Theor Appl Genet (1995) 90: 675–682

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Inheritance of osmotic adjustment to water stress in three grain sorghum crosses

Received: 30 June 1994 / Accepted: 4 August 1994

Abstract Water stress is one of the major constraints to the grain yield of sorghum in tropical and sub-tropical areas of the world. Osmotic adjustment has been widely proposed as a plant attribute that confers adaptation to water stress. The inheritance of osmotic adjustment to water stress was investigated in a series of generations derived from the three possible bi-parental crosses between two inbred sorghum lines with a high capacity for osmotic adjustment (Tx2813 and TAM422; high-OA lines) and one with a low capacity (QL27; low-OA line). Broad-sense heritability on a single-plant basis was generally found to be high. Analysis of segregation ratios by the mixture method of clustering identified two independent major genes for high osmotic adjustment. The line Tx2813 possessed a recessive gene which is given the symbol *oa1*; the line TAM422 possessed an additive gene which is given the symbol *OA2*. There was some evidence that there may be other minor genes which influence the expression of osmotic adjustment in these crosses as two putative transgressive segregants, with higher osmotic adjustment than the parents, were identified from the cross between Tx2813 and TAM422. Populations of recombinant inbred lines were developed and characterised for osmotic adjustment for two of the crosses (QL27 × TAM422, low-OA × high-OA; Tx2813 × TAM422, high-*oa1* × high-*OA2*). These will be used to conduct experiments which test hypotheses about the contribution of the high-osmotic-adjustment genes to the grain yield of sorghum under a range of water-stress conditions.

Communicated by G. Wenzel

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Key words Sorghum · Water stress
Osmotic adjustment · Inheritance · Major genes
Mixture method of clustering

Introduction

Water stress is one of the major constraints to the grain yield of sorghum in tropical and sub-tropical areas of the world. During crop growth, the occurrence of drought leads to depletion of soil moisture and decreasing availability of water to the plant. Some physiological mechanisms that could reduce the deleterious effects induced by water limitation have been reviewed for grain sorghum by Ludlow and Muchow (1990, 1992). They recommended that some of these traits should be incorporated as selection criteria for plant-breeding programs which target regions prone to drought. However, the adoption of these physiological criteria in breeding programs has been slow. A number of factors have contributed to this, including (1) an inadequate understanding of the inheritance of these traits, (2) insufficient information on the contribution of these traits to higher yield over the range of environments encountered in the target region, and (3) inability to rapidly and precisely screen for appropriate levels of these traits in breeding populations (Blum 1988).

Osmotic adjustment to water stress has been identified as an important physiological mechanism contributing to improved adaptation in a number of crop species grown under water-limited conditions (Ackerson et al. 1980; Morgan 1980; Ludlow and Muchow 1990, 1992). It has been claimed that growth and yield under water-limited conditions can be improved by selecting for lines with higher levels of osmotic adjustment in wheat (Morgan 1980), sorghum (Ludlow and Muchow 1990, 1992), and barley (Blum 1989). The contribution of osmotic adjustment to higher grain yield in sorghum under water-stress conditions has been discussed by Ludlow et al. (1990) and Santamaria et al. (1990). While positive contributions of osmotic adjustment to grain yield have been reported, Munns

(1988) questioned the role of osmotic adjustment in adaptation to water stress.

Basnayake et al. (1993) reported genotypic variation for osmotic adjustment among sorghum lines. High levels of repeatability for the discrimination among lines for osmotic adjustment were achieved under controlled environmental conditions. While differences among lines have been quantified, there is limited information on the genetic control of the variation for this trait in sorghum. Tangpremsri et al. (1991) suggested that maternal effects were important in determining osmotic adjustment. Basnayake et al. (1994) identified differences in general- and specific-combining ability among inbred sorghum lines in a diallel analysis. Grumet and Hanson (1986) studied the inheritance of glycinebetaine accumulation as an indirect measure of osmoregulation in barley. They reported that there was no significant cytoplasmic effect for glycinebetaine accumulation and that additive gene effects were the main component of genetic variation. Morgan (1983, 1991) showed that variation for osmoregulation in a cross between two wheat lines was controlled by a single recessive gene. The chromosomal location of this gene was identified as 7A (Morgan 1991). In contrast, Galiba et al. (1992) reported that genes determining the expression of osmoregulation in wheat may be located on 5A and 5D.

An understanding of the inheritance of any trait would assist its manipulation in a breeding program. In this paper, the inheritance of osmotic adjustment is characterised in segregating generations developed from three grain sorghum lines specifically selected for high (Tx2813 and TAM422) and low (QL27) expression of osmotic adjustment from the studies by Basnayake et al. (1993, 1994). Genetic models for the inheritance of osmotic adjustment were investigated in the P_1, P_2, F_1, F_2, BCP_1, and BCP_2 generations derived from the crosses. In the cross between QL27 and TAM422 the F_3 and selfed progeny of the BCP_1 and BCP_2 generations was also examined. Recombinant inbred populations ($F_{2:5}$) from two of these crosses (QL27 × TAM422 and Tx2813 × TAM422) were developed to test the genetic models proposed for these crosses.

Materials and methods

Genetic material and development of populations

Three genetic populations were developed as a series of generations derived from the biparental crosses between two parents with high (Tx2813 and TAM422; high-OA lines) and one parent with low (QL27; low-OA line) capacity for osmotic adjustment to water stress (Basnayake et al. 1993). It was hypothesised that the inbred lines Tx2813 and TAM422 may possess different genes for high osmotic adjustment as they contrasted for combining ability for osmotic adjustment in a half-diallel analysis (Basnayake et al. 1994).

The F_2 population was developed by selfing the F_1 generation in controlled pollination rooms. The backcross (BC) generations were made by hand emasculation of each parent and pollination of these by their respective F_1 progeny. For the cross QL27 × TAM422, the F_3 and self of the backcross generations (BCS) were produced by bagging one F_2 and BC head, respectively, for each cross. For the two crosses QL27 × TAM422 (low-OA × high-OA) and Tx2813 × TAM422 (high-OA × high-OA), single-seed descent (SSD) was used to derive recombinant inbred lines (RILs) in the F_5 generation from a random sample of 50 individuals in the F_2 generation ($F_{2:5}$ RILs). These were grown in separate pots and their heads were bagged before anthesis. Two seeds were taken from each F_2 plant to produce two lines of descent. Therefore, 100 $F_{2:5}$ RILs were examined for both crosses.

Cultural conditions and experimental design

The populations were screened for maximum osmotic adjustment to water stress under constant conditions in the controlled environment (CE) facility at the CSIRO Cunningham Laboratory, St. Lucia, Queensland. The environmental conditions provided were: 500 μmol m^{-2} s^{-1} photo-irradiance, 14 h photoperiod, 27°C and 25°C day and night temperature, respectively, and 60–65% and 90–95% day and night relative humidity, respectively (Basnayake et al. 1993).

Plants were grown in plastic-lined, PVC cylinders (0.25-m diameter and 1-m height), each containing 50 kg of air-dried soil. The field capacity of the soil was 19% and the soil pH was 6.5–7.0. Adequate amounts of N, P, K and other micronutrients were applied 3 days before planting and at 21 days after planting to ensure that nutrient supply did not limit growth. In all experiments, pots were kept well-watered until 21 days after sowing, when the soil water content was raised to field capacity. At this point, the first measurements of leaf water status were made on the unstressed plants. Gradual water stress was then imposed by withholding water until the plants were severely stressed (approximately 38–55 days after withholding water).

For the evaluation of the P_1, P_2, F_1 generations, ten plants from each were tested, while 30 plants were tested from the F_2, F_3, BCP_1, BCP_2 and selfed backcross (BCP_1S, BCP_2S) generations. The capacity of the controlled environment room restricted the number of plants that could be screened at any one time. Therefore, the three populations (including P_1, P_2, F_1, F_2, BCP_1, BCP_2 generations from each cross) were tested in three consecutive CE experiments. For each population, two plants were grown in each pot and the pots were re-randomised at weekly intervals throughout the experiment. The two RIL populations were tested in consecutive CE experiments. To evaluate each RIL population, a randomised complete block design, with two replicates, was used. Two plants derived from the same F_2 line were planted in the same pot. The pots were re-randomised within replicates at weekly intervals throughout each experiment. Two CE rooms were used to test each RIL population.

While the genetic material was screened in a series of CE experiments, conditions were controlled to be the same for all experiments. Therefore, it was assumed that the relative expression of osmotic adjustment would be consistent across experiments and results could be compared between experiments.

Measurements

Leaf water potential (ψ), leaf osmotic potential (π), and relative water content (RWC) were measured three times during the stress cycle. The first measurements were taken at the commencement of the stress cycle and the final measurements were taken when one-third of the youngest fully-expanded leaf was necrotic, which approximated when 90% of the leaf area had died. The procedures used to take the measurements were the same as those used by Basnayake et al. (1993). Since the last measurement on osmotic adjustment was taken just before the plants died, it was considered to be the maximum value for each line.

Generation-means analysis

The differences among the means of the six generations, P_1, P_2, F_1, F_2, BCP_1 and BCP_2, for each of the three crosses were analysed by the joint scaling test (Cavalli 1952; Rowe and Alexander 1980; Mather and Jinks 1982) to test the fit of the additive dominance model to the generation means. The midpoint (m), net additive effect [a] and net dominance effect [d] were estimated by weighted least squares

and the predicted generation means were compared to the observed means using a chi-squared test. Broad-sense heritability (H) on an individual plant basis was estimated (Nyquist 1991) from the variance components of the F_2, F_1, and parent generations as

$$H = \frac{\sigma^2_{F_2} - \frac{\left(\sigma^2_{P_1} + \sigma^2_{P_2} + \sigma^2_{F_1}\right)}{3}}{\sigma^2_{F_2}} \quad (1)$$

where, σ^2_{F2}, σ^2_{P1}, σ^2_{P2}, and σ^2_{F1} are the variance components estimated for the F_2, P_1, P_2 and F_1 generations, respectively. Narrow-sense heritability (h^2) on an individual plant basis was estimated from the variance components of the F_2 and backcross generations following the procedure described by Warner (1952),

$$h^2 = \frac{2\sigma^2_{F_2} - \left(\sigma^2_{BCP_1} + \sigma^2_{BCP_1}\right)}{\sigma^2_{F_2}} \quad (2)$$

where, σ^2_{F2}, σ^2_{BCP1}, σ^2_{BCP2} are the variance components for the F_2, BCP_1 and BCP_2 generations, respectively.

Segregation ratios

Segregation ratios within the F_2 and BC generations were investigated. To test simple genetic models, allocation of individuals to genotype classes is difficult where discrete classes are not observed. In many cases, the choice of the boundaries between classes is subjective, resulting in a questionable allocation of individuals to putative genotypic classes. A more objective criterion for allocating individuals to genotypic classes was used in this study. The mixture method of clustering (Basford and McLachlan 1985; McLachlan and Basford 1988) can be applied to a wide range of situations where it is desirable to partition individuals into groups, assuming the population comprises a mixture of overlapping normal distributions. This is often the case in the study of segregation ratios where quantitative data are collected. The mixture method of clustering was used to group the individuals in the segregating generations into putative genotypic classes. This procedure was used for the F_2, BC, F_3, BCS, and $F_{2:5}$ generations. The numbers of individuals allocated to groups by the clustering method were tested for their goodness of fit to the expected segregation ratios for single- and two-gene models by a chi-square test.

Analysis of recombinant inbred lines (RILs)

An analysis of variance was performed on the osmotic adjustment data for the two available $F_{2:5}$ populations (QL27 × TAM422 and Tx2813 × TAM422). Genetic variance was partitioned into among- and within-family components of variance according to the model of Hanson and Weber (1960)

$$y_{ijk} = m + b_k + f_i + (l/f)_{ij} + \varepsilon_{ijk}, \quad (3)$$

where y_{ijk} is the phenotypic observation on line j within family i in replicate k; m is the overall mean; f_i is the effect of F_2-derived family i, assumed to be distributed as N $(0,\sigma^2_f)$; $(l/f)_{ij}$ is the effect of line j within F_2-derived family i, assumed to be distributed as N $(0,\sigma^2_{l/f})$; b_k is the effect of replicate k, assumed to be distributed as N$(0,\sigma^2_b)$; and ε_{ijk} is the random error effect associated with line j within F_2-derived family i and block k, assumed to be distributed as N $(0,\sigma^2_\varepsilon)$. Additive $[\sigma^2_{(A)}]$ and additive-by-additive $[\sigma^2_{(AA)}]$ components of genetic variance were estimated from the among-family (σ^2_f) and within-family $(\sigma^2_{l/f})$ components of variance as described by Hanson and Weber (1960). Heritability on a line-mean basis (h^2_L) in the RIL populations was estimated as

$$h^2_L = \frac{\sigma^2_{(A)}}{\sigma^2_{(A)} + \sigma^2_{(AA)} + \frac{\sigma^2_\varepsilon}{n_r}} \quad (4)$$

where n_r is the number of replicates.

The one- or two-gene models identified from the study of segregation ratios in the F_2 and BC generations of the crosses QL27 × TAM422 and Tx2813 × TAM422 were tested for consistency with the segregation ratios identified in their respective $F_{2:5}$ RIL populations.

Results

Generation-means analysis

There were significant ($P< 0.05$) differences for the expression of osmotic adjustment among the P_1, P_2, F_1, F_2, BCP_1 and BCP_2 generations derived from the cross between Tx2813 (high-OA) and QL27 (low-OA) (Table 1). The joint scaling test indicated that both additive [a] and dominance [d] genetic parameters were significant ($P< 0.01$). The magnitudes of [a] and [d] were similar. The net dominance effect was negative indicating that high osmotic adjustment was inherited in a recessive manner. The three-parameter model (m, [a], [d]) explained the differences among the generation means (χ^2=6.27, P=0.10) suggesting there was no epistasis influencing the differences in generation mean for osmotic adjustment.

There were significant ($P< 0.05$) differences for the expression of osmotic adjustment among the six generations derived from the cross between QL27 (low-OA) and TAM422 (high-OA) (Table 1). The joint scaling test indicated that both additive [a] and dominance [d] genetic parameters were significant ($P< 0.01$). The dominance effect was negative; however, its magnitude was small relative to that of the additive effect. Therefore, there was some directional dominance for osmotic adjustment in the QL27 × TAM422 cross with a slight tendency for recessive expression of high osmotic adjustment. The three-parameter model (m, [a], [d]) explained the differences among the generation means (χ^2=7.03, P=0.07) suggesting that there was no epistasis influencing the differences in generation-mean osmotic adjustment.

There were significant ($P< 0.05$) differences (Table 1) for the expression of osmotic adjustment among the six generations of the cross between Tx2813 (high-OA) and TAM422 (high-OA). The joint scaling test indicated that both additive [a] and dominance [d] genetic parameters were significant ($P< 0.01$). In this cross the dominance effect was again negative but was larger than the additive effect. The three-parameter model (m, [a], [d]) explained the differences among the generation means (χ^2=3.16, P=0.38) suggesting there was no epistasis influencing the differences in generation-mean osmotic adjustment.

Broad-sense heritability on an individual plant basis in the F_2 generation was high for each cross (Table 1). The estimates of narrow-sense heritability differed among the crosses. For the cross Tx2813 × QL27, narrow-sense heritability was high and for the other two crosses, QL27 × TAM422 and Tx2813 × TAM422, it was intermediate. The estimate of heritability on a line-mean basis for the $F_{2:5}$ RIL population derived from the cross QL27 × TAM422 was intermediate and that for the cross Tx2813 × TAM422 was high.

Table 1 Means and standard error of the means for parent (P), F_1, F_2, F_3, backcross (BC) and selfed-backcross (BCS) generations, the broad-sense heritability (H) and narrow-sense heritability (h^2) estimated on a line-mean and individual plant basis for each cross and the genetic parameters and their SE for the joint scaling test for osmotic adjustment measured in the generation means analysis (GMA) and recombinant inbred line (RIL) experiments

Generations/ genetic parameters	Crosses		
	Tx2813 (P_1) × QL27 (P_2)	QL27 (P_2) × TAM422 (P_1)	Tx2813 (P_2) × TAM422 (P_1)
P_1	1.565 ± 0.019	1.672 ± 0.014	1.665 ± 0.019
P_2	0.762 ± 0.036	0.789 ± 0.019	1.600 ± 0.018
F_1	0.792 ± 0.023	1.154 ± 0.018	1.242 ± 0.015
F_2	0.936 ± 0.060	1.120 ± 0.059	1.471 ± 0.046
BCP_1	1.203 ± 0.057	1.269 ± 0.062	1.479 ± 0.037
BCP_2	0.869 ± 0.034	0.936 ± 0.037	1.482 ± 0.038
F_3	[a]	1.216 ± 0.070	[a]
BCP_1S	[a]	1.558 ± 0.053	[a]
BCP_2S	[a]	1.164 ± 0.069	[a]
Heritability (GMA)			
(broad H)	0.933	0.972	0.952
(narrow h^2)	0.769	0.545	0.651
$F_{2:5}$ population mean	[a]	1.129 ± 0.037	1.333 ± 0.030
$F_{2:5}$ variance Component			
Within family [$\sigma^2_{(1/f)}$]	[a]	0.061 ± 0.018	0.035 ± 0.012
Among family[$\sigma^2_{(f)}$]	[a]	0.049 ± 0.021	0.033 ± 0.013
$\sigma^2_{(A)}$	[a]	0.087 ± 0.071	0.065 ± 0.046
$\sigma^2_{(AA)}$	[a]	0.023 ± 0.065	0.004 ± 0.042
σ^2_{ε}	[a]	0.053 ± 0.008	0.043 ± 0.006
Heritability h^2 (RILs)			
Line mean (h^2_L)	[a]	0.640	0.721
Genetic Parameters[b]			
m	1.179** ± 0.018	1.225** ± 0.012	1.637** ± 0.013
[a]	0.384** ± 0.018	0.442** ± 0.012	0.032** ± 0.013
[d]	–0.375** ± 0.030	–0.084** ± 0.021	–0.388** ± 0.020
χ^2	6.273	7.032	3.157
P	0.099	0.071	0.380

** Significant at $P< 0.01$

[a] Generation not measured

[b] Estimates of genetic parameters m, [a], [d] were based on the P1, P_2, F_1, F_2, BCP_1, and BCP_2 generations, P is the probability of obtaining the χ^2 value by chance

Segregation ratios

For the cross between Tx2813 (high-OA) and QL27 (low-OA), the F_2 frequency distribution was positively skewed and the BCP_1 generation showed a bimodal distribution (Fig. 1). Allocation of the individuals in the F_2 (Fig. 1d) and BCP_1 (Fig. 1e) generations into putative genotypic classes using the mixture method of clustering produced a partition in the F_2 generation which fitted a 3:1 segregation (χ^2=0.40, P=0.53) and a partition in the BCP_1 generation which fitted a 1:1 segregation (χ^2=0.13, P=0.72) (Table 2). The variation within BCP_2 (Fig. 1f) was slightly greater than that for the P_1, P_2 and F_1 generations (Fig. 1a, b, c, respectively) but no individuals with the high osmotic adjustment possessed by P_1 (Fig. 1a) were identified. The analysis of the segregation ratios indicated that there was a single gene for the expression of high osmotic adjustment possessed by Tx2813, which had a recessive expression in the segregating generations.

For the cross between QL27 (low-OA) and TAM422 (high-OA), the F_2 frequency distribution showed three distinct genotypic groups and both the BCP_1 and BCP_2 generations showed bimodal distributions (Fig. 2). Allocation of the individuals in the F_2 (Fig. 2d) and BC generations (Fig. 2e, f) into putative genotypic classes using the mixture method of clustering produced a partition in the F_2 which fitted a 1:2:1 segregation (χ^2=0.40, P=0.82) and partitions in the BCP_1 and BCP_2 generations which fitted a 1:1 segregation in both cases (χ^2=0.53, P=0.47 and χ^2=2.13, P=0.14, respectively) (Table 2). This indicated that there was a single gene for the expression of high osmotic adjustment possessed by TAM422, which had an additive expression in the segregating generations. The F_3 and BCS generations showed segregating ratios of 3:2:3 and 5:2:1, respectively (Fig. 3 and Table 2). These observed segregation ratios were consistent with the expected segregation ratios for a single additive gene expression in TAM422.

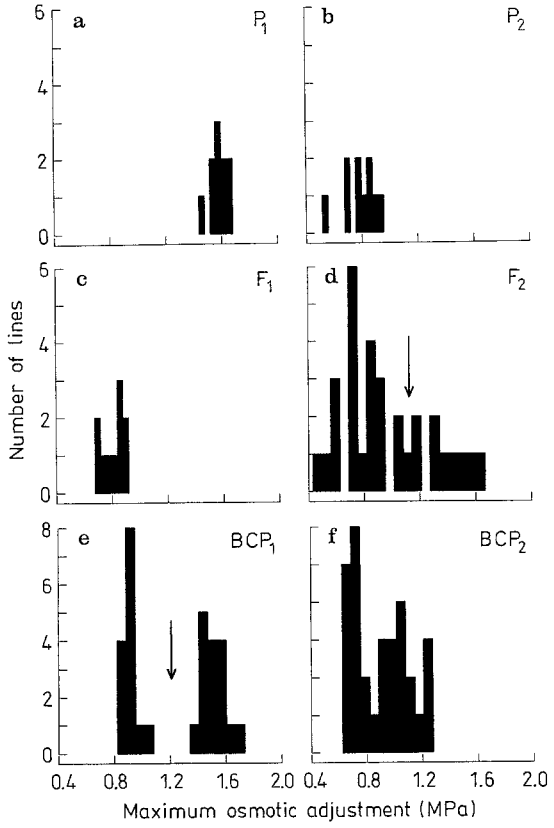

Fig. 1a–f Frequency distribution of osmotic adjustment in six generations derived from the cross Tx2813 × QL27: **a** Parent 1 (Tx2813), **b** Parent 2 (QL27), **c** F_1, **d** F_2, **e** backcross of F_1 to Tx2813, and **f** backcross of F_1 to QL27. *Down arrows* (↓) indicate the approximate boundaries of different classes

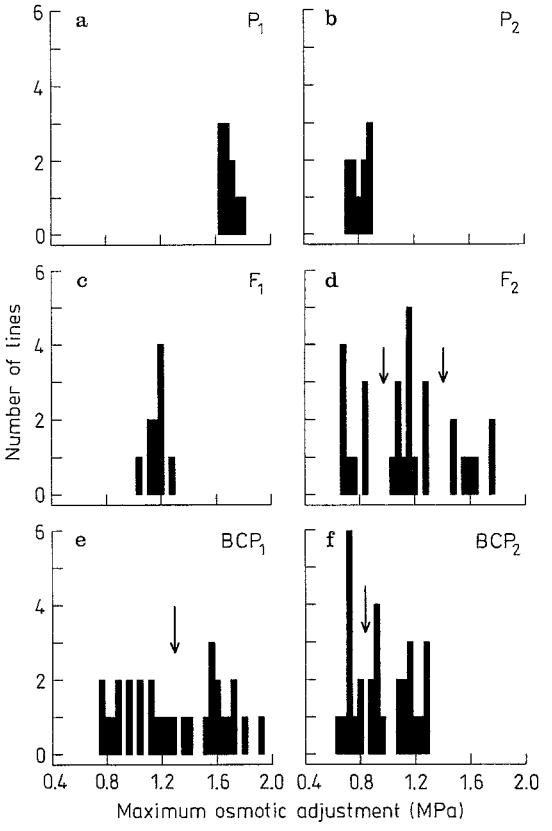

Fig. 2a–f Frequency distribution of osmotic adjustment in six generations derived from the cross QL27 × TAM422: **a** Parent 1 (TAM422), **b** Parent 2 (QL27), **c** F_1, **d** F_2, **e** backcross of F_1 to TAM422, and **f** backcross of F_1 to QL27. *Down arrows* (↓) indicate the approximate boundaries of different classes

Table 2 Summary statistics for evaluation of the goodness of fit of the segregation ratios to one- and two-gene models in the F_2, BCP_1, BCP_2, F_3, BCP_1S and BCP_2S generations derived from crosses between two lines with high osmotic adjustment (Tx2813 and TAM422) and one with low osmotic adjustment (QL27)

Generations	Observed distribution	Expected distribution[a]	Chi-square values
Tx2813 × QL27			
F_2	21:9	3:1	0.40, P= 0.53 ns[c]
BCP_1	14:16	1:1	0.13, P= 0.72 ns
BCP_2	[b]	[b]	[b]
QL27 × TAM422			
F_2	9:14:7	1:2:1	0.40, P= 0.82 ns
BCP_1	17:13	1:1	0.53, P= 0.47 ns
BCP_2	11:19	1:1	2.13, P= 0.14 ns
F_3	14:6:10	3:2:3	1.11, P= 0.57 ns
BCP_1S	3:5:22	1:2:5	1.55, P= 0.46 ns
BCP_2S	18:8:4	5:2:1	0.08, P= 0.96 ns
$F_{2:5}$ (RIL)	57:39	1:1	3.38, P= 0.07 ns
Tx2813 × TAM422			
F_2	2:9:19	3:6:7	5.41, P= 0.07 ns
BCP_1	11:19	1:1	2.13, P= 0.14 ns
BCP_2	6:7:17	1:1:2	0.60, P= 0.74 ns
$F_{2:5}$ (RIL)	22:78	1:3	0.13, P= 0.72 ns

[a] Expected segregation ratios for different generations based on a single- or two-gene model. Ratios are presented in the orders, low: intermediate, low: high, intermediate:high or low:intermediate: high

[b] For a single recessive gene there is only one phenotypic group expected for the BCP_2 generation

[c] P is the probability of exceeding the χ^2 value by chance and ns indicates the χ^2 is non-significant at the 5% probability level

The cross between Tx2813 (high-OA) and TAM422 (high-OA) involved two parents with high osmotic adjustment. The F_2, BCP_1 and BCP_2 frequency distributions were negatively skewed (Fig. 4). Allocation of the individuals in the F_2 (Fig. 4d) and BC (Fig. 4e, f) generations into putative genotypic classes using the mixture method of clustering produced a partition in the F_2 which fitted a 3:6:7 segregation (χ^2=5.41, P=0.07). The partition in the BCP_1 generation fitted a 1:1 segregation (χ^2=2.13, P=0.14), while the BCP_2 fitted a 1:1:2 segregation (χ^2=0.60, P=0.74) (Table 2). These observed segregation ratios were consistent with the expected ratios for two independent genes for high osmotic adjustment, with a recessive gene contributed by Tx2813 and an additive gene contributed by TAM422.

Recombinant inbred populations

The analysis of variance for the two available $F_{2:5}$ RIL populations (QL27 × TAM422 and Tx2813 × TAM422) identified significant (P< 0.01) among- and within-family genetic variation for the two crosses (Table 1). In both populations the estimate of additive variance among the RILs [$\sigma^2_{(A)}$] was much larger than that for additive-by-ad-

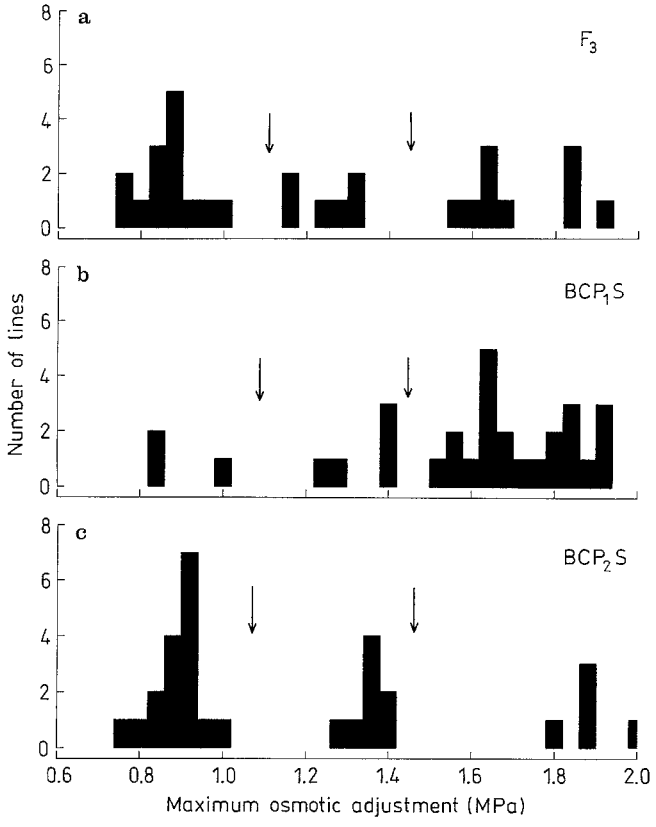

Fig. 3a–c Frequency distribution of osmotic adjustment in three generations derived from the cross QL27 × TAM422: **a** F_3 population, **b** self of backcross of F_1 to TAM422 (BCP_1S), and **c** self of backcross of F_1 to QL27 (BCP_2S). *Down arrows* (↓) indicate the approximate boundaries of different classes

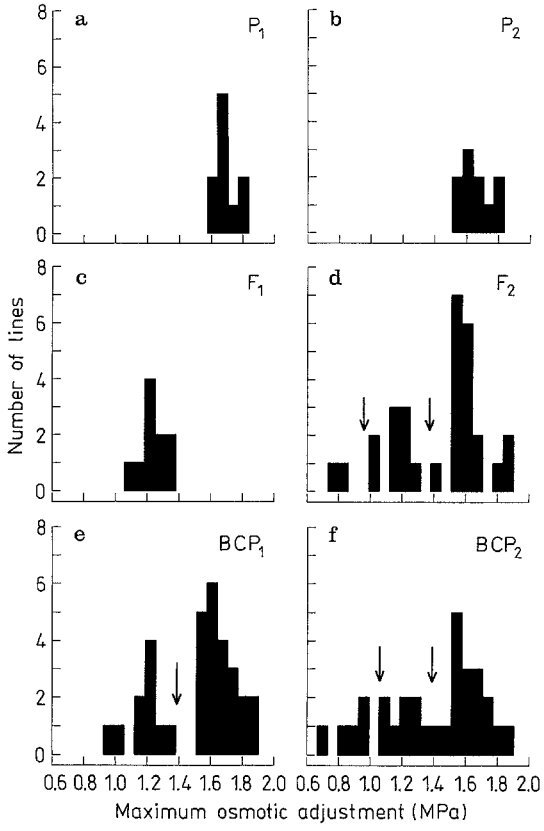

Fig. 4a–f Frequency distribution of osmotic adjustment in six generations derived from the cross Tx2813 × TAM422: **a** Parent 1 (TAM422), **b** Parent 2 (Tx2813), **c** F_1, **d** F_2, **e** backcross of F_1 to TAM422, and **f** backcross of F_1 to Tx2813. *Down arrows* (↓) indicate the approximate boundaries of different classes

ditive epistatic variance [$\sigma^2_{(AA)}$]. In addition, the standard error for the additive-by-additive epistatic components of variance was larger than the estimate of the component for both populations. This suggests it is unlikely that any additive-by-additive epistasis variance was expressed. The frequency distributions for osmotic adjustment in the two RIL populations were bimodal (Fig. 5). Allocation of the lines into putative genotypic classes by the mixture method of clustering identified a partition that fitted a 1:1 segregation ratio for cross QL27 × TAM422 (Fig. 5a) (χ^2=3.38, P=0.07) (Table 2). While there is an expectation of 6.25% heterozygosity in the F_5 generation, the identification of a fit to a 1:1 segregation ratio supports the presence of a single major gene for high osmotic adjustment in the QL27 × TAM422 cross. For the cross between Tx2813 and TAM422 (Fig. 5b), using the truncation point of 1.20 MPa between the high and low osmotic adjustment groups identified a partition which fitted a 1:3 segregation ratio (χ^2=0.13, P=0.72) (Table 2). This was the expected segregation ratio where the recessive (Tx2813) and additive (TAM422) genes were combined. However, further analysis using the mixture method of clustering identified a group comprising of two individuals with higher levels of osmotic adjustment than the mean value of the parents Tx2813 and TAM422. The pedigree records of these two lines identified that they were derived from the same F_2 plant. This provides preliminary information that, in addition to the two major genes identified, there may be minor genes segregating in this cross which influence the expression of osmotic adjustment.

Discussion

Previous genetic analysis (Basnayake et al. 1994) suggested that the two parents with a capacity for high osmotic adjustment (Tx2813 and TAM422) possessed different genes controlling high osmotic adjustment. The results of the current study confirmed this earlier hypothesis. The segregation patterns observed in the generations derived from the biparental crosses indicated that TAM422 possesses a single additive gene and Tx2813 a single recessive gene for high osmotic adjustment.

Analysis of variation among and within the parental, F_1, F_2, and BC generations derived from the three biparental

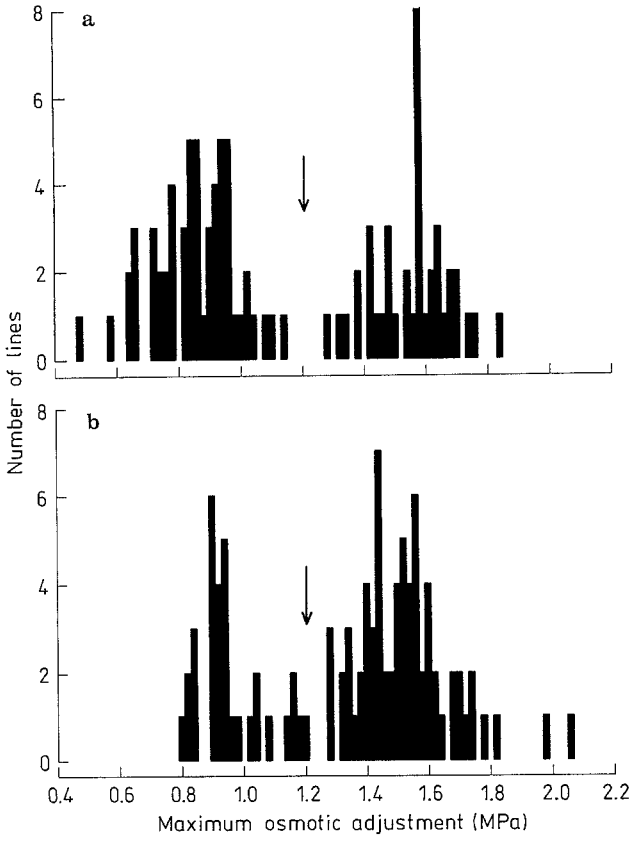

Fig. 5a, b Frequency distribution of osmotic adjustment in two $F_{2:5}$ recombinant inbred populations of crosses QL27 × TAM422 **a** and Tx2813 × TAM422 **b**. *Down arrows* (↓) indicate the approximate boundaries of different classes

crosses indicated that broad-sense heritability on an individual plant basis was high. Therefore, characterisation of the level of osmotic adjustment on an individual plant basis was possible with a high degree of confidence. The high heritability allowed inspection of frequency distributions in segregating generations to test for one- and two-gene models. Inspection suggested that the segregation patterns resulted from a mixture of overlapping genotypic classes due to either one or two genes. The mixture method of clustering provided an objective procedure for allocating the individuals into putative genotypic groups.

The analysis of the segregation ratios within the F_2, BCP_1 and BCP_2 generations for the cross between Tx2813 × QL27 identified a recessive gene for osmotic adjustment possessed by Tx2813. In the backcross generations, the individuals were grouped into two classes and the group means of these classes coincided with those of either their parents or F_1 generations. A similar analysis of the cross between QL27 × TAM422 identified a single gene with predominantly additive expression for high osmotic adjustment possessed by TAM422. In the F_2 generation three genotypic classes, showing a 1:2:1 segregation ratio, were identified. The segregation ratios observed for the F_3, BC, and BCS generations were consistent with a single additive gene for osmotic adjustment in TAM422.

The recessive gene for high osmotic adjustment possessed by Tx2813 is given the symbol *oa1* and the null allele *OA1*. The additive gene for high osmotic adjustment possessed by TAM422 is given the symbol *OA2* and the null allele *oa2*. Using these gene symbols the osmotic adjustment genotypes of Tx2813, TAM422 and QL27 can be denoted as *oa1oa1oa2oa2*, *OA1OA1OA2OA2* and *OA1OA1oa2oa2*, respectively. These three genotypes can explain the segregation ratios observed for the cross between the two parents (Tx2813, TAM422) with high capacity for osmotic adjustment. In the segregating generations, groups with high, intermediate and low osmotic adjustment were identified. The group with low osmotic adjustment would possess the genotypes *OA1_oa2oa2*. The mean osmotic adjustment of these genotypes was similar to that of the low-adjusting parent QL27. The high-osmotic-adjustment group resulted from the genotypes *oa1oa1_ _* and *_ _OA2OA2* and this group had a similar osmotic adjustment to the high parents Tx2813 and TAM422. The intermediate-osmotic-adjustment group resulted from the genotypes *OA1_OA2oa2*. The three genotypic classes (low:intermediate:high) were identified in the F_2 generation (3:6:7) and in the BCP_2 generation (1:1:2). Only the intermediate- and high-osmotic-adjustment classes were identified in the BCP_1 generation (1:1). These were the expected segregation ratios for two independent genes for high osmotic adjustment, where one gene is recessive and the other additive, when either parent possessed only one of the high-osmotic-adjustment genes.

The segregation ratios for the two available $F_{2:5}$ RIL populations were consistent with the gene models proposed from the segregation ratios of the other generations. While a small amount of heterozygosity is expected in the F_5 generation (6.25%), the expected and observed segregation ratio for the cross QL27 × TAM422 was 1:1 (low:high) which can be explained by the two expected homozygous genotypic groups; *OA1OA1oa2oa2* (low osmotic adjustment) and *OA1OA1OA2OA2* (high osmotic adjustment). The expected and observed segregation ratios for the cross Tx2813 × TAM422 were both 1:3 (low:high). These two groups were the result of accumulating four different homozygous genotypes with the genotype *OA1OA1oa2oa2* resulting in low osmotic adjustment and the three genotypes *oa1oa1oa2oa2*, *OA1OA1OA2OA2* and *oa1oa1OA2OA2* resulting in high osmotic adjustment. Analysis of the $F_{2:5}$ progeny from the cross between the two parents with high osmotic adjustment identified two lines derived from the same F_2 plant with osmotic adjustment which was higher than that of both parents. Further analysis of these lines is warranted since they may provide evidence of additional minor genes influencing the expression of osmotic adjustment.

Now that a set of RIL populations with known genotypes for osmotic adjustment have been developed, further experiments can be conducted to test hypotheses about the contribution of the high-osmotic-adjustment genes to grain yield under a wide range of water-stress conditions.

Acknowledgements The financial support of the Australian Rural Industry Research and Development Corporation is acknowledged.

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