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GENETIC AND PHYSIOLOGICAL COMPONENTS OF POST-FLOWERING DROUGHT TOLERANCE IN SORGHUM

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ABSTRACT

Four sorghum (*Sorghum bicolor* (L) Moench) parental lines RTx430, BTx3197, RTx7000, and B35; and their F_1, reciprocals, F_2, and backcross progenies were evaluated during the reproductive phase in two green house experiments to understand the number of genes involved in the expression of high water potential ψ and high osmotic potential π. The experiments were conducted at Texas A&M University during 1989 and 1990. Plants were well watered until anthesis, after which water was withheld, and measurements were then taken at two weeks interval. Cultivars differed significantly in all traits at all levels of drought stress. B35 and BTx3197 generally maintained higher ψ and π compared to RTx7000 and Rtx430. Crosses made with B35 resulted in hybrids which were lower in ψ and π, but higher in dry matter production. These were attributed to hybrid vigor and poor osmotic adjustment capacity. Crosses made with BTx3197 generally maintained higher ψ and π and were predominantly lower in dry matter, which was associated with better osmotic adjustment capacity. Comparison between F_1 and reciprocals indicated cytoplasmic influence. Depending on the parental combinations, the F_1 means showed higher or lower ψ and π when compared to either of their parents. Inheritance was proposed to be under multiple gene control with dominant and recessive epistatic effects. Heritability estimates indicated that either B35 or BTx3197 could be used as a parent for the improvement of plant water status. Correlations were found between dry matter accumulation and ψ and percent moisture loss, and between relative water content and ψ. These are unlikely to be useful in selection work because of expense and time required for measurement. Indirect selection would be more appropriate for improvement of drought tolerance.

Key Words: **Water potential, drought stress, osmotic adjustment, osmotic potential.**

RÉSUMÉ

Quatre lignée parentales de sorgo [*Sorgum bicolor* (L) Moench] du 'RTx430', du BTx3197', du 'RTx7000', et du B35; et leur F_1 reciproques, F_2 et leurs progénitures rétrocroisées ont été évalués durant la phase reproductive dans deux expériences en serre pour comprendre le nombre de gènes impliqués dans l'expression du haut niveau potentiel ψ et un fort potentiel osmotique π. Les expériences ont été menées à l'université A & M du Texas au cours de 1989 et 1990. Les plantes étaient bien arrosées jusqu'à l'anthèse, après quoi le ravitaillement en eau a été stoppé, et les mesurages de controles ont été faits à deux semaines d'intervalle. Les cultivars étaient différents sous tous les traits à tous les niveaux de contraintes causés par la secheresse. Le H35 et le RRTTx3197 maintenaient un plus haut ψ et π comparé au RTTx7000 et RTx430.

Les croisements produits du B35 ont donnés des hybrides qui étaient plus faibles en ψ et en π mais plus élevé en production de matières sèches. Cela était attribué à la vigueur de l'hybride et au mauvais ajustement osmotique. Les croisements produits du BTx3197 mainteneaient un plus haut ψ et π mais étaient surtout faibles en matières sèches, qui sont associées à une meilleur capacité d'ajustement osmotique. La comparaison entre F_1 et les réciproques indiquent une influence cytoplasmique dépendant de la combinaison parentale, la moyenne de F_1 a montré un ψ et π plus élevé ou plus faible quant il était comparé à un où l'autre parent. L'heritage a été proposé comme étant sous controle génétique multiple avec des effets épistatiques dominants ou rétrogrades. Les évaluations d'héritabilité indiquent que le B35 ou le BTx3197 pourraient l'un ou l'autre être utilisés comme parent pour l'amélioration de géstion de l'eau chez les plantes. Des correlations ont été trouvées entre l'accumulation de matières séches et ψ et le poucentage de perte d'humidité ainsi qu'entre le contenu relatif d'eau et ψ. Il est peu probable que cela soit utile au travail de sélection à cause des frais et du temps requis pour les mesurages de contrôles. La sélection indirecte serait plus appropriée pour l'amélioration de la tolérance à la secheresse

Mots Clés: **Potentiel osmotique, potentiel de géstion d'eau, contraintes de la secheresse, ajustement osmotique**

INTRODUCTION

Genetic variability for physiological components of drought tolerance has been documented. Sorghum (*Sorghum bicolor* (L) Moench) cultivars differ in their leaf water potential (ψ) when subjected to declining soil moisture (Blum, 1974; Madeleine *et al.*, 1980; Khizzah, 1991). Maintainance of high ψ is associated with better soil moisture extraction, osmotic adjustment, and stomatal activity Khizzah, 1991). Maintenance of high ψ is associated with bettter soil moisture extraction, osmotic adjustment, and stomatal activity. Variability for solute accumulation (osmotic adjustment) in whole sorghum plants as well as calli, grown under drought stress has been reported (Ackerson *et al.*, 1980; Newton *et al.*, 1986; Khizzah, 1991). Synthesis and accumulation of complex organi acids, amino acids, and sugars during drought stress are believed to aid reduced protein denuturation and cell wall degradation (Turner and Jones, 1980). It also has been proposed (Tenkunao, 1990; Khizzah, 1991) that solute accumulation is associated with resistance to charcoal rot [caused by *Macrophomina phaseolina* (Tassi) Goid] and the 'stay green' characteristics in some sorghum cultivars. However, the link between solute accumulation under declining soil moisture and dry matter production has not been establlished. Ackerson *et al.* (1980) contend that improved osmotic adjustment could result from smaller plants. The smaller size of plant organs either as source or sink could result in low potential yield.

Despite considerable accumulation of knowledge on high ψ and osmotic potential (π) as very important components of drought tolerance in sorghum, little is understood about their mode of inheritance. This is because their interactions with each other, with other morphological attributes, and with the environments as they relate to total resistance are not clearly understood. Also, timing of drought with respect to the plant's development stage is important to its stress responses. However, Morgan (1983) concluded that F_4 lines from a cross between high and low osmoregulating wheat varieties could be classified into high, medium and low osmoregulation, suggesting simple inheritance. Similar studies with sorghum (Hoffman *et al.*, 1984) indicated that the F_1 could be equal , lower or higher than any of 'stay green' characteritstics in 'B35' and 'R9188' sorghum. He reported that one major dominant gene and one recessive gene control leaf retention, while the 'stay green' was inhertianted independently from other leaf parmeters. Tenkouno (1990) concluded that the inhertiance of non-senescence in sorghum was under multiple genes with dominant and recessive epidstatic effects. Understanding the physiological and genetic basis of high ψ and π in post flowering sorghum during declining soil moisture provides information on the number of genetic factors involved in their expression. The major objectives of this investigation were: 1) to indentify the pattern of ψ and π in parental cultivars, and their F_1, F_2 and backcross progencies subjected to drought stress, 2) to determine the influence of ψ and π on dry, matter production, and 3) determine correlations among physiological components associated with post-flowering drought tolerance in sorghum.

MATERIALS AND METHODS

Four sorghum inbred cultivars RTx430, BTx3197, B35, and RTx7000, chosen for either contrasting post-flowering field drought tolerance (Rosenow, 1991; Miller, pers. com.) were used in this investigation. B35 and BTx3197 had earlier been reported to maintain relatively high ψ and π, while RTx7000 maintained low ψ and π and was very susceptible to drought stress and RTx430 showed moderate response (Khizzah, 1989).

Hand emasculated crosses were made between these cultivars following a full diallel mating system with reciprocals. Parents, F_1, F_2 and backcross progenies were evaluated in greenhouse experiments during the first rains (May-August) of 1989 and 1990. Conditions in the greenhouse remained similar to those of the surroundings. The experiment followed a complete randomized block design with three replications. Materials were planted in plastic containers, 50 cm high by 36 cm diameter, in freely drained clay soil. Thinning was done three weeks and four weeks after planting, to leave thirteen healthy looking plants per pot. Two containers were allocated to each genotype. Plants were well irrigated until anthesis, after which water was withheld and the containers allowed to dry slowly.

Component measurements were then taken once every two weeks and combined for analysis. Pre-dawn plant water potential ψ was measured on whole plants using a Scholander pressure bomb (model 3005, Soil Moisture Equip. Corp.), according to the method of De Roo (1969). The plant was cut at the base and placed in the pressure chamber through a rubber gland. The pressure in the chamber was increased by compressed air from a cylinder, until the sap just appeared at the severed end of the xylem. The pressure inside the chamber at that point was recorded as the plant water potential (MPa).

Relative water content (RWC) was determined by the method of Turner (1979). Samples (approx 20 cm^2) were cut from the first leaf below the flag leaf after the water potential measurement had been taken. Fresh weights (Wf) were determined before floating for 4 hr in distilled water in a well lighted room. Surfaces were blotted dry before the turgid weights (Wt) were taken. Samples were then placed in an oven at 75°C for 24 hr before dry weight (Wd) were taken. Relative water content was then determined by the relationship:

$$RWC = \{(W_f-W_d) / (W_t-W_d)\}\ 100$$

Osmotic potential was measured on the leaf discs obtained from the same leaf as above. Samples were sealed with a stopper in test tubes and frozen in liquid nitrogen. Osmotic potential was measured in the sap expressed from the plant material with a micropress after a 30-min. thawing period. An aliquot of expressed sap was placed on filter paper in the thermocouple phycrometer (WESCOR HR-33T Dew Point Microvoltameter and C-52 chamber) after equilibrating for 10 min. and colling for 10 sec. the osmotic potential was read exactly after 5 sec. (where the needle stalled for a moment while going down).

Percent moisture loss (PML) was estimated by the method of Wenzel and van de Berg (1987).

Percent cell damange was estimated by the method of Sullivan (1972) using a conductivity bridge and dip cell (YSI MODEL 32, Yellow Springs Instrument Co, Ohio). Leaf discs, 21 cm in diameter, were cut from each cultivar with a cork borer, placed in a test tube, and washed thoroughly with distilled water for 3 hr changing water four times. At the end of the washing, enough water was left to keep the discs wet during heating treatments. Tubes were covered with plastic wrap and placed in a water bath for 1 hr at 48°C. A duplicate sample was held as a control at room temperarure. After heating, 30 ml of distilled water was added to each tube and the tubes held overnight in an incubator at 10°C. Relative amount of electrolyte diffusing from the tissues was measured after equilibrating the tubes at 25°C. The samples were then completely killed by heating in a water bath at 90°C and cooling to 25°C before conductivity was again measured. Injury value was estimated from the elevated temperatures and calculated as percentage increased conductance over controls which provided an estimate of the plant's tolerance to heat.

Above groud dry matter (DM) was determined using the two remaining plants from each container. Whole plants were cut into small pieces, including leaves and panicles, and kept in the oven at 75°C for 24 hr, before dry weights (g) were obtained.

Analyses of variance were carried out to compare the effects of induced drought stress on cultivars. F_1 progencies were compared to both parents to establish dominance relationships. Also the frequency distributions of F_2 and backcross populations were used to confirm the relationships, and to estimate the number of genes, and the Chi-square tests for goodness of fit performed. Variances associated with each mean were used to determine genotypic (Vg), additive (Va), dominance (Vd), environmental (Ve), and narrow and broad heritability estimates.

RESULTS AND DISCUSSION

Significant differences were revealed for all traits at all levels of drought stress. Interaction effects for RWC, ψ and π were significant ($P<0.01$). Coefficient of variation (CV) values were low for RWC (9.5%) and high for ψ (54.5%), and were moderate for π, PML, DM, and cell damage. Mean RWC, cell damage, ψ, π, PML, and DM are given in Table 1. These were higher than the RWC, ψ and π values observed in most hybrids with B35. This could be explained by the plants hybrid vigour which is associated with better water use efficiency, resulting in larger plants (Quinby, 1972). More rapid depletion of soil moisture by larger plants could explain the early expression of drought symptoms (low RWC, ψ, and π) which were observed in crosses with B35. Smaller plants tended to conserve most of their tissue water, and maintained higher RWC, ψ and π. This was consistent with the observation made by Ackerson *et al.* (1980).

An attempt was made to study the pattern of distribution of high ψ and π, and for this, F_1 and reciprocal crosses were pooled to increase sample size. Data for RTx430 were excluded from this portion of the investigation because it could not be classified as having low or high ψ and π, respectively. The F_1 means for ψ were higher than their lower parents, while for π, F_1 means were lower than their lower parents, suggesting additive gene effects for both traits. The F_2 and

TABLE 1. Mean relative water content, cell damage, water potential (ψ), osmotic potential (π), percent moisture loss (PML), and dry matter (DM) for inbred parents and the F_1 progenies of selected sorghum cultivars grown in a greenhouse during the summers of 1989 and 1990 at College Station, Texas.

Pedigrees	RWC	Cell damage	ψ(MPa)	πPML	PML	DM (g)
B35	89.7ab	47.8c**	-0.20a	-1.50abc	21.1abcd	13.8bcde
BTx3197	87.5ab	39.5c	-0.51ab	-1.37a	18.7abcd	18.6abcd
BTx3197*RTx7000	87.2ab	75.5ab	-0.44ab	-1.36a	24.2e	11.9ed
RTx430	87.0ab	64.7c	-0.53abc	-1.81cde	23.1cd	18.5abcd
RTx7000*RTx430	86.7abc	84.6a	-0.39a	-1.78cde	19.5abcd	10.1e
B35*RTx430	86.3abc	78.3ab	-0.67abc	-1.88e	17.4abc	22.6a
BTx3197*RTx430	83.8abc	81.6a	-0.36a	-1.46abc	15.7ab	16.4abcde
RTx7000*BTx3197	82.5abc	77.8ab	-0.63abc	-1.50abc	22.8bcd	13.0bcde
RTx430*BTx3197	82.1abc	65.6b	-0.51ab	-1.40ab	17.4abcd	14.9bcde
RTx7000	82.1abc	72.9ab	-0.82bcd	-1.63cde	24.5e	12.6cde
B35*RTx7000	81.7abc	85.4a	-0.65abc	-1.77cde	21.7abcd	19.4abc
RTx430*B35	80.1bc	84.6a	-0.81bcd	-1.83de	16.4abc	18.0abcd
RTx430*RTx7000	78.2cd	87.4a	-0.61abc	-1.60bcd	18.6abcd	15.5bcde
BTx3197*B35	72.3ed	85.9a	-1.07d	-1.95e	14.8a	19.9ab
RTx7000*B35	71.2ed	77.0ab	-0.91cd	-1.87e	10.2a	18.1abcd
B35*BTx3197	69.8e	82.6a	-0.63abc	-1.77cde	14.6a	22.8a
LSD (0.05)	7.24	14.65	3.17	1.93	7.16	6.1

**Values within the column followed by the same letter(s) did not differ significantly at $P<0.05$ according to the Duncan Multiple Range Test. (Values are the means of 3 replications at 3 soil moisture levels) backcross frequency distributions suggested that two genes could be responsible for ψ and π, respectively. The following assumptions were made to explain the distributions.

(a) two loci, W_{pl}/W_{p2} with dominant effects and W_{pl}/W_{p2} with recessive effects
(b) either recessive homozygous was epistatic to the effect of W_{pl}/W_{p2}
(c) interaction: homozygous recessive at W_{pl} and W_{p2} produce high water potential.

Two loci assumed for π were O_{sl}/O_{s2} with dominant effects, and o_{sl}/o_{s2} with recessive effects. Using these assumptions, parental phenotypes, expected F_2 segregation ratios, and genotypes are described in Table 2. The Chi-square test of goodness-of-fit was conducted to test validity of these assumptions, and the resuts are summarized in Table 3. Data from F_2 and backcross-1 fit the theoretical expectations. Deviations from expectation in BC_2F_1 were possibly due to maternal effects and sample size. However, it could be reasonably argued that two genes each were responsible for the expressions of high ψ and π. However, assuming a regulatory gene to be involved in such a stress dependent physiological response, it would be tempting to speculate that maintenance of high ψ and π was under the control of a block of genes. Enzymes biossays would be a useful study to provide information on whether synthesis or biodegradation of some products result from induced water stress.

Variances and variance components associated with ψ and π, and heritability estimates are shown

TABLE 2. Theoretical expectation for crosses between parents of high and low water and high and low osmotic potential, respectively

Parents	Phenotypes	Segregation ratios			Genotypes	
		F_2	BC_1	BC_2	ψ	π
RTx7000	Low	1:3	1:1	0:1	$W_{p1}W_{p1}W_{p2}W_{p2}$	$O_{s1}O_{s1}O_{s2}O_{s2}$
B35	High	3:1	1:1	1:0	$w_{p1}w_{p1}W_{p2}W_{p2}$	$O_{s1}O_{s1}O_{s2}O_{s2}$
BTx3197	High	7:9	1:1	1:1	$W_{p1}W_{p1}w_{p2}w_{p2}$	$O_{s1}O_{s1}O_{s2}O_{s2}$

** high/low ψ = -0.3 to -1.5, and low < -1.6 MPa
..and high/low π (high π = 0.6 to -2.0, and low < -2.1 MPa)

TABLE 3. Segregation ratios of water potential osmotic potential in F_2 and backcross progenies derived from crosses between three sorghum cultivars grown in a greenhouse during 1989 and 1990 at College Station, Texas

Crosses		F_2		BC_1		BC_2	
		ψ	π	ψ	π	ψ	π
RTx7000*B35	Ob.†	14:30‡	26:28	17:23	23:17	17:40	22:34
	Exp.	11:33	33:11	20:20	22:22	0:40	0:57
	Chi²	1.091	5.121	0.900	0.912	249.070**	471.131**
RTx7000*BTx3197	Ob.	35:15	23:12	13:33	26:20	15:28	35:8
	Exp.	37.5:12.5	26.6:8.8	23:23	23:23	43:0	43:0
	Chi²	0.667	1.152	7.847*	0.543	802.233**	57.558**
BTx3197*B35	Ob.	21:37	16:42	22:17	19:20	5:37	26:16
	Exp.	24.4:32.6	25.4:23.6	18.5:18.5	19.5:19.5	21:21	21:21
	Chi²	1.341	5.518	0.784	0.000	22.881**	1.929

*, ** significant at 0.05, and 0.01 probability levels, respectively.
† Ob = observed, Ex = expected, Chi² = (I Ob high - Ex high 1-0.5)2/ Ex high + (I Ob low - Ex low 1-0.5)2/Ex low.
‡ high: low ratio
I, = absolute or positive value of the difference

TABLE 4. Variance components and heritability estimates for water potential and osmotic potential for some sorghum progenies grown in a greenhouse during 1989 and 1990 at College Station, Texas

Crosses	Variance components heritability									
	Additive		Dominance		Environment		Narrow		Broad	
	ψ	π	ψ	π	ψ	π	ψ	π	ψ	π
RTx7000*35	4.48	10.46	30.85	11.94	12.75	14.72	0.09	0.29	0.74	0.61
BTx3197*RTx7000	-2.03	6.64	13.03	13.83	22.26	26.94	-0.06	0.22	0.33	0.66
BTx3197*B35	53.15	18.99	-26.46	-3.92	24.67	13.41	1.04	0.67	0.52	0.53

in Table 4. Environmental variance was moderate, accounting for between 20 to 70% of the variability. Narrow-sense heritability values in some cases had values more than unity or were negative. In most cases, narrow sense heritability values conformed to the theoritical expectation, since they were slightly lower than broad sense values.

Strong negative correlations ($P<0.05$) were revealed between DM and π ($r=-0.364$) confirming that osmotic adjustment was accompanied by a decrease in growth rate (Munus, 1988). Other negative correlations were observed between DM and PML ($r=-0.398$). PML often had been considered to be a useful tool in selection for drought resistance (Wenzel and van den Berg, 1987). They reported correlations between PML and yield potential. The existance of correlations between DM, PML and π in this experiment confirmed those reported previously and further suggested its usefulness in selection work. This method is simple, cheap, and can be used with large numbers of cultivars. A negative correlation also was revealed between heat stress and RWC ($r=-0.306$), but between ψ and RWC the correlation was positive ($r=0.585$). Correlations were observed between ψ, π, and DM, and RWC, heat stress and PML, y and PML, but these were not significant. Efforts should be made to relate these measurements to simple phenotypic characters of drought resistance to make them more useful.

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