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CELL MEMBRANE STABILITY AS A MEASURE OF DROUGHT AND HEAT TOLERANCE IN GRASSES

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Abstract

Drought and heat tolerance test developed for Sorghum (Sullivan, 1972) were adapted with some modifications to evaluate some species of grasses grown under controlled environment during 1988-89. Significant differences were observed, between species for herbage growth rate (non-stressed, stressed and on recovery), percent heat injury as well their angular transformed data but not for injury due to dehydration stress. Even though severely affected during drought, cocksfoot rank the highest for herbage growth rate measured under all conditions followed by tall fescue, perennial ryegrass and Italian ryegrass. On an average, cocksfoot was found most resistant to membrane damage caused by both drought and heat stress in comparison with tall fescue and Italian ryegrass, where as perennial ryegrass came out as intermediate. None of the *in-vitro* drought and heat resistance test was found associated with drought resistance. However, negative association of recovery regrowth rate after drought with heat stability (0.531*) and positive association with droughted herbage growth rate (0.468*) indicated that the populations that recovered well after drought have less membrane damage due to heat injury as well as produce more herbage under water stress condition.

Key words : Membrane stability, Drought, Heat tolerance, Grasses.

Introduction

Genetic improvement of drought resistance in crop plants requires identification of relevant drought resistance mechanism and the development of suitable methodology for their measurement in large breeding population. A recent review by Bewley (1979) reported the critical role of membrane stability under condition of moisture stress as a major component of drought tolerance. The rate of injury to cell membranes by drought may be estimated through measurement of electrolyte leakage from the cells. The rate of injury to cell membranes is commonly used as a measurement of tolerance to additional plant stress, such as freezing (Dexter, 1956) and heat (Sullivan, 1972). Such a method of

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measurement for drought and tolerance developed for sorghum by Sullivan (1992) was modified by Dexter (1965). For drought and heat tolerance in grasses, a further modified method based on dehydration *in-vitro* of leaf discs by silica gel and a subsequent measurement of electrolyte leakage into deionized water was developed by Thomas (1987). The aims of the investigation were therefore, to find any relationship of these *in-vitro* methods with some growth parameters of grasses under both watered and droughted condition in controlled environment.

Materials and Methods

Sixteen ecotypes and three varieties of perennial ryegrasses of diverse European origin were studied. In addition three species with contrasting drought response (*Lolium multiflorum* L. var. RVP; *Festuca arundinacea* L. var. Dovey and *Dactylis glomerata* L. var. Cambria) were included to extend the range of expression.

The experiment was conducted at Welsh Plant Breeding Station. U.K. during 1988-89 in an unheated glasshouse which transmitted approximately 65% of incoming solar radiation. Seeds were pre-germinated on wet sand under laboratory condition. Six seedlings of each of the four replicated plastic pots (18 cm in diameter) filled with sterilized soil weighing 3.2 kg at field capacity. The pots were then watered on alternate days. On day 18 the pots were randomized into 4 blocks. The pots were then fertilized on day 28, and subsequently at 25 days intervals with commercial fertilizer Vitafeed -III (19 : 19 : 19 :: N : P : K) : 118 g of Vitafeed were dissolved in 2.7 litre of water and 30 ml of this solution were given to each pot.

Biological measurements

After 82 days of sowing fully grown plants in each pot were cut back to 30 mm above the soil level (Preliminary cut) and the herbage discarded and allowed to grow under well watered and fertilized condition. Herbage growth rate before drought adaptation was measured by cut back the plant to the same level 43 days after preliminary cut. Again allowed the plants to grow under watered and fertilized condition for further 35 days after which a brief drought treatments of 26 days was started when water was completely withheld. At the end of drought treatment, plants were cut back to the same level as a measure of herbage growth rate during drought adaption. The plants were then fully watered and fertilized and recovery herbage growth samples taken 21 days after the end of drought treatment following same method as above. All the herbage samples were oven dried at 80°C for 48 hours and dry weight were recorded.

In-vitro tests

The drought and heat tolerance tests were performed using leaf discs of 2 cm in length taken from the youngest fully expanded leaf blades. A single sample consisted of 0.075 to 0.085 g of leaf segments taken from six leaves per ecotype. Measurement of cell membrane stability due to heat and drought tolerance were made as mentioned by Thomas (1987).

Statistical analysis

Data were analyzed using appropriate analysis of variance model in the Genstat statistical package. Repeatabilities (broad sense heritability) were calculated following Becker (1975). Correlations were calculated using the Minitab statistical package.

Results and Discussion

The brief period of drought (only for 26 days) began after the plant had produced much herbage under well watered conditions; thus at the end of drought adaption, final herbage

Table 1. Genetic diversity in herbage growth rate (HGR, g pot^{-1} d^{-1}: (a) between 19 populations of perennial ryegrass (PRG) and (b) between all the species under watered (W), droughted (D) or recovery regrowth after drought (R) conditions.

Treatment	HGR W	HGR D	HGR R	D/W	R/W
a) PRG					
Mean	148.6	138.2	46.1	0.990	0.333
Min.	85.2	110.9	4.9	0.605	0.030
Max.	200.2	158.4	75.7	1.880	0.748
F-ratio	9.06 ***	3.01 ***	2.79 ***	14.18 ***	4.59 ***
b) All Species					
PRG mean	148.6 b	138.2 bc	46.1 b	0.990 a	0.333 b
RVP mean	129.2 b	122.0 c	10.7 c	0.953 a	0.083 c
Dovey mean	151.3 b	157.7 ab	87.5 a	1.042 a	0.582 a
Cambria mean	242.7 b	168.9 a	105.5 a	0.694 b	0.435 ab
% Repeatability	74.85	38.68	57.68	76.78	54.41
S.E.D	13.08	11.58	13.42	0.1140	0.1025
F-ratio	12.90 ***	3.52 ***	6.41 ***	14.22 ***	5.77 ***

Significant at * = P<0.05, ** = P<0.01, *** = P<0.001

growth rate was reduced by 22% of the control (Table 1). However, herbage growth rate for both watered and drought adapted treatments differed significantly ($P < 0.001$) between species. In both treatments, herbage growth rate was the highest for cocksfoot followed by tall fescue, perennial ryegrass and Italian ryegrass which were very similar to the field result obtained by Hughes *et al.* (1977). With respect to control, cocksfoot was most affected among all the species which agreed with the glasshouse experiment of Norris and Thomas (1982) where *Lolium perenne* was least affected by drought and *Dactylis glomerata* was most affected. This is because of little opportunity for differences in root growth to be expressed in small pots and of rapid drying leads to little adaption to drought. On recovery regrowth, the species also differed significantly ($P < 0.001$) with cocksfoot ranked the highest followed by tall fescue, perennial ryegrass and Italian ryegrass (Table 1) which were similar to the glasshouse experiment of Norris and Thomas (1982) and to the field experiment of Hughes *et al.* (1977).

Significant differences were not observed (Table 2) between species as well as within perennial ryegrass for either percent injury (Damage) due to stress, or their angular transformed data (Ang. damage). However, significant differences were obtained between species ($P < 0.01$) and within perennial ryegrass ($P < 0.05$) for both percent heat injury (Damage) and their angular transformed value (Ang. damage) with genetic component of variance of 35.39%. As observed in the present observation, cocksfoot was found most resistant to membrane damage caused by both drought and heat stress in comparison with tall fescue and Italian ryegrass, whilst perennial

Table 2. Genetic diversity in cytoplasmic membrane stability measured under drought stress (CMSD) and heat stress (CMSH) in temperate forage grasses: (a) between 19 populations of perennial ryegrass (PRG), and (b) between all the species.

Treatment	CMSD		CMSH	
	% Damage	Ang.damage	% Damage	Ang. Damage
a) PRG				
Mean	28.5	31.4	36.6	37.0
Min.	11.8	20.0	22.1	27.7
Max.	56.8	49.3	72.1	60.6
F-ratio	0.97 (NS)	1.00 (NS)	2.06 *	2.10 *
b) All Species				
PRG mean	28.5 a	31.4 a	36.6 b	37.0 a
RVP mean	32.8 b	34.2 b	48.1 b	43.9 a
Dovey mean	75.7 a	61.5 a	31.6 a	34.1 a
Cambria mean	39.9 ab	38.5 b	6.2 b	14.1 b
% Repeatability	17.42	17.97	35.39	38.80
S.E.D	15.43	9.77	12.27	7.75
F-ratio	1.63(NS)	1.66 (NS)	2.64**	2.90 **

Significant at * = P < 0.05, ** = P < 0.01, *** = P < 0.001

ryegrass come out as intermediate between these species.

Correlation studies (Table 3) revealed that herbage growth rate after drought adaption was significantly positively correlated with recovery regrowth rate (0.468*). Such results indicated that the populations that produced more herbage under drought conditions tended to recover well which agreed with the finding of Thomas and Evans (1990) in perennial ryegrass populations. This implies that selecting grasses for high herbage yield during drought should not in

Table 3. Correlation co-efficient between herbage growth rate (HGR) under watered (W), droughted (D) and recovery regrowth (R) conditions with membrane damage due to drought (CMSD) and heat stress (CMSH).

Characters	HGR (W)	HGR (D)	HGR (R)	CMSD
HGR (D)	-0.079			
HGR (R)	0.264	0.468 *		
CMSD	0.274	0.154	0.256	
CMSH	-0.348	-0.531 *	-0.531 *	-0.076

Correlation co-efficient significant at * = P<l0.05, ** = P<0.01

temperate climates at least, lead to deleterious effects on recovery from drought or on growth when well watered. Recovery rate was also found significantly negatively correlated with heat stability(-0.531*) which indicated that the populations which can withstand damage by heat tend to have higher recovery regrowth rate, possibly because photosynthesis (leading ultimate herbage production) is sensitive to high temperature (Alexandrov, 1964).

The outcome from this work revealed that although no association were observed between membrane damage by drought and heat stress with drought resistance but if we look at the herbage growth rate under different conditions (stress and recovery) it seems that studied *in-vitro* tools might be important in preliminary screening of large breeding population for their response to stress. In addition to this, looking at the better recovery potential after drought might help to isolate population that might performed well also under actual water stress as well as show some degree of heat tolerance.

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