### Characterization of High Yielding and Drought Tolerant RILs Identified from Wheat Cross WL711 x C306 RIL Mapping Population using Drought Susceptibility Index (DSI) as Selection Criteria

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Wheat cultivars WL711 and C306 were shown to be drought susceptible and drought tolerant, respectively, in an earlier study. These two cultivars were crossed to obtain a RIL population comprising 206 RILs. The parents and the RIL mapping population was tested for drought tolerance *via* field trials conducted at Delhi from 2007 to 2010. Water stress was created by withholding irrigation. Phenotyping of the RIL population was done for phenology, yield and yield components under irrigated (Irr) and water stress (WS) treatments. The range of days to flowering in the RIL population was 78-98 and 74-96 days after sowing (DAS) under Irr and WS conditions, respectively. The range of grain yield, biomass, harvest index, 1000 grain weight, grain number in the RIL population was 335.1-720.0g/m<sup>2</sup>, 1316-2413g/m<sup>2</sup>, 22.3-58.6%, 24.0-45.1g, 9497-22763/m<sup>2</sup> and 219.2-568.1 g/m<sup>2</sup>, 992-1759g/m<sup>2</sup>, 17.3-54.8%, 23.1-39.7g, 8807-17509/m<sup>2</sup> under Irr and WS conditions respectively. Analysis of variance revealed significant interaction between genotypes and treatment for all the traits except 1000-grain weight and harvest index. Drought Susceptibility Index (DSI) was used as criteria for drought tolerance. DSI of yield and yield components of the medium to late flowering RILs, 8 RILs were identified for combining yield higher than C306 with yield stability. These RILs maintained better water relations and tougher membranes under WS treatment, like C306.

# Key Words: Drought susceptibility index, Recombinant inbred lines, Water stress, Wheat, Yield, Yield components

#### Introduction

Bread wheat (Triticum aestivum L.) is an important food crop in India. Drought is by far the most important environmental stress in agriculture (Cativelli et al., 2008; Mir et al., 2012). In India, although 80% of the wheat is grown under irrigated conditions, only one-third receives full irrigation while the remainder is cultivated under partial irrigation with 1-2 irrigations over the cropping season. It is likely that water will become a limiting factor for sustained production of wheat in India (Joshi et al., 2007). The duration and intensity of drought is location specific and can vary from year-to-year because of rainfall pattern. When monsoon and winter season rainfall in India are considered, the probability of water deficit is predictable. Wheat crop can experience water deficit stress during growth and development both in irrigated and limited irrigation environments depending upon the water availability (Sinha et al., 1982). In fact wheat crop often experiences both drought and heat stress

in the post-anthesis period as it matures in increasing temperatures in North India. Growing food demand and global warming would further push wheat crop to drought and heat stress environments (Araus *et al.*, 2008; Semenov and Helford, 2009; Sinclair, 2011). Therefore, breeding for drought tolerance in wheat has been a major aim of many breeding programs both nationally and internationally in order to improve crop productivity under water-limiting conditions (Araus *et al.*, 2002; 2008; Chaves *et al.*, 2003, Richards *et al.*, 2010). If the breeding programs address factors of stress-adaptation in addition to yield under stress, then it may be possible to combine higher yield potential and drought tolerance (Blum, 2005; Richards *et al.*, 2010).

Wheat cultivars WL711 and C306 were shown to be drought susceptible and drought tolerant respectively in an earlier study involving *Triticum aestivum*, *T. durum* and *Triticale* germplasm (Sinha *et al.*, 1986). With the aim of combining drought tolerance with high yield, a

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program was initiated at the Stress Physiology Laboratory, Water Technology Centre, IARI, New Delhi in which these two varieties were crossed in order to obtain a RIL population (Patil and Khanna-Chopra, 2006). The aim of the research reported in this paper was to identify RILs combining drought tolerance with yield higher than C306 from the cross WL711 x C306 based on DSI and to analyse the basis of stability in yield in relation to physiological traits such as water relations and membrane stability in the post-anthesis stage.

#### **Materials and Methods**

#### Material and Location

The parent cultivars WL711 [(S308 x Chris) x Kalvansona] and C306 [(Regent x Ch23) x C591 (P19 x C28)] have several unique morphological and physiological differences in addition to yield and yield components. WL711 is a semi-dwarf, high yielding, drought susceptible, medium flowering variety with medium seedling vigour, erect growth habit and medium grains which are dull brown in colour while C306 is a tall, medium yielding, drought tolerant, late flowering variety with high seedling vigour, spreading growth habit and bold grains which are light amber in colour. The present investigation comprises of 206 RILs, F<sub>10</sub> generation onwards of the WL711 x C306 cross. The experiments were conducted for three consecutive years from 2007 to 2010 in the fields of Water Technology Centre (IARI), New Delhi (77°12'E, 28°40'N; 228 msl). The data presented is an average of the three trials at Delhi

#### Soil Characteristics

The soil at Water Technology Centre (IARI) is sandy loam with average bulk density of  $1.55 \text{g cm}^3$ . The water retention capacity is 18% at field capacity (-1.5MPa) and 4.5% at permanent wilting point (-1.5Mpa) on w/w basis (Sinha *et al.*, 1986). The contribution of ground water through capillary action is almost negligible because the depth of water table is 4m.

#### Sowing

The parent cultivars WL711, C306 and 206 RIL population were sown in the field during winter seasons of 2007-08, 2008-09 and 2009-10. Seed rate was approximately 200 seeds per m<sup>2</sup>. Pre-sowing irrigation was applied to obtain sufficient moisture for germination. Sowing was done on 25<sup>th</sup> November in the three trials in a Randomized Complete Block Design (RCBD)

with four replications. The plot size was  $3 \times 2m^2$  with 6 rows with a distance of 25cm between the rows. A fertilizer dose of 100:50:50 kg NPK ha<sup>-1</sup> was applied in the form of urea, single super phosphate and murate of potash, respectively, as a single basal dose at the time of sowing. Weeds were removed from the field as and when they appeared.

#### Irrigation Treatments

There were two irrigation treatments, full irrigation (Irr) and water stress (WS). Irr treatment received 4 irrigations at tillering (DC 2.0), jointing (DC 3.0), flowering (DC 6.0), and grain filling stages (DC 7.0) while WS treatment received 2 irrigations at tillering (DC 2.0) and flowering (DC 6.0). Each irrigation was approximately equivalent to 4 cm of water. The Irr treatment received 37.7, 37.1 and 37.8cm while WS treatment received 28.9, 28.3 and 29.5cm of total available soil water in 2007-08, 2008-09 and 2009-10 respectively.

#### **Parameters Recorded**

#### Phenology

Days of flowering was recorded for all RILs under both water treatments as the date when 50% of the shoots had reached this stage by observing RILs every 1-2 days from February to the first week of March. Days to flowering was calculated as days after sowing (DAS).

#### Physiological Parameters

Relative water content (RWC), and cell membrane stability (CMS) were measured in the parents and the selected RILs in 2009-10. RWC and CMS were measured in flag leaves at post-anthesis stage (A+14) under both Irr and WS treatments following the procedure given by Barrs and Weatherly (1962) and Blum and Ebercon, (1981), respectively. The results of CMS were expressed as % injury using the formula:

% Relative injury =  $1 - [1 - (T_1/T_2)]/(1 - (C_1/C_2)) \times 100$ .

Where, C and T refer to mean of treatment and controls, respectively, and the subscripts 1 and 2 refer to initial and final conductivity respectively.

#### Yield and Yield Components

One meter square area was harvested of each RIL at maturity for biomass  $(g/m^2)$  and grain yield  $(g/m^2)$ . There were 3 replicates per treatment. 500 grains were counted at random and their weight was recorded and expressed as 1000 grain weight. Harvest index (HI) was

calculated by the formula- HI = grain yield/total aboveground biomass x 100. Number of grains per meter square was obtained by dividing grain yield  $(g/m^2)$  by grain weight and multiplied with 1000.

#### Drought Susceptibility Index (DSI)

Drought susceptibility index (DSI) and stress intensity (D) was calculated using the formula of Fischer and Maurer, (1978).

DSI = (1 - Y/Yp)/D

Where, Y- Mean yield or yield component of a RIL under water stress condition, Yp- Mean yield or yield components under irrigated condition. D- Stress intensity

D=1-X/Xp

Where, X- Mean Y of all RILs, Xp- Mean Yp of all **RILs** 

DSI is a measure of drought tolerance. If DSI is < 1, the cultivar is drought tolerant and DSI > 1 indicates that cultivar is drought susceptible.

#### Statistical Analysis

Analysis of variance (ANOVA) was estimated for all traits separately for estimating coefficient of variation (CV) and critical difference (CD) for evaluation the significance of treatment and trial effects on the parents and the WL711 x C306 RIL population. ANOVA was done using the three factor factorial analysis of the statistical programme MSTAT-C, version 1.41, Michigan State University, USA. Correlation analysis was performed using MSTAT-C.

#### **Results and Discussion**

#### Weather

The average  $T_{max}$  and  $T_{min}$  during the crop season were 24.83±1.56°C and 10.96±0.85°C respectively. Total rainfall recorded during the 2007-08, 2008-09 and 2009-10 crop seasons were was 25.6 mm, 37mm and 30.6 mm, respectively. There was no rainfall in the month of March and April i.e. during grain filling to maturity. Meager rainfall and higher ET in both the years helped in development of severe water stress during post-anthesis period.

#### **Phenology**

WL711 and C306 flowered 88 DAS and 96 DAS under Irr treatment and 82 DAS and 92 DAS under WS treatment. Flowering in the RIL population started from mid February (78 DAS) and continued up to the first week of March (98 DAS). Under WS treatment, both parents and the RIL population flowered earlier than the irrigated control (Table 1). Phenology plays an important role in response to drought stress as growth duration determines water requirement and probability of exposure to water stress (Araus et al., 2008). Water stress developed in early March and consequently medium to late flowering RILs (85 -98 DAS) are likely to experience water deficit stress, in the post-anthesis period more than the early flowering RILs.

#### Yield and Yield Components

The parents, WL711 and C306, differed significantly in yield and yield components (Table 1). WL711 is a high yielding variety  $(673.9 \pm 57.3 \text{g/m}^2)$  while C306 is a medium vielding variety  $(435.8 \pm 23.2 \text{g/m}^2)$ . WL711 showed higher biomass ( $1856 \pm 120.7 \text{g/m}^2$ ), grain number  $(18657 \pm 825 / m^2)$  and HI  $(39.5 \pm 1.7\%)$  than C306 (1688  $\pm$  102.0 /m<sup>2</sup>, 11017 $\pm$ 541 /m<sup>2</sup> and 26.7  $\pm$ 0.9% respectively). C306 has bolder grains and higher 1000-grain weight  $(37.52 \pm 0.31g)$  than WL711  $(33.31 \pm$ 0.62g). The RIL population showed normal distribution and transgressive segregation for grain yield under Irr and WS treatments (Fig 1). The mean value of RILs



Fig. 1. Frequency distribution of grain yield (g/m<sup>2</sup>) of WL711 x C306 wheat RIL population under irrigated and water stress conditions based on the mean values of the three trials conducted at Delhi (2007-2010). The histograms with the solid and the open bars represent the frequencies of grain yield of RILs under irrigated and water deficit stress conditions respectively. W- WL711, C- C306

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Table 1.	Means (± SE) and ranges of grain yield and yield components in the parents, the selected RILs and the WL711 x C306 wheat RIL
	population under irrigated and water stress environments at Delhi from 2007-2010. Irr- irrigated treatment, WS- water stress
	treatment; NS -non-significant

Selected RILs	Days to flowering (DAS)		Grain yield (g/m <sup>2</sup> )		Biomass (g/m <sup>2)</sup>		Harvest index (%)		1000-grain weight (g)		Grain number (/m <sup>2</sup> )	
	Irr	WS	Irr	WS	Irr	WS	Irr	WS	Irr	WS	Irr	WS
WL711	87±2	81±1	673.9 ± 57.3	435.8 ± 23.2	1856 ± 120	1329 ±72	39.5 ± 1.7	27.6 ± 2.6	33.3 ± 0.6	28.1 ± 0.5	18657 ± 825	14375 ± 1018
C306	96±3	89±2	435.8 ± 23.2	347.2 ± 17.7	1688 ±102	1412 ± 63	26.7 ± 0.9	$\begin{array}{c} 26.7 \\ \pm  2.1  {}^{NS} \end{array}$	$\begin{array}{c} 37.5 \\ \pm \ 0.3 \end{array}$	36.4 ± 1.0	11017 ± 541	$10642 \pm 876^{NS}$
R-19	91±2	86±3	$578.8 \\ \pm 36.3$	458.3 ± 36.1	1855 ± 109	1473 ± 59	32.2 ± 3.8	30.4 ± 2.2	33.8 ± 1.5	32.4 ± 1.9	$\begin{array}{c} 16161 \\ \pm 830 \end{array}$	13994 ± 766
R-46	94±3	91±2	$540.0 \pm 28.1$	405.0 ± 16.9	1633 ± 74	1424 ± 84	31.6 ± 1.5	29.5 ± 2.0	37.6 ± 0.6	34.2 ± 1.1	$\begin{array}{c} 14302 \\ \pm \ 683 \end{array}$	13539 ± 921
R-61	91±3	87±3	614.8 ± 43.8	421.0 ± 13.1	2040 ± 129	$\begin{array}{c} 1480 \\ \pm 40 \end{array}$	31.9 ± 1.6	29.1 ± 1.6	33.5 ± 0.5	32.5 ± 1.3	18286 ± 982	15092 ± 745
R-208	90±2	86±4	617.6 ± 36.4	471.6 ± 16.8	2109 ± 163	1478 ± 69	34.3 ± 4.1	33.1 ± 1.0	36.1 ± 0.4	34.7 ± 1.3	16443 ± 795	14831 ± 820
R-25	89±3	84±3	659.9 ± 40.7	461.6 ± 9.7	2008 ± 130	1701 ± 75	35.7 ± 3.0	27.3 ± 1.7	$\begin{array}{c} 36.7 \\ \pm \ 0.8 \end{array}$	$^{36.5\pm}_{1.0^{ m NS}}$	17562 ± 887	14309 ± 829
R-109	91±2	84±4	673.6 ± 53.1	$\begin{array}{c} 498.2 \\ \pm 43.2 \end{array}$	2285 ± 153	1530 ± 61	33.0 ± 2.5	31.2 ± 2.5	35.5 ± 1.0	35.0± 1.7 <sup>NS</sup>	17721 ± 836	14575 ± 1154
R-128	94±1	88±2	$\begin{array}{c} 603.1 \\ \pm 44.0 \end{array}$	427.2 ± 27.2	1903 ± 118	1393 ± 42	33.6 ± 2.7	28.2 ± 2.3	38.0 ± 1.5	36.0 ± 1.5	15955 ± 584	12617 ± 1035
R-7	86±3	80±5	642.1 ± 35.3	460.2 ± 21.6	2375 ± 209	1699 ±122	35.5 ± 1.6	26.2 ± 2.5	37.4 ± 0.6	36.3 ± 1.9	17437 ± 496	14692 ± 956
RIL Population Mean	86±3	82±2	538.0 ± 27	368.6 ± 21	1729 ± 65	1324 ± 43	41.2 ± 2.8	34.2 ± 2.4	34.3 ± 1.7	31.6 ± 1.8	14706 ± 678	12273 ± 735
RIL Population Range	78–98	74-95	335.1 -720.0	219.2 - 568.1	1316 - 2413	992 -1759	22.3 - 58.6	17.3 - 54.8	24.0 - 45.1	23.1 - 39.7	9497– 22763	8807 - 17509

## Table 2. Analysis of variance for yield and yield components in the WL711 x C306 RIL population at Delhi 2007-2010. NS-non-significant; CV-coefficient of variation; CD-critical difference at 0.05% significance level

			Mean squares	1			
Source of variation	df	Grain yield (gm <sup>-2</sup> )	Biomass (gm <sup>-2</sup> )	Harvest index (%)	1000-grain weight (g)	Grain number (m <sup>-2</sup> )	Days to flowering
Replicates	3	58715.4	216255.9	3.64	1.13	12149013.1	28.6
Genotypes	210	90164*	278255.3*	61.5*	119.6*	63619182*	151.2*
Treatment	1	18895550.8*	125641914.9*	1614.3*	3148.2*	5293351989*	28729.7*
Genotype x treatment	210	14896.6*	73081.7*	55.7 <sup>NS</sup>	$10.76^{NS}$	23821076.3*	16.2*
Trial	2	2384898*	23368523.1*	6919.8*	6005*	3087407217.1*	20662.0
Genotype x trial	420	16485.9*	91768.6*	64.1*	29.37*	22114740.9*	39.0*
Treatment x trial	2	219518.6*	8409674.1*	11054.3*	757.5*	2707242163.7*	2048.5*
G x treatment x trial	420	7256.7*	48390.6*	60*	11.1*	2722673.9*	11.1*
Error	1267	1779.8	17612.8	41	0.304	11783228.3	0.62
CV (%)		9.40%	8.81%	10%	5.20%	24%	0.92%
CD		48.07	151.2	6.06	1.8	1815.5	0.92

was intermediate of the parents for most of the traits. Transgressive segregation was observed for all the traits in the RIL mapping population (Table 1). Analysis of variance revealed significant interaction between genotypes and treatment for all the traits except 1000grain weight and harvest index (Table 2).

#### Drought Susceptibility Index (DSI)

C306 was more stable  $(0.71 \pm 0.17)$  than WL711  $(1.39 \pm$ 0.15) for grain yield. Hence C306 is drought tolerant while WL711 is drought susceptible as reported earlier (Sinha et al., 1986; Table 3). DSI has been used as a criterion for evaluating genotypes and mapping populations for drought tolerance in wheat (Ehdaie et al., 1988; Blum et al., 1989; Lazar et al., 1995, Dencic et al., 2000; Foulkes et al., 2007; Kirigwi et al., 2007; Semenov et al., 2009). Considerable variation for DSI for grain yield was observed in the RIL population. Variation for grain yield stability has been observed in other wheat RIL populations under water stress environment (Foulkes et al., 2007; Kirigwi et al., 2007). C306 showed more stability for all yield components compared to WL711. The RILs also showed considerable variation in DSI for biomass, HI, 1000-grain weight and grain number (Table 3). DSI showed higher correlation with grain yield under Irr condition ( $r = 0.364^{**}$ ) than WS condition (r = 0.113 \*\*).

To identify RILs combining higher yield than C306 with yield stability under WS conditions, only medium and late flowering RILs were analyzed. Out of 182 RILs, 48 RILs were found to be stable for grain yield. Among these RILs, only 8 RILs exhibited higher yield than C306 and yield stability (Table 1, Fig 2). RIL R-19 was stable for four yield components, RILs R-46, R-61, R-208, R-25 and R-109 were stable for three yield components i.e. grain number, 1000-grain weight and HI while RILs R-128 and R-7 showed stability



#### Grain yield (g/m<sup>2</sup>)

Fig. 2. Relationship between grain yield (g/m<sup>2</sup>) and drought susceptibility index (DSI) of grain yield of medium to late flowering RILs from the wheat cross WL711 x C306. Symbol (D) indicates selected RILs, P1-WL711, P2-C306

Table 3. Means (±SE) of DSI of grain yield and yield components of the parents and the selected RILs from the wheat cross WL711 x C306 at Delhi from 2007-2010

Drought susceptibility index									
Parents and selected RILs	Grain yield (g/m <sup>2</sup> )	Biomass (g/m <sup>2</sup> )	Harvest index (%)	1000grain weight (g)	Grain number (/m <sup>2</sup> )				
WL711	$1.39 \pm 0.15$	$1.18 \pm 0.08$	$2.26\pm0.11$	$1.99\pm0.03$	$1.29\pm0.09$				
C306	$0.71 \pm 0.17$	$0.69 \pm 0.06$	$0.20 \pm 0.05$	$0.29 \pm 0.11$	$0.69\pm0.10$				
R-19	$0.63 \pm 0.08$	$0.79 \pm 0.13$	$0.36 \pm 0.10$	$0.24 \pm 0.11$	$0.73 \pm 0.11$				
R-46	$0.80 \pm 0.10$	$0.62 \pm 0.06$	$0.34 \pm 0.06$	$1.23 \pm 0.02$	$0.31\pm0.06$				
R-61	$0.82 \pm 0.04$	$1.16 \pm 0.06$	$0.99\pm0.09$	$0.24\pm0.09$	$0.97\pm0.19$				
R-208	$0.67\pm0.14$	$1.06 \pm 0.14$	$0.70\pm0.08$	$0.55 \pm 0.17$	$0.55\pm0.04$				
R-25	$0.92\pm0.16$	$0.65 \pm 0.17$	$2.30\pm0.03$	$0.11\pm0.08$	$1.03 \pm 0.12$				
R-109	$0.92\pm0.04$	$1.18\pm0.05$	$0.29\pm0.02$	$0.24\pm0.10$	$1.00 \pm 0.15$				
R-128	$0.98\pm0.12$	$0.92 \pm 0.14$	$1.06\pm0.07$	$0.66 \pm 0.19$	$1.23 \pm 0.11$				
R-7	$0.83\pm0.14$	$1.13 \pm 0.13$	$2.50\pm0.05$	$0.42 \pm 0.12$	$0.89\pm0.11$				
Range in RIL population	0.27 - 1.84	0.31 - 1.98	-1.8 - 2.6	0.12 - 1.69	0.34 - 1.90				

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for only two yield components (Table 3). The selected RILs, except RIL R-46 were able to maintain1000-grain weight under WS treatment and thus showed stability for this character like C306 (Table 1). C306 is known to mobilize pre-anthesis stored carbohydrates in the stems to the developing grains under post-anthesis water stress condition resulting in stable 1000-grain weight and consequently stable grain yield (Aggarwal and Sinha, 1984, Khanna-Chopra *et al.*, 1994). It may be inferred from the performance of selected RILs under WS treatment that some of them may have acquired this trait from C306. The range of grain yield, biomass, grain number, 1000-grain weight and HI of the selected RILs was 540.0-673.6 g/m<sup>2</sup>, 1633-2375 g/m<sup>2</sup>, 14302-18286 / m<sup>2</sup>, 33.5-38.0g and 31.6-35.7 % respectively.

RWC and CMS have been reported to be associated with drought tolerance (Blum, 2005). C306 had higher



Fig. 3. Relative water content (RWC, A) and cell membrane stability (CMS, B) in the flag leaf at post-anthesis stage (A+14) of the parents and the selected RILs from the wheat cross WL711 x C306 under water stress conditions. Vertical bars represent  $\pm$  SE (n=3)

RWC and membrane stability as compared to WL711 under WS treatment (Fig. 3A). RILs R-19, R-46, R-109 and R-128 maintained higher RWC than C306 under WS treatment. RILs R- 19, R-46, R-61, R-25, R-128 and R-7 were similar to C306 in CMS; however R-208 and R-109 maintained higher CMS than C306 under WS treatments (Fig. 3B). Grain yield of the selected RILs was significantly correlated with RWC ( $R^2=0.165$ , p<0.05), and CMS ( $R^2=0.461$ ) under WS treatment. In previous studies also, grain yield under water stress was shown to be significantly correlated to RWC (Sharma and Kumar, 2010) and CMS (Geravandi et al., 2011). Favourable expression of water relation traits are indicators of the capacity of the crop to access water from deeper layers of soil under water stress condition as a result of deep and dense root system (Reynolds et al., 2005; Blum et al., 1989). Deep root system increases the total water availability to the crop under water stress condition and is associated with improved drought tolerance (Reynolds et al., 2007).

In conclusion we report certain RILs from WL711 x C306 cross showing desirable recombination of traits resulting in yield improvement compared to C306 and showing stability in yield and some yield components under water deficit stress. The selected RILs are medium to late in flowering and some RILs also have amber coloured grains, a desirable character of grain quality. The selected RILs maintained high RWC and tougher membranes under water stress conditions like C306. Four of these RILs (R-7, R-61, R-19 and R-208) have been registered at NBPGR as drought tolerant wheat germplasm (Registration no. INGR11037, INGR11038, INGR11039 and INGR11040 respectively). These RILs can be used as genetic material for transferring the desirable drought tolerance traits acquired from C306.

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