralization of organically bound nutrients (Bloem *et al.*, 1992; Walworth, 1992), and nutritional transport by mass flow and diffusion (Seiffert *et al.*, 1995), and thus may diminish nutrient availability at the root surface.

Plants growing under drought stress reduce stomatal apertures to decrease the rate of water loss. Stomatal closure and leaf net CO_2 uptake decline in parallel during drought (Cornic, 1994). A direct inhibition of mesophyll photosynthesis in response to water deficit has been reported (Gunasekera and Berkowitz, 1993; Gimenez *et al.*, 1992), although impaired carbon assimilation may be the result of low availability of CO_2 to the chloroplast due to a water-stress induced closure of stomata. Thus, drought would severely modify the integrated processes of assimilation, translocation, storage and utilization of photoassimilated carbon. The alteration of carbohydrate metabolism in response to water-stress has been reported in several fruit trees (Düring, 1985; Rodrigues *et al.*, 1993; Wang *et al.*, 1995), in potato tubers (Geigenberger *et al.*, 1997; 1999) and forage species (Volaire *et al.*, 1998; Lucero *et al.*, 2000) as an adaptive mechanism. Carbohydrates are frequently associated with active osmotic adjustment (Premachandra *et al.*, 1992; Zhang and Archbold, 1993). Water-stressed mature leaves usually accumulate more sorbitol and less starch and sucrose than unstressed leaves (Wang and Stutte, 1992). However, most experiment has concentrated on osmotic adjustment and carbohydrate metabolism under water deficit in mature leaves, with only a few reports on whole plant.

The aim of this study was to analyze the main developmental and physiological traits that might contribute to persistence of white clove during prolonged drought. Particular attention was paid to composition and pool size of carbohydrate, since there is strong evidence that these contribute to survival of plants when photosynthetic leaf area is lost under severe natural drought (Volaire, 1995; 1998).

MATERIALS AND METHODS

Plant culture and experiment procedure

Sod of white clover (*Triforium repens* L.) at full vegetative stage was transplanted to 3 L pot containing a mixture

of sand and fritted clay. During 2 weeks of adaptation, water was supplied daily to maintain constant soil water potential (Ψ_s) close to -0.04 MPa. Thereafter, drought stress was imposed by withholding water from pots until Ψ_s of -0.12 MPa, and was achieved. Soil water potential (Ψ_s) was determined by tensiometer (Tensiometer model 2725, Soilmoisture equipment Corp., U.S.A). The range of Ψ_s was chosen to expose plants to moderate to severe level of drought stress, showing leaf wilting at midday. Pots with the non-drought stress plants were maintained at Ψ_s of -0.04 MPa (near field capacity). Each treatment lasted for 28 days. Plants samples were harvested at 14 and 28 days after treatment, respectively. Harvested plants were separated into leaves, stolon and roots. Tissue samples were immediately frozen in liquid nitrogen. Freeze-dried samples were finely ground and stored under vacuum for further analysis.

Carbohydrate analysis

About 25 mg of finely ground sample was extracted with 1 mL of 92% (v/v) ethanol. Tubes were shaken for 10 min at room temperature, centrifuged at 14,000 rpm at 4°C for 10 min. The ethanol extraction was repeated three times and the combined supernatant was diluted to a final volume of 10 mL with 92% (v/v) ethanol. The glucose concentration in the ethanol extracts was determined with anthrone reagent (Van Hande, 1968) using glucose as a standard. Fructose concentration in ethanol extracts was determined by the method of Davis and Gander (1967) as standard using fructose. Sucrose concentration in the ethanol extracts was determined by the method of Van Hande (1968) using sucrose as a standard. Starch was extracted from ethanol insoluble residue. The residue was dried at 80°C to evaporate ethanol. Starch was hydrolyzed with adding amyloglu-

cosidase (Sigma product A3514) and α-amylase (Sigma product A0273) in the 0.2 N Na-acetate buffer (pH 5.1) to each sample by incubating at 50°C for 24h with occasional shaking. Tubes were centrifuged as described previously and glucose in the supernatant was determined using glucose oxidase (Glucose Trinder, Sigma product 315-100). Starch concentrations were estimated as 0.9 × glucose concentration.

RESULT

Dry matter

The changes in dry matter of three plant tissues during 28 days are presented in Table 1. The dry weight of leaves at 0 day was 16.43 ± 0.21 g plant^{-1} in average, and remarkably decreased by 45% within 14 days and 74% within 28 days in drought-stressed plants compared to well-watered (control) plants. Dry matter of stolon was not affected by water treatment throughout experimental period. Dry matter of roots in drought-stressed plants was not significant difference compared to control within 14 days, thereafter a stimulated increase of drought-stressed roots appeared.

Glucose

The changes in glucose concentration in leaves, stolon and roots of control or drought-stressed plants over 28 days of treatment are shown in Fig. 1. Initial concentration of glucose in leaves was on average 83.9 ± 6.1 mg g^{-1} dwt. The glucose concentration at day 28 decreased to 73.3 mg g^{-1} dwt. in control plants, while tended to increase in drought stressed plants. In both stolon and roots of drought-stressed plants, the concentration at day 28 increased by 29.2% and

Table 1. Changes in dry weight of leaves, stolon and roots of well-watered or drought-stressed plants during 28 days. Each value is the mean ± S.E. for n=3.

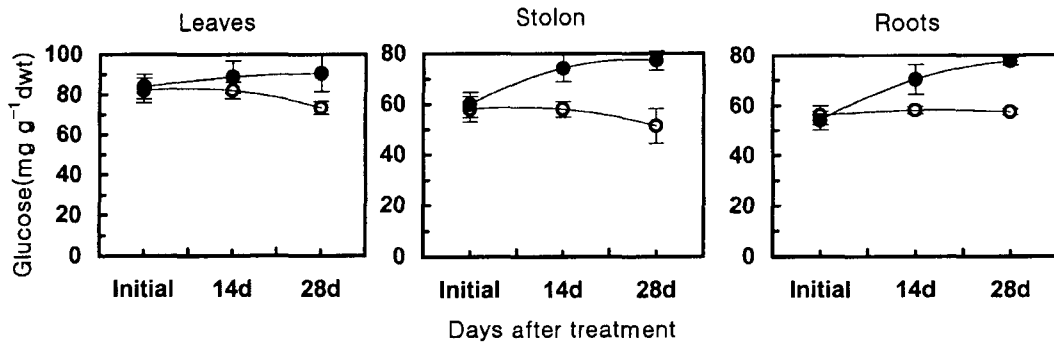| Organs	Days after drought treatment		
	Day 0	Day 14	Day 28
Leaves			
Well-watered	16.64 ± 0.47	20.94 ± 0.42	31.39 ± 3.26
Drought-stressed	16.23 ± 0.52	11.44 ± 0.03	8.31 ± 0.76
Stolon			
Well-watered	9.54 ± 0.08	11.97 ± 1.26	11.82 ± 1.17
Drought-stressed	9.62 ± 0.13	11.63 ± 1.43	10.73 ± 1.26
Roots			
Well-watered	5.65 ± 0.10	6.35 ± 0.22	8.97 ± 0.91
Drought-stressed	5.45 ± 0.31	6.25 ± 0.53	11.03 ± 1.06

Fig. 1. Changes in glucose concentration of leaves, stolon and roots of well-watered (○) or drought-stressed (●) plants during 28 days. Each value is the mean ± S.E. for n=3.

43.6%, respectively, compared to initial level. However, glucose concentration in control plants was less varied without significant difference for 28 days of experimental period.

Fructose

Changes of fructose concentration in each organ during 28 days after treatments are shown in Fig. 2. Fructose concentration in leaves was not changed for both treatments within 14 days, thereafter significantly decreased in control plants but relatively constant in drought-stressed plants. The initial

fructose concentration in stolon was 31.1 ± 2.8 mg g^{-1} dwt. In stolon, the concentration in control plants sharply decreased to 62.1% of the initial level, while relatively stable in drought-stressed plants. In roots, fructose concentration remained same level in control plants, whereas a sharp increase within 14 days occurred in drought-stressed plants.

Sucrose

Changes of sucrose concentration in leaves, stolon and roots of control or drought-stressed plants over 28 days of
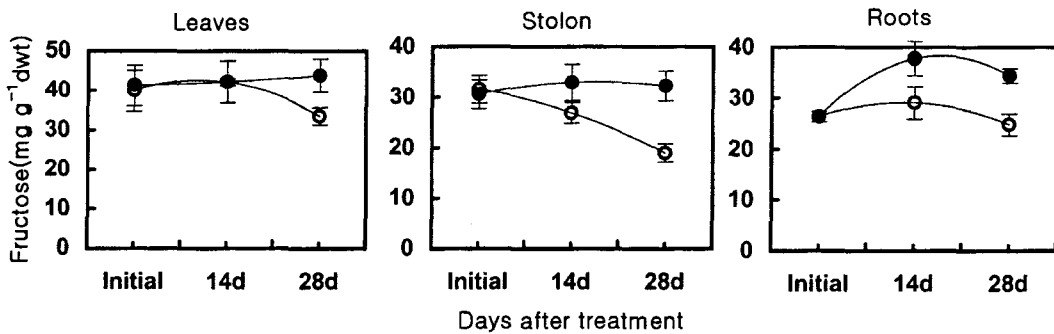

Fig. 2. Changes in fructose concentration of leaves, stolon and roots of well-watered (○) or drought-stressed (●) plants during 28 days. Each value is the mean ± S.E. for n=3.
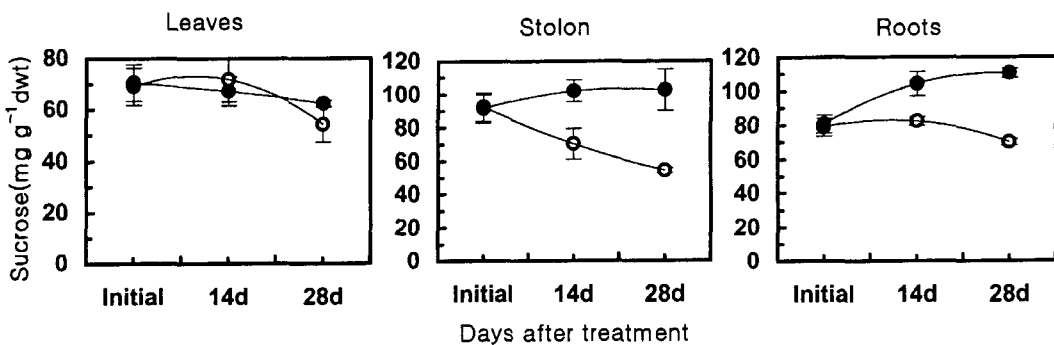

Fig. 3. Changes in sucrose concentration of leaves, stolon and roots of well-watered (○) or drought-stressed (●) plants during 28 days. Each value is the mean ± S.E. for n=3.
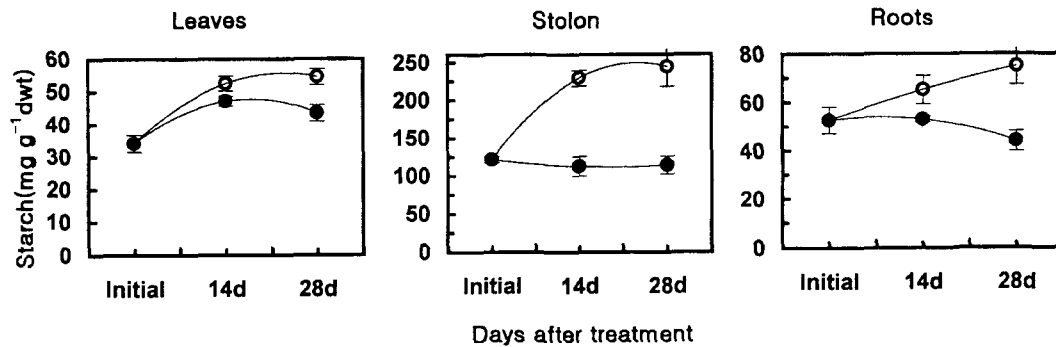
Fig. 4. Changes in starch concentration of leaves, stolon and roots of well-watered (○) or drought-stressed (●) plants during 28 days. Each value is the mean ± S.E. for n=3.

treatment are shown in Fig. 3. Sucrose concentration of leaves in drought-stressed plants was relatively constant, while a sharp decrease was observed from 14 days in control plants. In stolon, sucrose concentration in control plants was largely decreased by 23.1% within 14 days and 40.5% within 28 days compared to initial level, whereas the concentration was not significantly changed by drought-treatment. In contrast, the concentration in roots was significantly increased by drought-stress. The difference in sucrose concentration between control and drought-stressed plants was the largest in stolon. The sucrose concentration in drought-stressed stolon and roots at day 28 was 1.9 and 1.6 times higher than those of control plants.

Starch

The concentration of starch in leaves, stolon and roots of control or drought-stressed plants over 28 days of treatment are shown in Fig. 4. Starch concentration in leaves of both treatments tended to increase. The increasing rate was apparently lower in drought-stressed plants. The concentration in stolon was less changed in drought-stressed plant, whereas it increased to more than 2.0 times within 14 days in control plants, suggesting that starch accumulation was largely depressed under drought stress especially in a main reserve organ. The depression of starch accumulation in roots was also observed. Comparing the values at 28 day after treatment, it was estimated that drought depressed the starch accumulation in stolon and roots by 53.4% and 39.0%, respectively.

DISCUSSION

The most important agronomic problem for perennial forage species growing under prolonged drought is the decrease of production and survival ability. This is confirmed in the present study. The dry weight of leaves in drought-stressed

plants decreased by 45% within 14 days, and 74% within 28 days compared to that of control plants (Table 1). It has been reported that in several cultivars of forage grasses leaf expansion rate was halved within 5 days of drought, and had almost ceased within 10 days (Volaire et al., 1998). Dry matter of stolon was not significantly different between control and drought-stressed plant. In this experiment, root growth was not significantly affected by drought-stress within 14 days, while slightly increased by 28 days of drought. The effect of soil drought on root growth is species specific in relation to the intensity of drought. Drought-stress increases the number and growth of lateral roots in perennial ryegrass (Jupp and Newman, 1987). However, severe and long lasting drought decrease root growth due to a reduction in both cell division (Sacks et al., 1997) and cell extension (Frensch, 1997). On the other hand, the drought-induced increase of deposition of hydrophobic substances in the walls of epidermal roots cells has even been found when soil moisture was only locally decreased in basal root zones, and thus did not affect the root growth (Watt et al., 1996). Deposition of hydrophobic substances in the walls of root cells might affect apoplastic nutrient transport and accessibility of the membranes for nutrients (Peterson, 1987).

Our results showed that all organs contained higher concentration of soluble sugars such as glucose (Fig. 1), fructose (Fig. 2) and sucrose (Fig. 3). The concentrations of soluble sugars in leaves was less affected by drought within 14 day, while those of stolon and roots remarkably increased as drought was prolonged. It could be assumed that the translocation of mono- and disaccharides from source (leaves) to sink organs (stolon and roots) is accelerated, possibly due to low utilization of these compounds for leaf growth under soil drought condition. Dry matter response to drought-stressed plants (a sharp decrease in leaves, non-significant change in stolon and a slight increase in roots; Table 1) supported this assumption. Meyer and Boyer (1981) indicated that sugar accumulation occurred as a consequence of

the reduction in growth. Sum of four carbohydrate fractions in control and drought-stressed plants at 28 day of treatment was 808.5 and 833.7 mg g^{-1} dwt., respectively. Soluble sugars accounted for 76% of total non-structural carbohydrate in drought-stressed plants. These results indicate that a large part of C assimilates accumulates in the soluble form, allowing to adjust the osmotic potential of the cells (Bohnert et al., 1995). The accumulation of soluble sugars as an osmotic tolerance mechanism has been widely discussed in many species (Thomas, 1997). Also the accumulation of sugars under stress could have a protective function. Soluble sugars accumulate in the leaves of many species (Munns and Weir, 1981; Morgan, 1984; Zrenner and Stitt, 1991), including potato (Moorby et al., 1975). Different results occurred in mature leaves of other plants. In mature apple leaves, the concentration of sorbitol increased and that of sucrose either decreased or remained relatively stable as water stress lasted (Wang et al., 1995). An increase in mannitol and a decrease in sucrose concentration with increased salt concentration were also reported in a salt-tolerant celery species (Kann et al., 1993). Therefore, the type of carbohydrate associated with the osmotic adjustment differed among plant organs. A higher variation in sucrose in stolon and roots (Fig. 3) is expected to have a major effect on osmotic adjustment.

Starch concentrations in response to drought stress (Fig. 4) are in direct contrast to those of soluble sugars. Starch accumulation in all three organs of drought-stressed plants significantly depressed. The data obtained showed that the difference in the concentration of starch, which would be a primary storage C compound, between control and water-stressed plants was the greatest in stolon. Stolon has a characteristic of main reserve organ, showing 3-5 times higher concentration of starch comparing to leaves or roots (Fig. 4). Much active conversion of sucrose to starch in the main reserve organs, as like potato tubers, was observed (Geigenberger et al., 1997). Our results showed a continuous increase in sucrose synthesis and a depression of starch accumulation under drought stress. An increase of sucrose synthesis is probably a consequence of the inhibition of starch synthesis and the resulting accumulation of mono- and disaccharides under drought-stress. Decrease in the starch concentration in leaves under water-stress have been well documented in many plant species, including apple (Wang and Stutte, 1992), grape (Rodrigues et al., 1993), spinach (Quick et al., 1989), and bean (Vassey and Sharkey, 1989). Geigenberger et al. (1997) proposed that this sucrose cycle also modulated starch-sucrose interconversions during water stress. The increase of sucrose concentration may be also associated with an inhibition of unidirectional sucrose degradation under drought stress, although this study does not provide the evidence for a build up of products of sucrose synthase

reaction. Geigenberger et al. (1999) showed that operation of sucrose-phosphate synthase (SPS) and sucrose cycle might lead to marginal decrease in starch accumulation in non-stressed plants, but the activation of SPS and stimulation of sucrose synthesis resulted in a decrease of 3-Phosphoglycerate (3PGA). This decrease of 3PGA correlates with a partial inhibition of starch synthesis. In conclusion, the present results indicated that the shift in carbon partitioning from in-soluble carbohydrate (starch) to soluble carbohydrates and an increased breakdown of starch (Dance et al., 1990; Chaves, 1991) could greatly contribute to osmotic adjustment capabilities by increasing the number of molecules like glucose and fructose, thereby maintaining tissue pressure.

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