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Molecular Tagging of Drought Resistance in Wheat: Osmotic Adjustment and Plant Productivity

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Molecular Tagging of Drought Resistance in Wheat: Osmotic Adjustment and Plant Productivity.

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Abstract (one page maximum, single spaced), include:

List the original objectives, as defined in the approved proposal, and any revisions made at the beginning or during the course of project.

Background to the topic.

Major conclusions, solutions, achievements.

Implications, both scientific and agricultural.

Achievements

Significance of main scientific achievements or innovations

Drought stress is a major limitation to bread wheat (*Triticum aestivum L.*) productivity and its yield stability in arid and semi-arid regions of world including parts of Israel and the U.S. Currently, breeding for sustained yields under drought stress is totally dependent on the use of yield and several key physiological attributes as selection indices. The attempt to identify the optimal genotype by evaluating the phenotype is undermining progress in such breeding programs.

Osmotic adjustment (OA) is an effective drought resistance mechanism in many crop plants. Evidence exists that there is a genetic variation for OA in wheat and that high OA capacity supports wheat yields under drought stress. The major objective of this research was to identify molecular markers (RFLPs, restriction fragment length polymorphisms; and AFLPs, amplified fragment length polymorphisms) linked to OA as a major attribute of drought resistance in wheat and thus to facilitate marker-assisted selection for drought resistance.

We identified high and low OA lines of wheat and from their cross developed recombinant inbred lines (RILs) used in the molecular tagging of OA in relation to drought resistance in terms of plant production under stress.

The significant positive co-segregation of OA, plant water status and yield under stress in this RIL population provided strong support for the important role of OA as a drought resistance mechanism sustaining wheat production under drought stress. This evidence was obtained in addition to the initial study of parental materials for constructing this RIL population, which also gave evidence for a strong correlation between OA and grain yield under stress. This research therefore,provides conclusive evidence on the important role of OA in sustaining wheat yield under drought stress.

The measurement of OA is difficult and the selection for drought resistance by the phenotypic expression of OA is practically impossible. This research provided information on the genetic basis of OA in wheat in relations to yield under stress. It provided the basic information to indicate that molecular marker assisted selection for OA in wheat is possible.

The RIL population has been created by a cross between two agronmic spring wheat lines and the high OA recombinants in this population presented very high OA values,

not commonly observed in wheat. These recombinants are therefore an immediate valuable genetic recourse for breeding well-adapted drought resistant wheat in Texas and Israel.

We feel that this work taken as a whole eliminate the few previous speculated doubts about the practical role of OA as an important mechanism of drought resistance in economic crop plants. As such it should open the way, in terms of both concept and the use of marker assisted selection, for improving drought resistance in wheat by deploying high osmotic adjustment.

Agricultural and/or economic impacts of the research findings

Not known yet

Details of cooperation:

Cooperation in performing this research was very close and well synchronized. Its basis was twofold: (a) a close personal relationship and professional compatibility between the two main PI's, and (2) the interface of two domains of expertise – crop stress physiology and agronomy on one hand and plant molecular genetics on the other. Furthermore the corresponding facilities and expertise of the two PI's allowed a very clear-cut division of the areas of responsibility in carrying out this research project. Crop stress physiology, agronomy and field studies were performed in Israel where facilities and climate were compatible with the task. Molecular genetics work was done in the excellent laboratory in Texas.

List of Publications:

Blum A., Zhang J.X. and Nguyen H.T. 1999. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crops Res. 64:287-291.

Appendix

Table of Contents:

Title	Status
Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production.	Published
Molecular tagging of osmotic adjustment as a drought resistance mechanism in wheat. I. Phenotypic expression of drought resistance	To be submitted
Molecular tagging of osmotic adjustment as a drought resistance mechanism in wheat. II. Molecular markers conferring osmotic adjustment.	To be submitted

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Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production

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Abstract

Osmotic adjustment (OA) is generally considered an important component of drought resistance. Several reports by J.M. Morgan [Morgan, J.M., 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Aust. J. Agric. Res. 34, 607–614; 1992. Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. Aust. J. Plant Physiol. 19, 67–76; 1995. Growth and yield of wheat lines with differing osmoregulative capacity at high soil water deficit in seasons of varying evaporative demand. Field Crops Res. 40, 143–152; Morgan, J.M., Condon, A.G., 1986. Water-use, grain yield and osmoregulation in wheat. Aust. J. Plant Physiol. 13, 523–532] from Australia concluded that consistent genetic differences in OA existed among wheat cultivars and that high OA cultivars tended to yield better than low OA cultivars under drought stress. Our study was performed to assess his results with his and other genetic materials.

Two of Morgan's spring wheat lines with high OA ('H.Osm-134') and low OA ('L.Osm-136') capacity in addition to eight other diverse spring wheat cultivars were tested for OA and plant production when grown in small plots under a rain exclusion shelter at Bet Dagan, Israel in 1996. OA of five of these cultivars (including Morgan's lines) was also measured in two independent greenhouse tests in 1997 (Israel) and 1998 (Texas).

The five cultivars differed significantly and ranked consistently for OA in all tests. No significant cultivar by test interaction for OA was revealed. OA was well correlated across cultivars between tests. The significantly higher OA capacity of H.Osm-134 as compared with L.Osm-136 was repeated in all tests. OA of all ten cultivars was positively correlated with biomass ($r = 0.73$; $p = 0.02$) and yield ($r = 0.55$; $p = 0.09$) under pre-flowering drought stress in the rain exclusion shelter. H.Osm 134 line performed significantly ($p \leq 0.05$) better than L.Osm 136 line for both biomass and yield under drought stress. We therefore support Morgan's results and conclude that consistent differences in OA exist among wheat cultivars and that these differences can be associated with plant production under pre-flowering drought stress.

Keywords: Wheat; Drought resistance; Osmoregulation; Osmotic Adjustment; Yield

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1. Introduction

Osmotic adjustment (OA) is generally considered an important component of drought resistance (e.g. Ludlow and Muchow, 1990). Morgan (1983, 1992, 1995) and Morgan and Condon (1986) demonstrated that consistent genetic differences in OA existed among wheat cultivars and that high OA cultivars perform better than low OA cultivars under drought stress. Despite this evidence, arguments were occasionally raised verbally to the effect that these results were limited to the genetic materials and the test conditions used in these experiments.

The study reported herein was performed to assess Morgan's results in order to ascertain whether indeed consistent and repeatable differences may exist in OA among wheat cultivars and whether indeed OA may be associated with wheat production under drought stress.

2. Materials and methods

Two of Morgan's spring wheat (Triticum aestivum L.) lines representing high ('H.Osm-134') and low ('L.Osm-136') OA capacity were selected for study, in addition to eight diverse spring wheat cultivars (Table 1). These ten cultivars were tested for OA and plant production under a rain exclusion shelter in 1996. A selected sub-sample of five cultivars (including Morgan's lines) was tested for OA under greenhouse conditions also in 1997 and 1998.

The rain exclusion study was performed in 1996 at Bet Dagan in the coastal region of Israel. The shelter consisted of a large greenhouse structure. All walls consisted of strong PVC curtains, which could be rolled up or down according to weather. Soil under the shelter was dry to a depth of at least 1.8 m, at planting. Ten cultivars (Table 1) were planted on 5 December, 1995 into fully fertilized soil in a split plot design. Main treatments were stress and non-stress (controls) and sub-treatments were cultivars, in three replications. Each experimental plot consisted of five rows 1.5 m long and 15 cm apart. Overhead low-capacity sprinkler irrigation allowed all the experiment or only the controls to be irrigated. Plants were germinated by irrigation, which continued at weekly intervals as needed. When plants reached the full tillering growth stage (Feekes stage 3), irrigation was discontinued in the stress treatment, until OA measurements were performed, after which irrigation was resumed as in the control. Upon maturity three central rows 1 m in length in each plot were harvested to determine total above-ground biomass (dry weight) and grain yield per plot.

The methods used for OA measurement are discussed in detail elsewhere (Chandra Babu et al., 1999). Briefly, OA was measured in the rain exclusion experiment by the 'rehydration method'. Periodic measurements of midday relative water content (RWC) (Barrs

Table 1
Osmotic adjustment (OA) (in MPa) of diverse wheat cultivars as measured in 1996 (Bet Dagan), 1997 (Bet Dagan) and 1998 (Texas). In all tests measurements were performed by the dehydration method and in 1997 also by Morgan s method

Cultivar	Origin	1996	1997		1998
			Morgan	Rehydration	
K3	Israel	0.56	1.25	0.90	1.35
H.Osm.-134	Australia	0.63	0.74	0.64	1.12
Chinese Spring	USA	0.49	0.31	0.23	0.77
Chenab-70	CIMMYT	0.47	0.27	0.36	0.91
L.Osm.-136	Australia	0.39	0.14	0.16	0.63
Gutha	Australia	0.61	–	–	–
Nirit	Israel	0.59	–	–	–
Attila	CIMMYT	0.58	–	–	–
V52	Israel	0.57	–	–	–
Nesser	CIMMYT	0.53	–	–	–
LSD ($p \leq 0.05$)		0.11	0.52	0.22	0.21

and Weatherley, 1962) were made as stress developed. In each cultivar uppermost fully expanded leaves were sampled for OA determination when their RWC was between 65 and 70%, which was 38 days (on average) after the last irrigation. Leaves were then detached, wrapped in a PVC sleeve, immediately placed with their cut end in water and then placed at 10°C for a 12 h rehydration period. Previous tests performed with rice (Chandra Babu et al., 1999) and wheat (Blum, unpublished) showed that the duration of this rehydration period of up to 12 h did not affect OA estimates. Similar leaves were sampled from the control plots and treated as detailed above. Upon rehydration the central part of each leaf was detached and immediately frozen, thawed and measured for osmotic potential (OP) by the psychrometric method, using the Decagon (Pullman, WA) thermocouple psychrometer. OA was calculated as the difference in OP between rehydrated stressed and controls leaves.

The 1997 and 1998 studies for the determination of OA were both performed under similar protocols with potted plants of five cultivars (Table 1) grown in the greenhouse, at Bet Dagan Israel and Lubbock Texas, respectively. Three (in 1997) or four (in 1998) plants were germinated from seed in 18 l pots containing a pre-fertilized potting mixture of high water-holding capacity. At least five pots (replicates) per cultivar were used. Plant were grown under full irrigation until the early jointing growth stage (Feekes stage 4) when drought stress was applied for a mean period (depending on cultivar) of 29 and 34 days in 1997 and 1998, respectively. RWC was measured periodically as stress progressed. Once plants of a given cultivar were at 65 to 70% RWC the pots were irrigated in the evening and the rehydrated leaves were sampled and frozen next morning for OP determination. OP was also measured in freeze-thawed non-stressed leaves that were sampled immediately after the last irrigation before stress commenced. OA was calculated as the difference in OP of rehydrated leaves that were sampled before and after stress. This protocol is based on the early work of Jones and Turner (1978) and it is further elaborated by Chandra Babu et al. (1999).

In 1997 OA was also measured according to Morgan's method (Morgan, 1992), where consecutive daily measurements of RWC and OP were made during the stress cycle to a RWC of at least 70%. OA estimates were derived from the regressions of RWC on OP in each replicate. For this measurement five additional pots per cultivar were established.

3. Results and discussion

In all tests, OA was measured at about the same plant water stress, namely RWC of 65 to 70%, and after a prolonged period of water stress (>28 days on average). In each of the four tests cultivars differed significantly ($p \leq 0.05$) for OA (Table 1). For the five cultivars present in all four tests no significant test by cultivar interaction in OA was revealed. The relative OA capacity of cultivars was consistent across tests, as can be seen in the correlations presented in Table 2. Spearman rank correlation across cultivars between tests gave very similar results (not shown). The consistent and repeatable superiority of H.Osm-134 over L.Osm-136 in OA as reported by Morgan (1983) and Morgan and Condon (1986) is confirmed here (Table 1). Cultivar K3 ranked consistently high for OA while Chenab-70 and Chinese Spring were relatively low in this respect.

We ascribe at least partially, the consistent results for OA as observed here to the measurement of OA at a sufficient and a similar rate of leaf water deficit in all cultivars.

Grain yield and biomass data for the rain exclusion shelter study are presented here on a per plot basis because yield or biomass calculated from a small plot into a per hectare basis is usually biased upwards. The importance of yield and biomass data in this experiment is in their relative and associated values with respect to cultivars and OA capacity. Biomass of cultivars ranged significantly ($p \leq 0.05$) from 1.51 to 2.27 kg/plot in the controls and from 0.86 to 1.71 kg/plot under drought stress. Grain yield ranged

Table 2
Correlations (r) across 5 cultivars between osmotic adjustment values in four different assays

	1996	1997 (Morgan)	1997 (Rehydration)
1997 (Morgan)	0.74		
1997 (Rehydration)	0.79*	0.98**	
1998	0.81*	0.96**	0.99**

* and **Correlation coefficient (r) significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

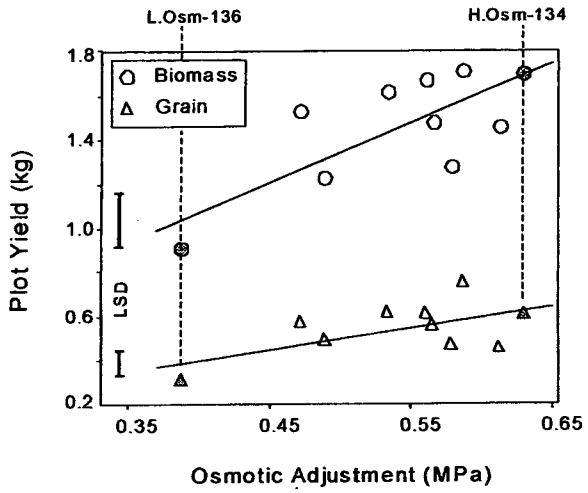

Fig. 1. The linear regression of biomass and grain yield under pre-flowering stress conditions on osmotic adjustment under the same conditions over 10 wheat cultivars tested in a rain exclusion shelter at Bet Dagan in 1996. For biomass $y = 0.025 + 2.64x$; $R_2 = 0.53$. For yield $y = 0.06 + 0.90x$; $R_2 = 0.31$. Vertical bars represent LSD at $p \leq 0.05$ for biomass and yield.

significantly ($p \leq 0.05$) from 0.50 to 0.78 kg/plot in the controls and from 0.32 to 0.76 kg/plot under drought stress. There were significant treatment by cultivar interactions for biomass ($p = 0.006$) and yield ($p = 0.050$) in the rain exclusion shelter test in 1996.

OA as measured in that test was not correlated across cultivars with either biomass ($r = 0.15$) or yield ($r = 0.14$) under irrigated conditions (data not shown). OA was positively correlated across cultivars with biomass ($r = 0.73$; $p = 0.02$) and yield ($r = 0.55$; $p = 0.09$) under drought stress conditions (Fig. 1). It could be expected that the correlation between OA and yield would be smaller than the correlation with biomass, whereas yield is a more complex trait than biomass and because stress occurred pre-flowering while grain development was free of stress. From the LSD estimate for biomass and the regression equation as presented in Fig. 1 it can be deduced that about 0.1 MPa is the minimal effective increase in OA that would affect biomass when OA is above 0.35 MPa.

OA was specifically and positively associated with plant production under drought stress but not with plant production under irrigated conditions. Morgan's line H.Osm-134 performed significantly ($p \leq 0.05$) better than line L.Osm-136 for both biomass and yield under drought stress (Fig. 1), concurrently with their respective levels of OA.

The association between OA and plant production under drought stress is not to be expected as a universal occurrence. Under the conditions of this rain exclusion shelter plants did not have any deep soil moisture reserve when irrigation was stopped. Where such deep soil moisture reserves are available, the variation in plant production under drought stress may be controlled mainly by variation among cultivars in deep root development and deep soil moisture extraction which may override any effect of OA capacity (e.g. Blum et al., 1999). Alternatively, when drought stress develops after flowering, grain yield may be controlled largely by stem reserve mobilization and not necessarily by OA capacity during that time.

Our results were derived from test conditions that ascribe an advantage to OA capacity. They lend full support to results presented by Morgan, showing that wheat cultivars can differ consistently for OA and that such differences among cultivars can be associated with differences in plant production under pre-flowering drought stress. It is therefore concluded that OA can be an important component of drought resistance in wheat within a relevant environmental context.

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Molecular tagging of osmotic adjustment as a drought resistance mechanism in wheat. I. Phenotypic expression of drought resistance.

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ABSTRACT

Osmotic adjustment (OA) is a major mechanism of drought resistance shown to be associated with sustained yield under drought stress in many crops. Despite its recognized importance OA has not been improved by direct phenotypic selection in breeding programs because it requires a tedious protocol unsuitable for selection in large populations. Molecular markers such as RFLP provide a powerful potential technology to enable efficient selection for OA. The objective of this research was to identify molecular markers (RFLP and AFLP) linked to OA as a major attribute of drought resistance in bread wheat (*Triticum aestivum*).

The first part in this series was performed to establish the genetic variation for and the role of OA in a recombinant inbred lines population (RILs) in relation to field performance under drought stress. 150 RILs were developed by the single seed descent method from a cross between high OA (K3) and low OA (Chenab-70) lines. F_7 Lines were measured for OA in the greenhouse (defined hereon as OA capacity). 98 lines and their parents were grown under Mediterranean type drought stress in the field as compared with fully irrigated controls. Grain yield was measured in two years [1999 (F_8) and 2000 (F_9)] while biomass, phenology and plant water status were measured in one year (1999).

Variation in OA capacity was large, ranging from 0.02 MPa to 1.0 MPa. Mean grain yield under stress was 51.7% and 63.0% of that in the controls in 1999 and 2000 when total rainfall was 289 mm and 485 mm, respectively. A significant ($p<0.01$) positive regression of yield under stress on OA capacity was found across RILs in both years ($R^2=0.39$ and $R^2=0.29$, respectively). No such associations were seen for yield under irrigation. RILs varied significantly for flowering date and contrary to expectations later flowering RILs tended to yield relatively better under stress. RILS varied significantly for midday canopy temperature and relative water content (RWC) under stress and later flowering RILS tended to be less stressed in terms of having lower canopy temperature,

higher RWC and higher OA capacity. Multiple regression analysis indicated that 46% of the variation in RILs yield under stress was accounted for by OA capacity and canopy temperature, whereas day to heading had no significant contribution in this respect. The results are interpreted to indicate that OA may have been genetically or developmentally associated with late flowering in this population and that assumed deeper roots in later flowering RILs may have helped to sustain better plant water status and grain yield under stress.

INTRODUCTION

Drought stress is a major limitation to wheat productivity and yield stability in arid and semi-arid regions of the world. Significant developments were achieved in understanding of the physiology of drought resistance and in developing physiological screening techniques for drought resistance (Blum, 1988; Ludlow and Muchow, 1990; Fukai and Cooper, 1995; Boyer, 1996; Turner, 1986, 1997; Bruce et al., 2002).

While disagreement and even confusion may characterize some of the discussions on what constitutes a significant and an effective mechanism of drought resistance in crop plants, osmotic adjustment (OA) is receiving an increasing recognition as a major mechanism. The importance of OA in drought resistance could not be an evolutionary coincidence, since it also constitutes an important component of tolerance to freezing (Olien and Smith, 1981) and salinity (Bohnert et al., 1995) stresses - both of which involve a component of osmotic stress.

Wheat has a moderate capacity for OA, as compared with extreme cases such as sorghum (high) on one hand and cowpeas (low) on the other. There is repeated evidence on significant genetic variation for OA within wheat (Morgan, 1977; Johnson *et al.*, 1984; Gupta and Berkowitz, 1987; Schonfeld et al., 1988; Blum and Pnuel, 1990). Morgan (1983) performed direct selection for OA in wheat and was able to show that (a) the improvement of OA by direct selection is experimentally possible; and that (b) OA in his tested cultivar was controlled by a single recessive gene most likely on chromosome 7A (Morgan, 1983, 1984 and 1991; Morgan and Tan, 1996).

Briefly (see reviews by Zhang et al., 1999 and Blum, 1988), OA allows plants to retain higher turgor at a given level of plant water deficit and subsequently support carbon assimilation and growth under stress; OA support extended root growth when soil moisture became limited; OA is involved in protecting the functional integrity of cellular membranes and proteins; OA is important in supporting tiller survival and recovery after drought stress; and assimilates used for cellular osmotic adjustment during stress may be partially used for re-growth upon recovery.

Consequently, OA has been consistently found to be associated with sustained yields under drought stress in peas (Rodriguez-Maribona *et al.*, 1992), chickpea (Morgan *et al.*, 1991), Pigeon pea (Subbarao et al., 2000), Brassica (Kumar *et al.*, 1984; Wright et al., 1997), sorghum (Wright *et al.*, 1983; Ludlow *et al.*, 1990; Santamaria *et al.*, 1990), sunflower (Chimenti et al., 2002), barley (Gonzalez et al., 1999) and wheat (Morgan

and Condon, 1986; Morgan *et al.*, 1986; Blum and Pnuel, 1990; Morgan, 1995; Ali et al., 1999; Blum et al., 1999).

Despite its importance, the capacity for high OA has not been readily applied in practical breeding programs for drought stress conditions, mainly because of serious methodological constraints. Firstly, since OA is proportional to the intensity of water stress (*e.g.* Ludlow and Muchow, 1990), its measurement in different genotypes must be performed when all plants are subjected to the same rate of dehydration and plant water deficit. This involves a complicated and time-consuming protocol. Secondly, measuring OA by the current psychrometric or other alternative methods is too slow for the routine selection work. Therefore, the selection for improved OA by measuring the phenotype is tedious and may be considered impractical by most breeders.

Molecular markers such as RFLP provide a powerful approach to tag major genes and quantitative trait loci (QTL). After molecular markers for OA are identified, screening for these markers can be used to improve the selection efficiency for OA.

The objective of this research was to identify molecular markers (RFLP, restriction fragment length polymorphism; and AFLP, amplified fragment length polymorphism) linked to OA as a major attribute of drought resistance in bread wheat (*Triticum aestivum*) and to demonstrate a relationship to QTLs controlling plant productivity under stress.

MATERIALS AND METHODS

Plant population

Plant material used in these studies was a recombinant inbred lines (RIL) population developed by the single-seed-descent (SSD) method from a cross between two wheat cultivars: K3 (Novosteponkaya/SieteCerros) and Chenab-70 (C271/WLT//SN64). These two cultivars were previously found (Blum et al., 1999) to differ in osmotic adjustment and yield under stress, with K3 being better in this respect than Chenab-70. Mean OA over 4 independent tests was 1.01 MPa and 0.50 Mpa for K3 and Chenab-70, respectively. 94 RIL were randomly selected for OA measurement and 98 lines were randomly selected for field trials.

Measurement of osmotic adjustment

94 RILs and their parent were measured for OA. Methods are detailed in part II of this report.

Field Trials

98 RILs were tested in 1999 (F_8) and 2000 (F_9) at Bet Dagan field station located on the Coastal Plain of Israel. In both years the same experimental design and plot size were used. The crop was managed for optimum growth, including fertilization, weed and disease control. Trials were laid out in a randomized split-plot design with two replications where main plots were water regime treatments and sub-plots were RILs. Plots were drill-planted at 15 cm row spacing. Plot size was 1.4 by 3 m. Rainfall was lower than normal in both years, especially in 1999 (Table 1). The trial was planted on Dec 27, 1998 which was a normal planting date and later than normal on Feb 22, 2000, in order to assure drought stress at least during the latter part of the crop cycle. Water regime treatments consisted of dryland (only rainfall) and well-watered conditions where total calculated crop evapotranspiration was replenished by sprinkler irrigation every 10-14 days, until physiological maturity.

In both years grain yield was determined by harvesting the central area (2.4 m^2) of each plot. In 1999 the following additional data were collected for each plot. Heading date, plant height at maturity, biomass as total aboveground dry matter per 2.4 m^2. Harvest index was calculated as the ratio of grain yield to total biomass. Midday canopy temperature as a measure of plant water stress (Blum et al., 1982) was measured with an infrared thermometer in each plot as plant water deficit symptoms

appeared during late tillering to the late boot growth stage. Only the last measurement taken under the more sever stress during April 1999 is reported here.

Broad-sense heritability for OA was computed as $h^2 = \delta^2_g/(\delta^2_g + \delta^2_e/n)$ where δ^2_g and δ^2_e were the estimates of genetic and residual variances, respectively, derived from the expected mean squares of the analysis of variance and n was the number of replications.

RESULTS AND DISCUSSION

Osmotic adjustment

For 94 F_7 RILs assayed in the greenhouse at a relative water content (RWC) of 60% to 65% mean OA was 0.43 MPa, ranging from 0.02 MPa to 1.0 MPa. Distribution of RILs for OA (Fig.1) was not normal according to Shapiro-Wilk W-test. Differences among RILS in OA were very significant ($F= 0.24$; $p<0.01$). As expected, the variation for OA in this population was high, representing a wide range of values that can be found in common wheat (*e.g.* Morgan, 1977; Blum et al., 1999). Broad-sense heritability for OA was high, estimated at 0.93.

Grain yield and biomass

In 1999 mean grain yield of 98 F_8 RILs under dryland conditions (Table 2) was 309 g m-2, which was 51.7% of mean yield under irrigation. Dryland yield of all RILs ranged from 70 to 689 g m-2. Yield differences were significant ($p<0.01$). Dryland distribution of the various RILs (Fig.2) was normal according to Shapiro-Wilk W-test. Parental dryland yield differed significantly ($p<0.01$) ranging from 181 to 510 g m-2 in Chenab-70 and K3, respectively. Chenab-70 was the low OA parent while K3 was the high OA parent (Blum et al., 1999). Grain yield under irrigation was about two-fold of that under dryland conditions. Grain yield distribution under irrigated conditions was not normal according to Shapiro-Wilk W-test. Grain yield under irrigation was lower in Chenab-70 than in K3. However, yield under dryland as a percent of that under irrigated conditions was 38.8% and 68.7% in Chenab-70 and K3, respectively. Hence, Chenb-70 was more drought susceptible than K3 in terms of yield and rate of yield reduction under stress.

Mean dryland biomass of RILs in 1999 (Table 2) was 1010 g m-2, which was 63.0% of mean biomass under irrigation. For the different RILs dryland biomass ranged from 258 to 2047 g m-2. Its distribution in the various RILs (Fig.2) was

normal according to Shapiro-Wilk W-test. Parental dryland biomass differed significantly ($p<0.01$), ranging from 484 to 1433 g m-2 in Chenab-70 and K3, respectively.

In 2000 mean grain yield of 98 RILs under dryland conditions (Table 2) was 353 g m-2, which was 62.0% of mean yield under irrigation. Dryland yield of all RILs ranged from 108 to 503 g m-2. Yield differences were significant ($p<0.01$). Dryland yield distribution of the various RILs (Fig.3) was normal according to Shapiro-Wilk W-test. Parental dryland yield differed significantly ($p<0.01$) ranging from 480 to 820 g m-2 in Chenab-70 and K3, respectively. Grain yield distribution under irrigated conditions was not normal according to Shapiro-Wilk W-test. Dryland yield was 321 and 354 g m-2 in Chenab-70 and K3, respectively. Irrigated yield was 771 and 536 g m-2, respectively. Dryland yield as percent of irrigated yield was 41.6% and 66.2% in Chenab-70 and K3, respectively, confirming 1999 results regarding the relatively greater drought resistance of K3 in terms of yield response to stress.

Mean yield under stress was lower in 1999 than in 2000 (Table 2), corresponding to the total seasonal rainfall in the two years, respectively (Table 1). A reduction of mean yield under stress to 51.7% and 62.0% of that under non-stress conditions also represent the respective difference in rainfall between 1999 and 2000.

The level of yield reduction caused by the dryland conditions in these trials can be considered to represent an appreciable drought stress pressure.

Phenology and plant stress (1999 trial)

Mean RILs number of days from emergence to heading under stress was 70.5 (Table 2). It was shorter by 1.2 days as compared with the mean under irrigated conditions. However, the range for days to heading under dryland minus days to heading under irrigation over all RILs was variable and ranged from –6 days to 6 days. Therefore, when compared with non-stress conditions some RILs flowered earlier under stress while others flowered later, as can be seen also for the two parental lines. There was a significant though low negative correlation across all RILs between days to heading under non-stress conditions and days under stress minus days under irrigation ($r=-0.48$; $p<0.01$) indicating a tendency for late flowering lines to advance in flowering under stress while the very early lines tended to delay

flowering time under stress. This could also be seen in Chenab-70 and K3 where the later cultivar tended to advance flowering under stress by 6 days.

Most cereals (*e.g.* rice, sorghum) express a distinct delay in flowering when subjected to drought stress pre-flowering. Wheat has long been shown to be variable in this respect (Angus and Moncur, 1977), with no good explanation. This study appears to underline the dilemma.

Plant water deficit under dryland conditions as expressed by midday RWC measured at the boot stage averaged 83.8% over 20 randomly selected RILs. RILs differed significantly ($p<0.01$) in this respect, ranging from 74.3% to 93.4% (Table 2). This is a large difference, ranging from near wilting (RWC of around 70%) to almost full turgor (around 98%). Such variation could be easily recognized in the field by symptoms of water stress and leaf rolling, as seen in Fig.4.

Canopy temperature as a measure of plant water deficit ranged significantly ($p<0.01$) from 28.6^0 to 30.8^0 over all RILs (Table 2). It was higher in Chenab-70 than in K3. This difference between the two parental lines corresponds to their relative drought resistance in terms of yield, whereas the more resistant parent had lower canopy temperature.

Associations among traits

Grain yield under drought stress but not under irrigated conditions was positively associated with OA across 98 RILS in both years (Fig.5). The regression of dryland yield on OA in both years was significant, but OA could not explain more than 39% of yield variations among RILs in both years. Factors other than OA were apparently also important in determining yield variation in this population under stress. Possible effects of variations among RILs in root depth and soil moisture extraction cannot be not be overruled (see below). OA was also significantly correlated with dryland biomass ($r=0.54$; $P<0.01$) but not with irrigated biomass ($r=0.23$; $p>0.05$) in 1999.

The correlation matrix across 98 RILs for the main traits measured in 1999 and including OA is presented in Table 3. Grain yield under stress was positively correlated with days to heading, indicating a yield advantage to late flowering genotypes in this trial. This was not the case for yield under irrigation, where no correlation with days to heading was in evidence. Days to heading under stress was negatively correlated with canopy temperature and positively correlated with OA and RWC. Hence, the later flowering RILs that had relatively higher yield under stress

also had relatively high OA and better plant water status under stress. Canopy temperature was also negatively correlated with grain yield under drought stress and biomass under stress and nonstress conditions. The relative importance of traits in affecting yield under stress is reflected also in the multiple regression of dryland yield on OA, canopy temperature and days to heading (Table 4). This analysis indicated that OA (and to a lesser extent canopy temperature) account for the greater part of the variation in yield under stress in this population while days to heading has a minor and a non-significant effect in this respect.

It is almost a consensus (Blum, 1988) that under Mediterranean dryland conditions typical of this study early flowering genotypes have a relative yield advantage under stress because they escape the increasing water shortage developing during the latter part of the growing season (Table 1). In this study the opposite was seen. Grain yield under stress was associated with later flowering. The analysis of the associations among traits support the assumption that late flowering RILs had a relative yield advantage under stress because of their better water status. This was seen in terms of the lower canopy temperatures and the higher RWC in the later flowering RILs. One reason for the better water status of the later flowering RILs could be in their relatively better osmotic adjustment. Another reason could be in that the later flowering RILs may have had a relatively larger root system which allowed continued soil moisture extraction from deep soil, as compared with early flowering RILs. Having larger biomass, later flowering RILS may be suspected to have also a large root. Further analysis may perhaps indicate if the associations between phenology, OA and plant water status under stress arise from a genetic basis or if it is derived from developmental-physiological associations. Studies with sorghum lines isogenic for maturity genes that control flowering time demonstrated a pleiotropic effect of maturity genes on root size, as root size and depth increased in late flowering genotyps (Blum et al., 1977). In wheat, the vernalization requirement gene Vrn1 that may be present in late flowering cultivars, was found to carry a peiotropic effect on sugar accumulation (Galiba et al., 1997), which may enhance OA (*e.g.* Kameli and Losel, 1995; Bajji et al., 2001). The genetic analysis performed in the second part of this research should shed light on some of these issues.

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Table 1. Rainfall received at Bet Dagan during 1999 and 2000

Year	Oct-Nov	Dec	Jan	Feb	Mar	April	May-Sept	Total
1998/99	8.6	43.7	153.9	51.5	16.4	14.5	0	289
1999/00	24.0	32.2	340.3	42.2	46.4	0.1	0	485

Table 2. Grain yield, biomass, phenology, canopy temperature and relative water content in 98 RILs and their parental lines tested at Bet Dagan.

Variable	Units	Mean of RILs	Range of RILs	Chenab-70	K3
Dryland grain yield (1999)	$g\ m^{-2}$	309	70 to 689	181	510
Irrigated grain yield (1999)	$g\ m^{-2}$	612	205 to 794	474	727
Dryland biomass (1999)	$g\ m^{-2}$	1010	258 to 2047	484	1433
Irrigated biomass (1999)	$g\ m^{-2}$	1618	933 to 2272	1223	1808
Dryland grain yield (2000)	$g\ m^{-2}$	353	108 to 503	321	354
Irrigated grain yield (2000)	$g\ m^{-2}$	576	480 to 820	771	536
Days to heading (stress)	Days	70.5	64 to 80	77	71
Days to heading (stress minus irrigated)	Days	-1.2	-6.0 to 6.0	-6	1
Canopy temperature at peak stress	^{0} C	30.4	27.1 to 33.5	30.8	28.6
RWC *	%	83.8	74.3 to 93.4	..	..

(*) Only in 20 randomly selected RILs

Table 3. Phenotypic correlations (r) among traits as measure in the field experiment in 1999 and including osmotic adjustment, over 98 RILs. Bold values are significant at $p<0.01$.

	Drydays	DaysStress-Irr	DryYield	DryBiomass	Dry HI	Irrdays	IrrYield	IrrBiomass	Irr HI	Temp_18/4	OAMean
Drydays	1.00										
DaysStress-Irr	0.22	1.00									
DryYield	0.29	-0.16	1.00								
DryBiomass	**0.35**	-0.02	**0.72**	1.00							
Dry HI	-0.05	-0.16	**0.49**	-0.21	1.00						
Irrdays	**0.75**	**-0.48**	**0.39**	**0.33**	0.07	1.00					
IrrYield	-0.15	-0.20	0.30	0.20	0.14	0.01	1.00				
IrrBiomass	0.14	-0.17	**0.41**	**0.46**	0.00	0.24	**0.71**	1.00			
Irr HI	**-0.38**	-0.06	-0.08	-0.25	0.20	-0.29	**0.55**	-0.19	1.00		
Temp	**-0.37**	-0.05	**-0.34**	**-0.62**	**0.32**	-0.30	-0.12	**-0.36**	0.24	1.00	
OAMean	**0.52**	-0.05	**0.62**	**0.54**	0.18	**0.50**	0.05	0.23	-0.20	-0.12	1.00
RWC-II (n=20)	**0.58**	0.00	0.38	0.40	0.21	**0.56**	0.37	0.33	0.03	-0.40	0.39

Table 4. The multiple regression of dryland yield in 1999 on OA, canopy temperature and days to heading.

Model R^2=0.46

	Multiple regression Coefficient	t value	p value
OA	374.9	6.63	<0.0001
Canopy temperature	20.4	-3.34	0.0012
Days to heading	1.5	0.57	0.56

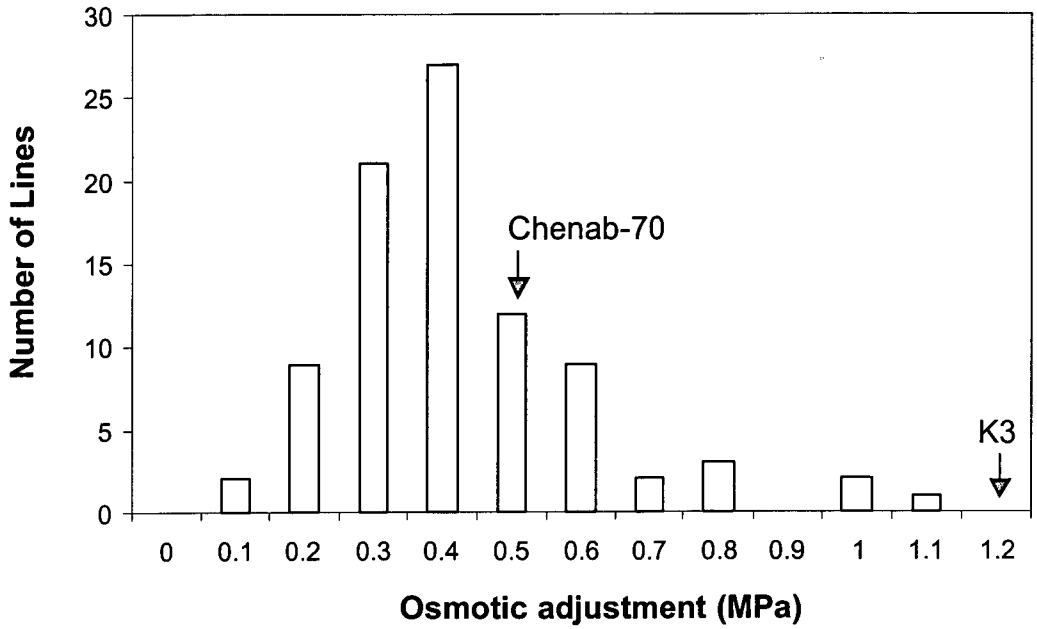

Fig.1. The distribution of osmotic adjustment in 94 F_7 RILs of common wheat and the respective values of the two parents.

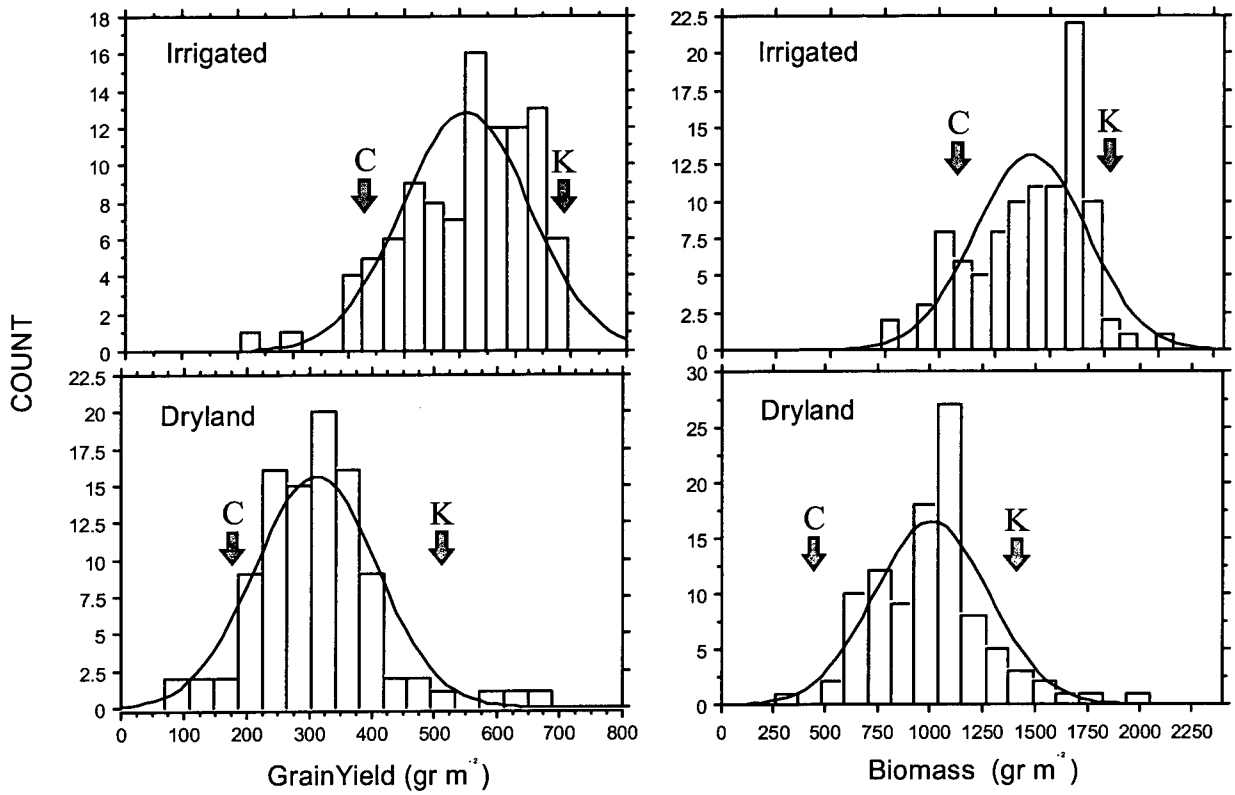

Fig.2. The distribution of 98 RILs with respect to grain yield and biomass under dryland (stress) and irrigated (non-stress) conditions at Bet Dagan, 1999. C and K denote yield of Chenab-70 and K3 parents, respectively.

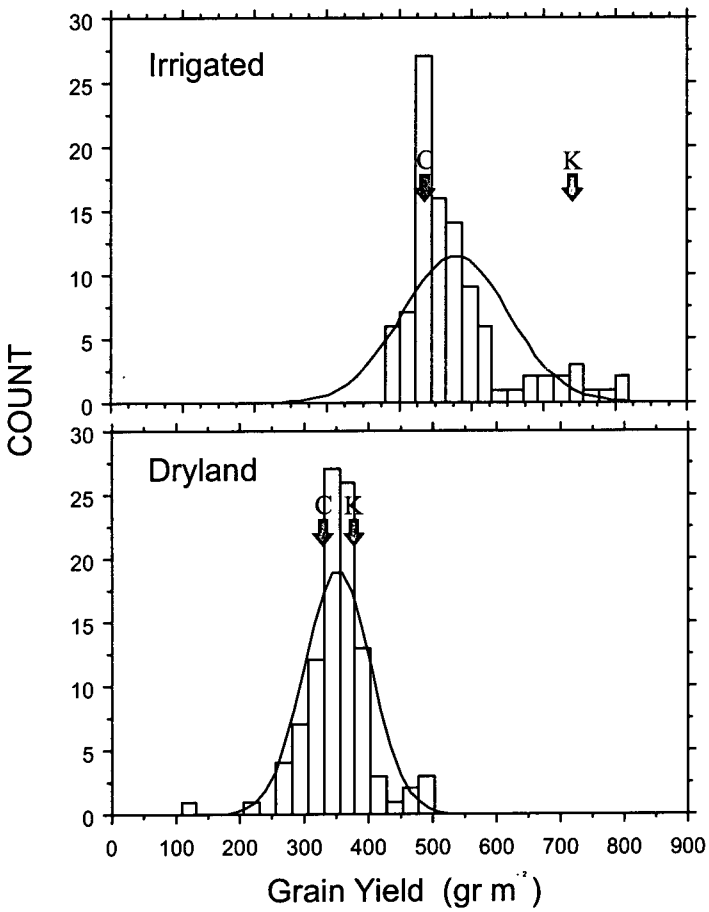

Fig.3. The distribution of 98 RILs with respect to dryland and irrigated grain yield at Bet Dagan, 2000. C and K denote yield of Chenab-70 and K3 parents, respectively.

Fig.4. A difference between two RILs in water deficit symptoms under dryland conditions at Bet Dagan, 1999. Left- advanced leaf rolling; right- hardly any leaf rolling. Notice soil cracking between plots.

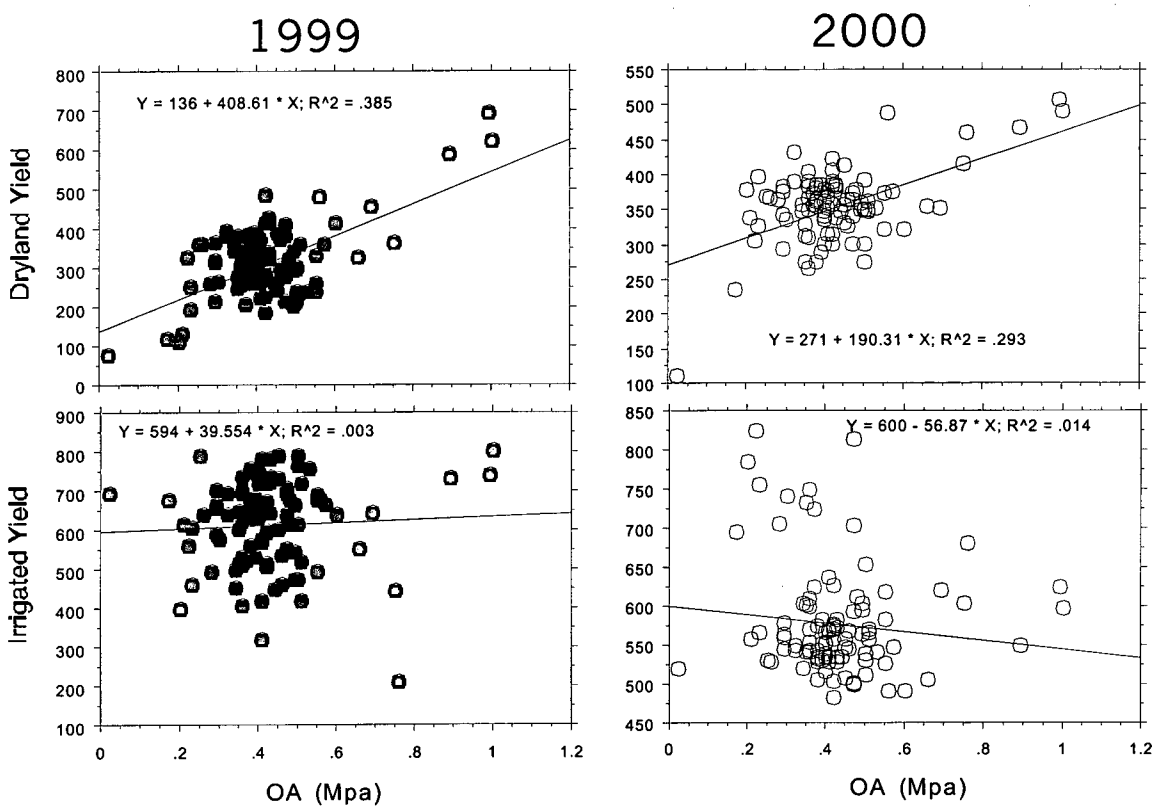

Fig.5. The regression of yield under dryland conditions or under irrigated conditions on osmotic adjustment over 98 RILs, in 1999 and 2000.

Molecular tagging of osmotic adjustment as a drought resistance mechanism in wheat. II. Molecular markers conferring osmotic adjustment.

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ABSTRACT

Osmotic adjustment (OA) is considered one of the major drought tolerance mechanisms in wheat. To identify genomic region associated with OA, 94 wheat F_7 recombinant inbred lines (RILs), developed from a cross between K3 and Chenab-70, were studied in a temperature-controlled greenhouse. Drought stress was imposed on pot grown 35 day-old plants by withholding water. Stress developed slowly as monitored by measuring leaf relative water content (RWC). OA was measured in leaves by the rehydration method when RWC was within 60 to 65%. A significant difference was observed for OA between the two parents and among 94 RILs. The continuous distribution of RILs for OA and broad-sense heritability of h=0.64 indicates that multiple genes control OA. A partial linkage map was constructed with 127 markers (63 SSR and 64 AFLP). Using single marker analysis, 20 markers (18 SSR and 2 AFLP) were found significantly linked with OA at the level of $p<0.0001$. R-square for significant individual marker ranged from 11% to 34%. Six markers were found on linkage group 2A, four in linkage group 3B, three in linkage group 5A, and one each in linkage groups 2D, 3A, 4A, 5D, 6A, 6B, and 7B. In stepwise regression analysis three markers Xgwm186, Xgwm339, and Xgwm493 showed significant association for OA at the level of $p<0.05$ on chromosome 5A, 2A, and 3B respectively. Three markers were significantly linked with grain yield under drought stress and one marker Xgwm493 found common with OA. Three markers were found to be linked with biomass production under drought stress and marker Xgwm186 found common with OA. Two markers Xgwm5 and Xgwm114 found common in grain yield and biomass production under drought stress.

Galiba et al. (1992) detected two genes controlling OA in wheat, which were also located in chromosome 5A and 5D. In the present study, marker Xgwm186 located on chromosome 5A showed significant association with OA at $p<0.0001$ with a highest R-square of 16.8. One ABA

QTL was also mapped by Quarrie *et al.*, (1994) in the long arm of wheat chromosome 5A. These common significant markers among three different traits indicate positive contribution of OA to grain yield and biomass production under drought stress.

INTRODUCTION

Among different abiotic factors, drought is the leading environmental factor that limits wheat production in the USA and rest of the world. In most breeding programs, the genetic improvement of adaptation to drought resistance is addressed through the conventional approach, which involves progeny testing over many locations and years for yield and its stability. It makes selection procedure expensive and slow in attaining progress, thus, success in traditional breeding is becoming a function of economic investment and time. Significant developments have been achieved in understanding the physiology of drought resistance and in developing physiological screening techniques for drought resistance (Blum, 1988; Ludlow and Muchow, 1990), which may reduce the extent of yield testing in selection programs.

Osmotic adjustment (OA) is a major component of drought resistance and recognized as an effective mechanism of drought resistance in different crops (Blum 1988; Ludlow and Muchow, 1990; Babu et al. 1999). Osmotic adjustment involves the net accumulation of solutes in a cell in response to drought stress. Under different environmental stresses, plants accumulate low molecular weight organic solutes know as compatible solutes, which include amino acids, betaines and sugars. Compatible solutes and osmotically active inorganic solutes of the cell help to lower osmotic potential and thus retain water and maintain cell turgor at given water potential. OA support root water uptake and shoot carbon assimilation and growth under stress. It is also involved in protecting functional integrity of cellular membranes and proteins, supporting tiller survival and recovery after drought stress (Paleg et al. 1985; Ludlow, 1987; Blum, 1989; White et al. 1992).

Wheat has a moderate capacity for OA. Significant genetic variation was found for OA in wheat (Morgan, 1977; Johnson et al., 1984; Gupta and Berkowitz, 1987; Schonfeld et al., 1988; Blum and Pnuel, 1990). OA has also been consistently found to be associated with yield and yield stability under drought stress in wheat (Morgan et al., 1986; Teulat et al., 1997; Blum et al., 1999). Although OA is considered as an important mechanism for drought tolerance, it has not been applied in practical breeding programs, mainly because of labor-intensive and time-consuming complex methods. Different methods have been used for measuring OA. Babu et al., (1999) have compared

different methods in measuring OA in 12 rice genotypes. Although Morgan's method is time and labor consuming, it seems to be comprehensive method for measuring OA in a small sample size. This method is not suitable for screening a large mapping population. With a large sample size, the re-hydration method (Blum and Sullivan 1986; Blum 1989) is considered more suitable
The development of molecular marker technologies allow breeders to track genetic loci controlling drought tolerance without having to measure the phenotype, thus reducing extensive field-testing over time and space. Molecular markers such as RFLP (restriction fragment length polymorphism), SSR (simple sequence repeat) and AFLP (amplified fragment length polymorphism) provide a powerful approach to tag major gene(s) and quantitative trait loci (QTL). After molecular markers for OA are identified, screening for these markers can be used to improve the selection efficiency for OA via marker aided selection. OA QTL has been mapped in rice (Lilley et al. 1995; Zhang et al. 2001), barley, (Teulat et al. 1998, Teulat et al. 2001). In wheat Morgan and Tan (1996) performed a limited linkage study of OA in a small population suggesting a probable loci position on the short arm approximately 13 cM towards the centromere from RFLP locus Xpsr119.

The major objective of this study is to identify molecular markers SSR and AFLP tightly linked to osmotic adjustment and to investigate relationships to QTLs controlling yield under water-limited conditions.

MATERIALS AND METHODS

Plant materials

A population of 156 recombinant inbred lines (RILs) were developed from a cross between two wheat cultivars, K3 (Novosteponkaya/SieteCerros) and Chenab-70 (C271/WLT//SN64) via single seed descent (SSD) method. Details are given in Part I of this report. A total of 94 RILs randomly selected from the population were used for phenotypic evaluation, genetic map construction, and QTL analysis of OA.

Measurement of osmotic adjustment

Phenotypic evaluation for OA capacity was conducted in the temperature-controlled greenhouse of Texas Tech University, Lubbock, Texas. Two parents and RI lines were grown in 20 L pots containing a potting mix (mixture of composted pine bark, vermiculite, peat, pertile and others; Ball Seed Co, Chicago, Illinois) of high water holding capacity, to allow stress to develop slowly for about 2 weeks. A randomized complete block design, with three replications. Two pots per genotype x

replication combination were used. Two plants were established per pot. Pots were watered regularly and fertilized with nutrient solution (Miracle-Gro liquid fertilizer; Port Washington, New York). OA was measured by the re-hydration method (Babu et al., 1999) and was calculated as the difference in osmotic potential at full turgor between non- stressed plants and drought stressed plants that were rehydrated to full turgor.

The last irrigation was applied on the 35th day after emergence. At this time, leaf samples were collected to serve as the non-stressed baseline. OA is affected by the rate of plant water deficit. Genotypes can be compared for OA capacity only if all are stressed to the same level. In order to sample all stressed leaves at the same level of leaf water deficit, we visually monitored plants under stress treatment and periodically measured their relative water content (RWC) (Barrs and Weatherley, 1962) at midday. When plants in each pot reached midday RWC of 60% to 65% the pot was irrigated that evening and leaf samples were collected in the next morning when fully turgid. Leaf samples consisted of the middle part of the youngest fully expanded leaf. Samples were wrapped in a piece of aluminum foil and kept on ice until brought to the laboratory where frozen in liquid nitrogen and stored in -80^0C for later use. Prior to measurement, samples were thawed for 30 minutes at room temperature. Sap was squeezed out of the sample under pressure using a 1 ml syringe. 10 µl of sap was used for the determination of osmotic potential (OP) using an osmometer. OA was calculated as the difference in OP at full turgor between non-stressed and stressed rehydrated plants.

Collection of field data on yield and biomass is described in part I of this report.

DNA isolation

Plants for DNA isolation were grown at the Texas Tech University greenhouse in October-November of 2001. Four-week old fresh leaf tissues (5-10 g) were collected in mesh bags from the 94 RILs along with two parents and samples were kept in thermo-cool box with ice. After coming back to the laboratory, leaf samples were quickly frozen with liquid nitrogen. After 10 minutes, leaf tissues were freeze-dried for three days in lyophilizer (Lyph-lock 6, Labconco, USA) where the chamber temperature was about -54^0C and pulling force was less than 10 X 10^{-3} M bar. Dried leaf tissues were ground to a fine powder with Cyclotec mill (Tecator Co, Sweden) and stored in plastic vials until use. Genomic DNA was isolated using phenol chloroform method to get high quality DNA. The DNA quality and concentration were checked with agarose gel electrophoresis and spectrophotometer.

Molecular marker analysis:

Microsatellite primers used are as described by Plaschke et al. (1995), and Roder et al. (1995, 1998) and primers were obtained from Research Genetics (Carlsbad, California). PCR reactions were performed in a volume of 25 µL. The reaction mixture contained 250 nm of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM of $MgCl_2$, 1 unit of Taq polymerase and 100 ng of template DNA. After 3 minutes at 94^0C, 45 cycles were performed with 1 min at 94^0C, 1 min at 50^0C, 55^0C, or 60^0C depending on the annealing temperature of each microsatellite, 2 min at 72^0C, and a final extension of 10 min at 72^0C. Fragment analysis was carried out in 4% super fine agarose (Amresco, Solon, Ohio, USA). The gels were run in 1X TE buffer (10 mM Tris, pH 8.0 and 1 mM EDTA pH 8.0) at 80 volts for two hours. The fragments were visualized, scored, and photographed under UV treating the gel with ethidium bromide. The gels were reused five to seven times.

Amplified fragment length polymorphisms analysis (AFLP) was performed as described by Vos et al. (1995). Five hundred ng of genomic DNA from each of the parental lines Chenab-70, K3, and 94 RILs were digested with EcoRI (E) and MseI (M) restriction enzymes and ligated with respective adapters. Each of these reactions was incubated for 3 hours at 37^oC. Pre-amplification was performed using primers having one selective nucleotide (E-N/M-N). Selective amplifications were performed using various combinations of EcoRI primers with three selective nucleotide and MseI primers with three selective nucleotides (E-NNN/M-NNN) (Table 1). The EcoRI selective primer was labeled with ^{33}P-ATP. Reaction mixture volumes were 10 µL and contained 2.5 µL of diluted pre-amplified products, 1.0 unit of Taq polymerase, 15 ng of M-NNN primer, 12.5 ng [^{33}P]-labeled E-NNN primer, 10 mM Tris-HCL pH 8.3, 15 mM $MgCl_2$, 50 mM KCL, and 0.2 mM of each of the four dNTPs. The selective amplifications were carried out using 36 cycles of a 30 second denaturation at 94^0C, a 30 second annealing at 65^0C and a 60 second extension at 72^0C. The annealing temperature was 65^0C for the first cycle and was reduced by 0.7^0C for each of next 12 cycles; for the remaining 23 cycles, the annealing temperature was 56^0C.

The PCR products were then separated on denaturing gel containing 5% polyacrylamide, 7.5 M urea, and 1X TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). A loading buffer (10 µl) consisted of 98% deionized formamide, 10 mM EDTA pH 8.0, 1 mg/ml bromophenol blue and 1 mg/ml xylene cyanol was added to each of the selective amplification products prior to gel loading. The mixture was heated at 94^0C for 5 min, and then quickly cooled on ice before 3.5ul of sample was loaded on the gel. The electrophoresis was carried out on a CBS sequencing gel apparatus using 1X TBE running buffer, with running parameters of 65 W (2030 V, 35mA) and a plate temperature

of 45^{0}C for about 2 h. The gel was dried at 80^{0}C for about 2h and then exposed to X-ray film for 24-48 h and visualized by autoradiography. Scoring was done based on presence or absence of bands.

Data analysis, partial map construction and QTL mapping

Data obtained from scoring the segregation pattern of SSR and AFLP markers of 94 RILs were analyzed using single marker analysis. All basic statistical analyses were performed using SAS package (PROC GLM, PROC CORR, PROC REG) (SAS Institute Inc, Cary, North Carolina). Single marker analysis helped to find out markers associated with trait of interest without construction of a complete genetic linkage map. Stepwise selection analysis was conducted with the markers that showed significant association of markers with agronomic traits in single marker analysis to identify major markers associated with traits. Regression analysis determined the strong association of marker and trait explaining the largest amount of phenotypic variation. Groups of two or more closely linked markers with significant association were assumed to identify the same QTL. A partial genetic map was constructed with 127 polymorphic marker loci (63 SSR and 64 AFLP) at a LOD score of 3.0 using Mapmaker V.2 (Lander et al. 1987) program. Broad-sense heritability at the genotypic mean level was calculated as $h^2= \sigma^2g/(\sigma^2g+\sigma^2e/n)$, where σ^2g is genetic variance, σ^2e is error variance and n is number of replicates.

RESULTS AND DISCUSSION

A large difference in OA was observed between two parents, K3 (range 0.82-1.75, with a mean 1.2 MPa) and Chenab-70 (range 0.38-0.61, with a mean of 0.53). RI lines showed significant variation for OA. Distribution of RI population ranged from 0.18 to 1.0, with a mean of 0.43. Segregation of most of the lines skewed towards Chenab-70 parents with low OA capacity. (Fig 1). The broad-sense heritability was calculated as 0.68. Continuous distribution of RILs for OA and broad-sense heritability indicated that control of OA is polygenic.

The degree of polymorphism for the 240 tested SSRs was about 37% (out of 88 polymorphic markers, 63 were mapped). The average ratio of polymorphic AFLP loci per primer combination was 5%, ranging from 1 to 12. Due to low polymorphism in RFLP probes (about 15%), none of the RFLP probes were used for mapping. SSR markers were selected to construct a framework map covering the whole genome, but due to low of polymorphic SSR markers,

there were not enough polymorphic markers to cover all linkage groups. SSR markers order was assigned based on wheat map of Roder et al. (1998).

Using single marker analysis, 20 markers (18 SSR and 2 AFLP) were found significantly linked with OA (table 2) at the level of 0.0001. R-square for significant individual marker ranged from 11% to 34%. Six markers were found on chromosome 2A, four on chromosome 3B, three on chromosome 5A, and one each on chromosome 2D, 3A, 4A, 5D, 6A, 6B, and 7B. Earlier Morgan and Tan (1996) suggested in a preliminary fashion one gene controlling OA located in short arm of chromosome 7A. Lilley et al. (1996) and Zhang et al. (2001) identified a major QTL for OA on chromosome 8 of rice. The region of rice chromosome 8 containing the OA QTL is homoeologous with a segment of wheat chromosome 7 (Ahn et al. 1993) where a single locus putatively associated with OA in wheat was identified. In the present study, there were not enough polymorphic markers to construct a framework map of chromosome 7A. Based on stress-induced free amino acid accumulation in a series of wheat substitution lines, Galiba et al. (1992) detected two genes controlling OA that were located on chromosomes 5A and 5D. In the present study, three markers (WMS154, Xgwm186 and Xgwm617) were identified in chromosome 5A showing significant association for the trait OA. In stepwise regression analysis with 20 markers detected earlier in single marker analysis with significant association with OA, only three markers (Xgwm186, Xgwm339 and Xgwm493) showed significant association for OA at the level of 0.05 (Table 3 and Fig 2). Marker Xgwm186 of chromosome 5A showed significant association at 0.0001 levels with R-square of 16.8. ABA accumulation in plants a distinctive drought responsive phenomenon to the same extent that OA is. Quarrie et al. (1994) detected an ABA QTL on the long arm of wheat chromosome 5A. This region was homoeologous to rice chromosome 9 (Ahn et al. 1993). The ABA QTL detected in wheat was also located in a similar genomic region where an ABA and OA QTL mapped in rice (reviewed by Zhang et al., 2001). It is to be determined whether QTL for OA and ABA are located in a similar genomic region in wheat.

Grain yield and biomass production

In single marker analysis, six markers found significantly linked with grain yield under drought stress (YDS). In stepwise selection, three markers (Xgwm493, Xgwm114 and Xgwm5) were linked with YDS (Table 3). Marker Xgwm5 was common for both the OA and YDS. Six markers found significantly linked with biomass production under drought stress (BDS) in single

marker analysis. In stepwise selection, three markers (Xgwm186, Xgwm114 and Xgwm5) found linked with BDS (Table 3). Markers Xgwm114 and Xgwm5 found common with YDS and marker Xgwm186 found common with OA. These common significant markers among three different traits indicate positive contribution of OA to biomass and grain yield under drought stress. This is in agreement with earlier finding by Blum et al. (1999) indicating a consistent phenotypic association between OA and plant production under drought stress.

In single marker analysis, 15 markers showed significantly linked with grain yield under full irrigation (YIR). In stepwise selection, only three markers three (Table 3) were significantly ($p<0.02$) linked with YIR. Among the three markers, Xgwm268 and Xgwm617 were located in chromosome 2A and 5A respectively. In chromosome 5A, marker xgwm617 was very close to significant marker Xgwm186 linked with OA. For biomass production under drought stress, two markers (WMS131 and Xgwm111) were significant correlation with the trait. No marker found common between the traits OA, YDS, BDS and YIR, BIR. This result indicated that no association of OA with plant production under irrigated condition.

So far little progress has been made to locate genes controlling OA in wheat using large mapping population. This is the first attempt to map OA and correlate it with field performance. Research is in progress with this material and data, adding more AFLP markers on the existing map and detecting more polymorphic SSR markers for framework map for unmapped chromosomes. This will help to detect chromosomal regions more precisely for those that contribute to OA. QTLs detected in this population which are common to other stress related QTLs in wheat and are homoeologous to other cereals are a valuable resource for improving drought tolerance in wheat.

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Table 1. Selective EcoR1 and MseI primer combinations were used to screen 94 RILs used for osmotic adjustment study in wheat.

EcoR1 selective primers	Primer designation	MseI selective primers	Primer designation
GACTGCGTACCAATTCA**CA**[1]	E1	GATGAGTCCTGAGTAA**CTG**	M1
GACTGCGTACCAATTCA**AG**	E2	GATGAGTCCTGAGTAA**CAC**	M2
GACTGCGTACCAATTCA**CG**	E3	GATGAGTCCTGAGTAA**CAA**	M3
GACTGCGTACCAATTCA**GC**	E4	GATGAGTCCTGAGTAA**CAG**	M4
GACTGCGTACCAATTCA**TA**	E5	GATGAGTCCTGAGTAA**CTT**	M5
GACTGCGTACCAATTCA**CC**	E6	GATGAGTCCTGAGTAA**CCA**	M6

[1] selective nucleotides in bold face

Table 2: Single marker analysis showing significant association of markers with osmotic adjustment at $p < 0.01$.

Marker	linkage group	R-square	Pr > F
WMS95	2A	0.23	0.0001
WMS154	5A	0.27	0.0001
Xgwm5	3A	0.23	0.0001
Xgwm71.2	2D	0.28	0.0001
Xgwm95	2A	0.11	0.0008
Xgwm133	6B	0.31	0.0001
Xgwm160	4A	0.31	0.0001
Xgwm186	5A	0.34	0.0001
Xgwm234	5D	0.15	0.0001
Xgwm248	2A	0.26	0.0001
Xgwm285	3B	0.27	0.0001
Xgwm333	7B	0.20	0.0001
Xgwm339	2A	0.08	0.006
Xgwm372	2A	0.23	0.0001
Xgwm425	2A	0.23	0.0001
Xgwm448	2A	0.22	0.0001
Xgwm459	6A	0.14	0.0001
Xgwm493	3B	0.31	0.0001
Xgwm617	5A	0.23	0.0001
E3m5-2	3B	0.11	0.0007
E2M4-3	3B	0.11	0.001

Table 3: Step-wise regression analysis showing significant association of markers with osmotic adjustment, yield and biomass production under drought stress, yield and biomass production under full irrigation at $p<0.05$ (Data from Bet Dagan field study)..

Marker	Linkage group	Partial R^2	Model R^2	Pr > F
Osmotic adjustment				
Xgwm186[b]	**5A**	**0.35**	**0.35**	**<0.0001**
Xgwm339	2A	0.04	0.40	0.01
Xgwm493	**3B**	**0.05**	**0.47**	**0.005**
Yield-drought stress				
Xgwm493	**3B**	**0.09**	**0.09**	**0.002**
Xgwm114	3D	0.07	0.16	0.009
Xgwm5	**3A**	**0.05**	**0.21**	**0.02**
Biomass-drought stress				
Xgwm186	**5A**	**0.12**	**0.12**	**0.0007**
Xgwm114	**3D**	**0.08**	**0.20**	**0.003**
Xgwm5	**3A**	**0.07**	**0.27**	**0.005**
Yield-irrigated				
Xgwm617	5A	0.47	0.47	<0.0001
Xgwm247	3B	0.06	0.50	0.001
Xgwm508	6B	0.02	0.57	0.02
Biomass-irrigated				
WMS131	7B	0.34	0.34	<0.0001
Xgwm111	7D	0.02	0.39	0.01

[b]common significant markers for traits osmotic adjustment, yield under drought stress and biomass production under drought stress shown in bold face.