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Research Information

Excised leaf water loss in wheat (*Triticum aestivum* L.) as affected by short periods of heat and water-deficit treatment followed by recovery

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Abstract

Drought and high temperature are especially considered as key stress factors with high potential impact on crop yield. Two contrasting wheat (*Triticum aestivum* L.) cultivars WH730 (high temperature tolerant) and UP2565 (high temperature sensitive) were tested for differential response to short periods of combined and individually applied high temperature (HT) and drought (D) stress as well as revival to examine differences for excised leaf water loss as drought and heat tolerance character. Assessment of water loss from excised leaves has shown promise for characterizing drought resistance and thermo-tolerance in wheat genotypes. The effects of high temperature and drought were additive. High temperature increased the degree of water stress and the combined effects of drought and high temperature were more severe than those of each individual treatment.

Key words: drought, tolerance, excised leaf water loss, wheat, high temperature

Abbreviations: ELWL: Excised leaf water loss; HT: high temperature; D: drought; HT + D: high temperature + drought; DAA: days after anthesis; DAS: days after sowing; PWP: permanent wilting point; RH: relative humidity

Introduction

Bread wheat (*Triticum aestivum* L.), due to its wide adaptability it can be grown under diverse agro-ecological conditions ranging from temperate to subtropical climates. Thus, considerable climatic differences in temperature and relative humidity exist in these areas and wheat crop experiences wide seasonal variations which causes large annual fluctuations in the yield (Munjal and Dhanda, 2005). Under field conditions wheat plants are often simultaneously exposed to soil drying and high temperature stress. These two stress factors could create water deficit

in plant tissues, which, in turn, may affect the yield. On a global basis, high temperature in conjugation with coincident drought poses the most important environmental constraint to plant survival and to crop productivity. Drought is often accompanied by relatively high temperatures, which increases the evapotranspiration, reduces photosynthetic capacity of plants consequently reducing crop yields (Reynolds and Ortiz, 2010). Production of plants tolerant to high temperature and drought stress is of immense significance in the light of global warming and climate change. Genetic improvement of wheat for drought/heat

resistance requires a search for possible physiological components of stress resistance and the exploration of their genetic variation (Passioura, 2010; Sinclair, 2011). Synchronization of growth duration with the expected or the predicted seasonal soil moisture supply is an important aspect of plant breeding for water-limited environments (Blum, 2009). A large number of plant water relation parameters have been identified for use in breeding programmes (Zaman-Allah et al., 2011). Assessment of water loss from excised leaves (ELWL) has shown promise for characterizing drought resistance and thermotolerance in wheat genotypes (Clarke and Richards, 1988; Clarke et al., 1989; McCaig and Ramagosa, 1991; Mir et al., 2012). This trait is moderately heritable and can be easily determined in large population (Dhanda and Sethi, 1998; Kumar and Sharma, 2007). Following excision, stomata close and after 20 to 30 min the rate of water loss enters a linear phase that lasts for several hours (McCaig and Romagosa, 1991). During this phase the water is lost from incompletely closed stomata. This trait also influences the recovery of plant from stress and consequently affects yield and yield stability. If water retention capacity of wheat genotypes is increased, the yield of rainfed wheat could be increased or at least stabilized. This parameter can also be easily determined, and is hence applicable for use in large populations. Since identification of germplasm having drought/heat tolerance is of paramount importance to develop new stress tolerant cultivars, the systematic characterization of differences in physiological responses to stress among elite lines may lead to a better understanding of underlying mechanisms.

Materials and Methods

a) Plant materials and growing conditions:

Two contrasting wheat (*Triticum aestivum* L.) cultivars WH730 (thermo-tolerant) and UP2565 (thermo-sensitive) were tested for differential response to high temperature and drought tolerance for excised leaf weight loss. Plants were raised in earthen pots (30 cm in diameter) lined with polythene bags and each containing 5 kg of dune sand (*Typic torripsammments*) [93.3% sand + 3.0% silt + 3.7% clay, saturation capacity 25 %, pH 8.2, EC_e 0.8 dS m⁻¹ at 25°C, 10.3 mg (N) kg⁻¹, 2.5 mg (P) kg⁻¹, 180 mg (K) kg⁻¹] under natural conditions of a screen house. After thinning four healthy plants were maintained in each bag. The experiment was three factors complete randomized design (CRD). CD was calculated at

5% level of significance.

b) High temperature and water deficit treatments:

- Control [Field capacity (20-22%) and ambient temperature (100-260C) during growth of crop]
- High temperature stress (by shifting the pots to polyhouse for one week with maximum temperature 5-80C > than ambient)
- Drought [Drought was imposed by withholding water supply till permanent wilting point (PWP, gravimetric soil moisture 6-7%) was attained.]. The plants were re-irrigated (600 ml water per pot to attain field capacity) after PWP.
- High temperature along with drought [Combined stress was given to plants by shifting pots to polyhouse for one week and simultaneously drought conditions were maintained by withholding water supply].

Temperature, relative humidity (RH) and soil moisture (gravimetrically) were recorded during treatment period under screen house and polyhouse (Fig. 1 and Table 1).

c) Treatment imposition stages:

The plants of both varieties of wheat viz. WH730 and UP2565 were exposed to high temperature, drought and the combination of both stresses at the following stages:

- Booting stage (60-65 DAS)
- Post anthesis stage (90-95 DAS)
- (i) + (ii)

d) Sampling:

The plants were observed for temporary wilting in the evening and only those plants which did not recover during the night were measured on the following day. The excised flag leaves were placed in polythene bags and transported to the laboratory as quickly as possible in order to minimise water losses due to evaporation. The plants were sampled at the termination of stress and one week after the revival period.

e) Excised leaf weight loss (mg h⁻¹):

Three flag leaves of each variety per treatment were excised from the plant and their fresh weight was immediately recorded. These leaves were then kept in an incubator at 28°C at 50% relative humidity and their weights were recorded after every hour to determine the loss in weight per hour. The weight loss (mg h⁻¹) from the excised leaves in the form of water vapours was calculated for each genotype as:

Rate of ELWL (1st h) = Initial weight excised leaf weight after 1st h
Rate of ELWL (2nd h) = Excised leaf weight after 1st h – excised leaf weight after 2ndh
And so on up to five h.

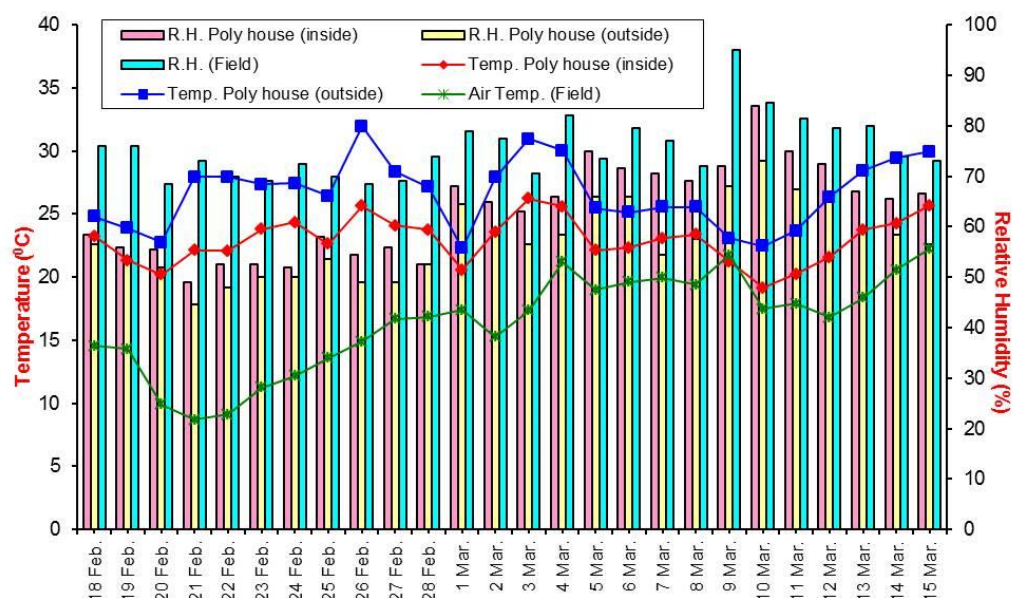


Fig. 1. Daily temperature and relative humidity conditions in polyhouse and field during the imposition of individually applied high temperature (HT), drought (D) and combined (HT+D) stress on wheat genotypes.

Table 1. Average gravimetric soil moisture percentage in sand at the termination of individually applied high temperature (HT), drought (D) and combined (HT+D) stress treatments in var. UP2565 and WH730 at different stress imposition stages.

Treatment imposition stage	Treatments	Soil moisture (%) in UP2565	Soil moisture (%) in WH730
Booting stage	Control (C)	21.60 (1.42)	21.68 (1.35)
	High temperature (HT)	20.42 (1.97)	21.46 (1.71)
	Drought (D)	6.05 (1.02)	6.15 (1.12)
	High temperature + Drought (HT+D)	5.95 (0.92)	5.48 (1.41)
Post-anthesis stage	Control (C)	20.68 (1.49)	21.79 (1.37)
	High temperature (HT)	21.02 (1.62)	20.00 (1.48)
	Drought (D)	6.17 (1.12)	6.02 (1.22)
	High temperature + Drought (HT+D)	5.70 (1.69)	5.24 (1.04)
Booting + Post-anthesis stage	Control (C)	22.51 (1.88)	21.60 (1.82)
	High temperature (HT)	20.13 (1.99)	21.54 (1.68)
	Drought (D)	6.02 (1.52)	6.45 (1.42)
	High temperature + Drought (HT+D)	5.75 (1.42)	5.42 (1.51)

Values are means with S.E. in parenthesis.

Results and Discussion

ELWL in var. UP2565: Fig. 2 represents the rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment imposed at booting stage in var. UP2565. Maximum loss in water vapours from the excised flag leaf was observed during the 1st h of excision which was very fast and then slowly declined up to five h. The rate of weight loss was different because of differential rate of stomatal closure during different treatments. Interactive HT+D stress resulted in minimum (25.90 mg) ELWL. After revival, rate of ELWL during 1st h was less than the stress treatments, however maximum reduction was observed during 1st h of excision. Slope was low as compared to termination of stress treatments. Interactive HT+D stress resulted in maximum loss of water vapours during all the five h of observation in comparison to HT and drought revived plants. At post-anthesis stage (Fig. 3) the loss was very rapid, except interactive HT+D stress treatment. Out of all the stress treatments, maximum rate of ELWL during first h was recorded in HT stress (75.5 mg) while HT+D resulted in minimum loss (22.1 mg). After revival, rate of ELWL due to drought stress was higher than that of other stress treatment. Magnitude of ELWL (booting+post-anthesis stage) was same to post-anthesis stage (Fig. 4). Highest rate of ELWL was observed in HT stress (67.45 mg) while lowest in interactive HT+D stress (33.56 mg) during 1st h of observation. After revival, reversal of this situation was observed where interactive HT+D stress resulted in maximum rate of ELWL (47.7 mg) and HT stress led to minimum ELWL (28.25 mg) when compared to control (54.4 mg) during 1st h of observation. This indicates differential stomatal conductance during different stress treatments.

ELWL in var. WH730: Overall magnitude of ELWL was less at booting stage (Fig. 5). Rate of ELWL was lower than var. UP2565. Similar to results observed in UP2565 (Fig. 2) rate of ELWL in WH730 declined progressively during 2nd to 5th h of observation indicating slow stomatal closure. Out of all the three stress treatments, interactive HT+D stress resulted in maximum decline during all the five h of observation in comparison to HT and drought revived plants. At post-anthesis stage (Fig. 6) maximum rate of ELWL was recorded due to HT stress (55.4 mg) in comparison to control, however the same was less when compared to var. UP2565 (Fig. 3). ELWL of plants relieved from drought stress was higher (43.7 mg) during all the five h of observation in comparison to other two stresses;

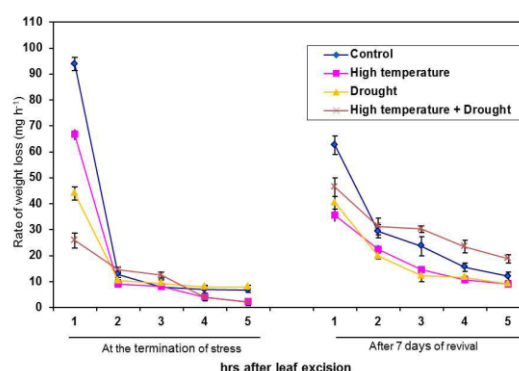


Fig. 2. Rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment given at booting stage in var. UP2565.

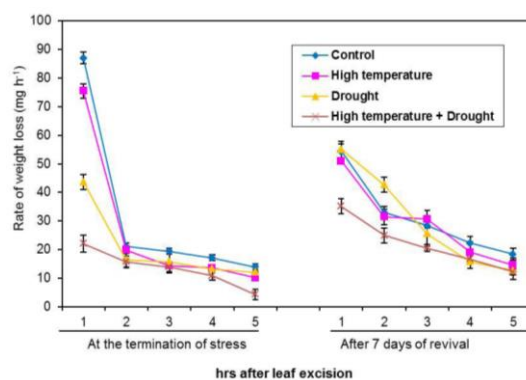


Fig. 3. Rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment given at post-anthesis stage in var. UP2565.

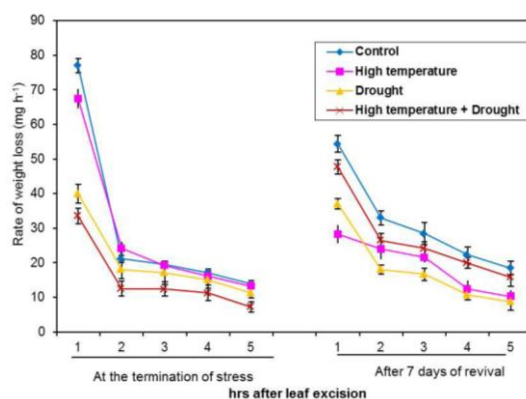


Fig. 4. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting + post-anthesis stage in var. UP2565.

however it was still lower in comparison to var. UP2565. Results from Fig. 7 reveal rate of ELWL when stress was imposed at booting +

post-anthesis stage. ELWL was high in drought (63.15 mg) and interactive HT+D (64.85 mg) than

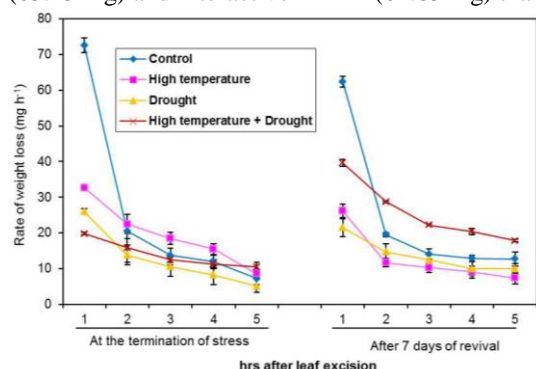


Fig. 5. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting stage in var. WH730.

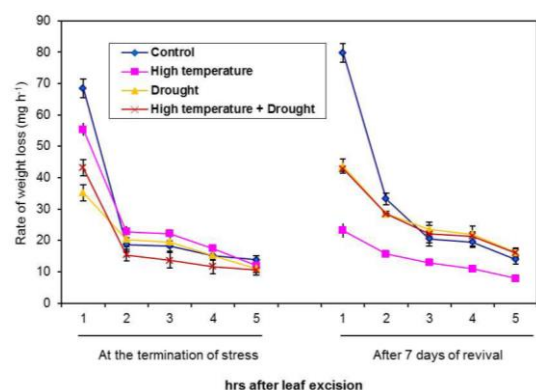


Fig. 6. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at post-anthesis stage in var. WH730.

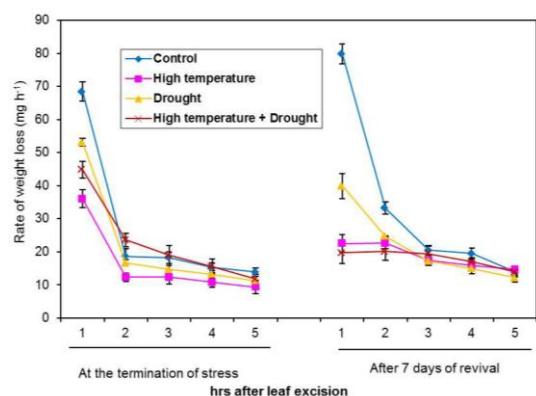


Fig. 7. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting + post-anthesis stage in var. WH730.

HT (36.05 mg) during 1st h of observation however, the same was less than that observed in UP2565 (Fig. 4). After revival, the ELWL of

drought relieved plants was higher (39.9 mg) in comparison to other two stress treatments.

The results show higher excised leaf water loss in UP2565 compared to WH730. This indicated that closing of stomata was not as rapid in UP2565 and continued to lose more water through transpiration than WH730. The result of stress treatments were more acute at post anthesis stage relative to other two stages for plants sampled after stress as well as revival. Relative to stress termination, decline in ELWL was noted after revival at each hour of observation (Fig. 5-7) thus suggesting persisting effects of stress treatments after one week of revival period.

Conclusion

ELWL may more closely reflect the balance between water supply to the leaf and transpiration rate. This improves the ability of the plant to recover from stress and consequently the grain yield and its stability. Genotypes indicating low excised leaf-water loss under drought or heat stress have better capability to maintain water balance in their leaves seems to be attributable to stress tolerance indicating considerable scope for selection under stress conditions. This parameter can be easily determined and is hence applicable for use in large populations.

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Research Information

Diversification of cytoplasmic male sterility in Indian varieties through back cross breeding

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Abstract

25 CMS lines received from CIMMYT, Mexico and 5 CMS lines from IARI, New Delhi were used for parental diversification in Indian wheat backgrounds through backcross breeding. Indian varieties were used as recurrent parent in order to recover their agronomic background. The extent of male sterility was taken into consideration during each backcross. A number of diversified CMS lines showing complete male sterility were evaluated and based on plant height and heading, 56 CMS lines were found promising for further utilization in hybrid wheat development programme.

Key words: bread wheat, hybrid cultivars, cytoplasmic male sterility, maintainer line, diversification

Introduction

India has a remarkable success story in wheat production and at present it is second largest wheat producing country after China. Wheat recorded all time highest production in India during 2013-14 but the yield levels are hovering around 3tonnes/hectare. At present the estimated wheat production of wheat in India is 93.50m tones (Anonymous, 2016). Major wheat areas of the country are now facing stagnation in yield levels that needs newer approaches to break yield barriers. Exploitation of heterosis and development of hybrids is an innovative approach in cereals for breaking yield barriers and realizing higher yields. Indian hybrid wheat programme has re-oriented in 1995 with introduction of some cytoplasmic male sterile (CMS) lines from CIMMYT, Mexico but these could not be utilized due to their agronomic background. In 2005, the diversification of these CIMMYT lines was initiated with the objective to develop new CMS lines in the agronomic background of Indian wheat varieties so that these can be further utilized in hybrid wheat development programme.

Materials and methods

25 CMS lines received from CIMMYT, Mexico and 5 CMS lines received from IARI, New Delhi were used as source of cytoplasmic male sterility. The lines from CIMMYT at CIMMYT were developed using *Triticum Timopheevii* cytoplasm in the base varieties MTSA 2A, Chuan 13A and Chuan 18A. CMS lines from IARI were also based on *T. timopheevii* cytoplasm. These CMS lines were used as female parent in first cross with Indian advanced varieties and elite material as male parent. Thereafter 8 generations of backcrosses were made with Indian varieties as recurrent parent in order to recover the respective agronomic background. In each cross, more than 100 spikes were pollinated. In every generation, five spikes of recipient population were bagged just after emergence from the flag leaf in order to ensure complete male sterility as indicated by no seed set (Virmani *et al.*, 1997).

After eight backcross generations, the resultant 73 male sterile lines were planted in the field along with their recurrent parent as maintainer line. These CMS lines were pollinated

Table 1. Performance of diversified new CMS (A) lines and their maintainers (B) lines for male sterility, heading days and plant height.

S No.	Line ID	Base CMS line	CMS (A) Line			Maintainer (B) line		
			Male sterility (%)	Days to heading	Pl. ht (cm)	Base line	Days to heading	Pl. ht. (cm)
1.	CMS 1A/8*PBW343	MTSA 2A/BCN	100	100	94	PBW343	100	96
2.	CMS 10A/8*PBW343	CHUAN 18A/CHUAN 18B//7*KAUZ/HEVO	100	101	91	PBW343	100	97
3.	CMS 11A/8*PBW343	CHUAN 18A/CHUAN 18B//7*PARUS	100	101	101	PBW343	100	95
4.	CMS 12A/8*PBW343	CHUAN18A/CHUAN18B/3/7*SERI/NKT//2*KAUZ	100	101	101	PBW343	100	99
5.	CMS 13A/8*PBW343	CHUAN 18A/CHUAN 18B//7*CMH80A542/ CNO79	100	102	98	PBW343	102	101
6.	CMS 17A/8*PBW343	CHUAN18A/3/7*KAUZ*2/MNV//KAUZ	100	102	95	PBW343	102	97
7.	CMS 20A/8*PBW343	CHUAN18A/3/7*HE1/5*CNO79//BORL95	100	99	95	PBW343	103	98
8.	CMS 3A/8*DBW17	CHUAN 18A/PRINIA	100	97	86	DBW17	97	88
9.	CMS 5A/8*DBW17	CHUAN 13A/CHUAN 13B/4/7*KAUZ/PFAU/VEE 5/3/KAUZ	100	97	85	DBW17	97	92
10.	CMS 8A/8*DBW17	CHUAN13A/5/7*ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	100	97	90	DBW17	97	91
11.	CMS 10A/8*DBW17	CHUAN 18A/CHUAN 18B//7*KAUZ/HEVO	100	97	89	DBW17	97	92
12.	CMS 12A/8*DBW17	CHUAN18A/CHUAN18B/3/7*SERI/NKT//2*KAUZ	100	97	82	DBW17	97	85
13.	CMS 13A/8*DBW17	CHUAN 18A/CHUAN 18B//7*CMH80A542/ CNO79	100	97	86	DBW17	97	89
14.	CMS 14A/8*DBW17	CHUAN18A/6/7*WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS*55/ALD	100	97	91	DBW17	97	87
15.	CMS 15A/8*DBW17	CHUAN18A//7*ATTILA/3BCN	100	97	84	DBW17	97	85
16.	CMS 18A/8*DBW17	CHUAN18A/4/7*KAUZ//VORONA/CNO79/3/KAUZ	100	97	85	DBW17	97	92
17.	CMS 21A/8*DBW17	CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	100	97	86	DBW17	97	88
18.	CMS22A/DBW17	CHUAN18A/ 2*CHUAN 18B/3*HE1/ 3*CNO79// *SERI/ 3/ATTILA	100	97	86	DBW17	97	87
19.	CMS 25A/8*DBW17	CHUAN18A/ 2*CHUAN 18B/3*URES /BOW//OPATA	100	97	85	DBW17	97	85
20.	CMS 26A/8*DBW17	Pusa 2019A-11	100	97	87	DBW17	97	83
21.	CMS 30A/8*DBW17	Pusa 2338A-20	100	97	86	DBW17	97	87
22.	CMS 2A/8*DBW16	MTSA 2A/8*RAYON	100	98	94	DBW16	98	98
23.	CMS 8A/8*DBW16	CHUAN13A/5/7*ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	100	98	96	DBW16	98	92

Table 1 (continued)

24.	CMS 10A/8*DBW16	CHUAN 18A/CHUAN 18B//7*KAUZ/HEVO	100	98	91	DBW16	98	96
25.	CMS 11A/8*DBW16	CHUAN 18A/CHUAN 18B//7*PARUS	100	98	88	DBW16	98	90
26.	CMS 12A/8*DBW16	CHUAN18A/CHUAN18B/3/7*SERI/NKT//2*KAUZ	100	98	95	DBW16	98	96
27.	CMS 15A/8*DBW16	CHUAN18A//7*ATTILA/3BCN	100	98	96	DBW16	98	97
28.	CMS 18A/8*DBW16	CHUAN18A/4/7*KAUZ//VORONA/CNO79/3/KAUZ	100	98	91	DBW16	98	99
29.	CMS19A/8*DBW16	CHUAN18A/4/7*ATTILA//ALTAR84/AOS/3/ATTILA	100	98	92	DBW16	98	90
30.	CMS21A/8*DBW16	CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	100	98	91	DBW16	98	89
31.	CMS 23A /8*DBW16	CHUAN18A/ 2*CHUAN 18B/3*KONKITU	100	98	90	DBW16	98	88
32.	CMS 1A/8*PBW502	MTSA 2A/BCN	100	102	98	PBW502	102	94
33.	CMS 6A/8*PBW502	CHUAN13A/CHUAN13B/3/7*OASIS/SKUAZ//4*BCN	100	102	102	PBW502	102	101
34.	CMS 21A/8*PBW502	CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	100	102	104	PBW502	102	95
35.	CMS 5A/8*DBW55	CHUAN 13A/CHUAN 13B/4/7*KAUZ/PFAU/VEE 5/3/KAUZ	100	99	94	DBW55	99	98
36.	CMS 9A/8*DBW55	CHUAN13A//7*OASIS/5*BORL95	100	99	92	DBW55	99	97
37.	CMS 15A/8*DBW55	CHUAN18A//7*ATTILA/3BCN	100	99	93	DBW55	99	98
38.	CMS 24A/8*DBW55	CHUAN18A/ 2*CHUAN 18B/3*SERI/KAUZ	100	99	95	DBW55	99	96
39.	CMS 21A/8*DBW55	CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	100	99	91	DBW55	99	90
40.	CMS 8A/8*DBW60	CHUAN13A/5/7*ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	100	93	98	DBW60	91	102
41.	CMS 20A/8*DBW60	CHUAN18A/3/7*HE1/5*CNO79//BORL95	100	93	101	DBW60	91	104
42.	CMS 23A/8*DBW60	CHUAN18A/ 2*CHUAN 18B/3*KONKITU	100	93	103	DBW60	91	106
43.	CMS 26A/8*DBW60	Pusa 2019A-11	100	94	101	DBW60	91	98
44.	CMS 2A/8*CBW38	MTSA 2A/8*RAYON	100	99	102	CBW38	99	105
45.	CMS 10A/8*CBW38	CHUAN 18A/CHUAN 18B//7*KAUZ/HEVO	100	99	106	CBW38	99	108
46.	CMS 15A/8*CBW38	CHUAN18A//7*ATTILA/3BCN	100	99	101	CBW38	99	102
47.	CMS 2A/8*RAJ3077	MTSA 2A/8*RAYON	100	95	103	RAJ3077	95	105
48.	CMS 8A/8*RAJ3077	CHUAN13A/5/7*ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	100	95	104	RAJ3077	95	110
49.	CMS 14A/8*RAJ3077	CHUAN18A/6/7*WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS*55/ALD	100	95	101	RAJ3077	95	98

Table 1 (continued)

50.	CMS 8A/8*DBW76	CHUAN13A/5/7*ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA	100	94	104	DBW76	91	111
51.	CMS 21A/8*DBW76	CHUAN18A/6/7*KAUZ*2/4/CAR//KAL/BB/3/NAC/5/KAUZ	100	93	103	DBW76	91	107
52.	CMS 2A/8*UP2338	MTSA 2A/8*RAYON	100	99	97	UP2338	99	104
53.	CMS 7A/8*GW411	CHUAN13A/CHUAN13B/3/7*URES/BOW//OPATA	100	100	103	GW411	98	101
54.	CMS 14A/8*PBW550	CHUAN18A/6/7*WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS*55/ALD	100	93	89	PBW550	91	90
55.	CMS 14A/8*RAJ4037	CHUAN18A/6/7*WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS*55/ALD	100	92	97	RAJ4037	90	101
56.	CMS 28A/8*PBW175	Pusa 2046A-8	100	95	103	PBW175	95	110

with maintainer lines for getting seeds of the CMS lines. During crop seasons 2014-15 and 2015-16, fifty six uniform cytoplasmic male sterile lines (A lines) were selected out of 73 and these were planted with respective maintainer lines (B lines) in 2B:4A:2B row ratio. All the recommended agronomic practices were adopted to raise a good crop. Five randomly selected spikes of these CMS lines were bagged at just after emergence from flag leaf and the seed set was observed in these un-pollinated spikes for calculating seed set percentage (Virmani *et al.*, 1997; Aruna *et al.*, 2013). The data were also recorded on days to heading and plant height for evaluating their suitability to hybrid wheat programme.

Results and discussion

The diversification of cytoplasmic male sterility in Indian varieties was initiated with varieties suitable for high fertility, timely sown irrigated conditions. New CMS lines were diversified in 14 different backgrounds namely, PBW 343, DBW 17, DBW 16, PBW 502, DBW 55, DBW 60, CBW 38, Raj 3077, PBW 550, DBW 76, UP 2338, GW 411, Raj 4037 and PBW 175. The seed set was observed among these lines and the results indicated no seed set in the bagged un-pollinated spikes of the CMS lines. This indicated complete male sterility in the new lines (Table 1). The heading among the new CMS lines was ranged between 92-102 days with mean value of 98 days. Compared to this, the heading among maintainer lines ranged between 90-103 days with mean value of 97 days. The plant height was also taken into consideration for identification of promising lines for use in hybrid programme. The plant height was ranged between 82-106 cm among CMS lines and 83-111 cm among maintainer lines. Mean plant height was 97 cm and 96 cm in new CMS lines and maintainer lines, respectively.

In hybrid development programme based on three line system, the maintenance of CMS lines is very crucial. An ideal CMS line should flower earlier than the maintainer line to get maximum seed set in the CMS line and it should be lesser in height for abundance availability of pollen grains. The results indicated that 44 lines out of 56 were earlier flowering than the respective maintainer lines and 41 CMS lines have less plant height. Among these, 32 lines were ideally had earlier flowering and dwarf plant stature compared to the respective maintainer lines. The diversified lines are in the agronomic backgrounds of PBW 343, DBW 17, DBW 16, DBW 55, CBW 38, Raj 3077, PBW 550, UP 2338 and PBW 175. These may be easily maintained by planting with maintainer

lines. Similar results were also reported by Venkatesh *et al.*, 2012, Aruna *et al.*, 2013. On the other hand, none of the new CMS line in the background of PBW 502, DBW 60, DBW 76, GW 411 and Raj 4037 had earlier heading and dwarf plants in combination. These lines may require additional support like rope pulling for facilitating pollination while maintaining them.

It may be concluded that these new diversified CMS lines may be used intensively for development of hybrid wheat for Indian conditions so that new breakthrough may be achieved in wheat productivity.

Acknowledgements

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Research Opinion & Topics

A report of the third term of the National BioResource Project-Wheat, Japan: Fiscal year 2015

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Key words: wheat, DNA marker, polymorphism, core-collection

Here I am summarizing the activities of the NBRP (National Bioresource Project) - Wheat in the fiscal year 2015, which was the fourth year of the third term of the NBRP. NBRP has been supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan and now supervised by the Japan Agency of Medical Research and Development (AMED, Japan). I am very grateful to the researchers directly and indirectly involving in the activities of NBRP-Wheat. Especially I would like to appreciate the tireless efforts given by Dr. Miyuki Nitta and Mr. Atsushi Ota, Graduate School of Agriculture, Kyoto University. They have been working on the new system to preserve seed stocks in a uniformed format. I shall summarize our activities in the year 2015 were a bit behind of the schedule that is mostly due to the unsystematic records of the stocks in the previous terms of NBRP-Wheat. We have been spending quite a lot of time to find out the proper bags of seeds to be distributed for the stocks that have been maintained at the Laboratory of Plant

Genetics. I hope we can complete the task to establish a seed storage and distribution systems in the remaining years of the third term.

At the beginning of the fiscal year 2015, Dr. Shotaro Takenaka left the team and he found a faculty position in the newly opened Faculty of Agriculture, Ryukoku University. I am most happy that he can continue working on wheat in the new position. At the end of the fiscal year 2015, Dr. Taihachi Kawahara left Kyoto University who has been the curator of the wild species and landraces for a long time. I would like to thank him from my deepest heart and hoping that he will keep his good health and enjoy his life in the rural area he moved after retirement.

The third term will be terminated in the fiscal year 2016. We will do our best to accomplish what we planned for the third term. I hope the Japanese government will keep supporting our activities to preserve, propagate and distribute the wheat genetic resources.



Research Opinion & Topics

The report of National Bioresource Project-Wheat III. Seed resources, 2015.

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Maintenance of seed resources: Regenerated seeds of total of 1,230 strains were harvested until early summer in 2015. Two hundred and eighty *Triticum araraticum* and *T. timopheevi* were grown in Kihara Institute for Biological Research, Yokohama City University (KIBR) and 950 strains consisting of several wild species and landraces were grown in Graduate School of Agriculture, Kyoto University. Total of 1,009

strains were sown in autumn 2015 at Laboratory of Crop Evolution (MOZUME), Graduate School of Agriculture, Kyoto University.

Distribution of seed resources: Total of 1,297 strains have been distributed to various researchers and institutions around the world (Table 1).

Table 1. Number of seed stocks distributed in 2015 (including in-house use)

Code (Institution) *	No. of strains distributed		
	Domestic	Overseas	Total
LPGKU	164	148	312
KU (MOZUME)	926	20	946
KT (KIBR)	20	19	39
Total	1,110	187	1,297

* LPGKU and MOZUME: Graduate School of Agriculture, Kyoto University,
KIBR: Kihara Institute for Biological Research, Yokohama City University



Research Opinion & Topics

National BioResource Project of Japan III : DNA resource of Wheat, 2015

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The third phase of the National BioResource Project of Japan “Wheat” has reached the fourth fiscal year in 2015. DNA resource team of NBRP-Wheat has continuously collected, maintained and supplied for cDNA clones of common wheat. At present, (1,252,563) ESTs (expressed sequence tags) were collected from the 55 cDNA libraries, in which 26,241 proof-read full-length cDNA sequences are included. BLAST search of those sequences can be applied from the “KOMUGI” site of National Institute of Genetics, Mishima, Japan. The wheat oligo DNA microarray harboring 38K gene probes is also available from the Agilent Technologies Co. Ltd.

Additionally, screening of target clones from TAC library of Chinese Spring wheat and cosmid library of goat grass (*Aegilops tauschii* KU2094) are under way in response to the request. In 2015 seasonal activity, 73 clones requested from five institutions inside and outside of Japan were supplied after DNA sequence check. Furthermore, 33 genomic clones of goat grass had been sent in requests of two institutions.

The work was supported by the National BioResource Project-Wheat from the Ministry of Education, Culture, Sports, Science and Technology, Japan.



Research Opinion & Topics

“Core-collection” Project of the National BioResource Project-Wheat, Japan: 2015 Progress report

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Key words: wheat, DNA marker, polymorphism, core-collection

National Bioresources Project-Wheat (NBRP-Wheat) stores nearly 12,000 accessions of wild species, landraces and historic cultivars of wheat and its relatives (*Triticum* and *Aegilops* species). The main body of the collections derives from the several scientific expeditions dispatched from Kyoto University. In the third term of NBRP-Wheat, we decided to establish core-collections of hexaploid wheat including *Triticum aestivum* and exotic hexaploid wheat, tetraploid wheat (both wild and cultivated, AABB and AAGG species), and diploid wheat (*T. monococcum*, *T. urartu*, and *T. boeoticum*). This is the annual report of the project summarizing the progress made in the fiscal year 2015. We believe that once the core-collections are established, they will facilitate wheat researches using genome data.

Hexaploid wheat core-collection

The hexaploid core-collection is open to public. The F₁ and F₂ seeds between the accessions (female parent) and Norin 61 or Chinese Spring wheat (male parent) are available with specific arrangement. Those who want to use the materials, please contact to nasushu@kais.kyoto-u.ac.jp. We propagated the seed of F₁ (six crosses) and F₂ (71 crosses) that were in short.

Tetraploid wheat core-collection

We have selected the tetraploid core-collection from the genotype data. In short, we genotyped a total of 2,008 AABB and 401 AAGG accessions

by the small number of DArT array markers. Based on the genotype data we established a tetraploid wheat core-collection with the 192 accessions representing the genetic diversity. The core-collection is extensively genotyped by the genotyping-by-sequencing approach (Poland et al., 2012) and the DArTseq available from Diversity Arrays Technology Pty. Ltd. We successfully propagated 190 of 192 accessions for seed delivery, and AABB accessions are also made crosses with durum cultivars Langdon and Kronos. In the fiscal year 2015, we made 190 crosses and got F₁ seeds in 134 combinations. We have recorded some agronomic characters for the accessions in the core-collection.

Diploid wheat core-collection

We finished DNA preparation for the 501 AA genome species. Those include 50 *T. monococcum* and 38 *T. urartu*, and 413 *T. boeoticum* accessions. We added 140 *T. urartu* and one *T. boeoticum* accessions introduced from USDA for genotyping. Genotyping was performed by DArTseq technology. As a result, we established a diploid wheat core-collection of a total of 160 accessions (3 *T. monococcum*, 93 *T. boeoticum*, and 64 *T. urartu*). We propagate them in the fiscal year 2015.

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two-enzyme genotyping-by-sequencing
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Others

Editorial Remarks

We are pleased to announce the publication of the Wheat Information Service volume 122.

Wheat Information Service as the electronic newsletter, namely eWIS, was launched in 2006 and has tried to promote the exchange of information among wheat geneticists and breeders for eleven years. Thanks to several authors, we have published a total of 126 articles to date, which consists of 63 articles (2.9 per volume) for Research Information, 46 articles (2.1 per volume) for Research Opinion & Topics, and 17 articles (0.8 per volume) for meeting reports. We will continue the effort to make this journal more active.

Since the launch of eWIS, the articles were first published in HTML format for its promptness. And then, the articles, collected and laid out in a PDF file, were published as one volume biannually. However, after continuous and constructive discussion for several years, we made a decision to modify the system for the convenience of authors and readers. We believe that these modifications will not prevent prompt publication. Following modifications have been applied from the latest volume 122.

1. The accepted article is edited and published as a PDF file which is suitable for printing. It appears in the “Latest Articles” section.
2. The articles are collected biannually, and are organized as a volume in “Archives” page. There, each article is downloadable separately.
3. Supplemental data (PDF or Excel), which cannot be included in the manuscript for the reason of space, can be submitted.
4. Each article is specified by article number, which is assigned after acceptance.

December, 2016

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Manuscript Categories

eWIS accepts the following categories of papers:

- (1) **Research information:** Original research articles in the field of wheat sciences

The manuscript should start with a title, the names of author(s), affiliation(s), abstract, followed by the text. Abstract may be omitted if not necessary. There is no fixed limit on the length but a concise presentation is encouraged.

- (2) **Research Opinion & Topics:** Reviews, minireviews, trends and topics in wheat research.

Authors who wish to submit a (mini-)review should contact the Editorial Office prior to submission.

- (3) **Meeting Reports:** Announcement of forthcoming meeting and reports on the meeting attended

- (4) **Others:** Any other information useful for wheat researchers

Title, Affiliation and Abstract

In the title page(s), the manuscript category (as mentioned above), a title, the names of the author(s), affiliation(s) and address(es) of the authors, and the e-mail address, telephone, and fax numbers of the corresponding author must be clearly indicated.

The Abstract (100-250 words) may not contain references.

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References should be cited in the text by the author(s) and year, and listed at the end of the text with the names of authors arranged alphabetically. When an article has more than two authors, only the first author's name should appear, followed by "et al.", in the text. The references should be formatted as follows.

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Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. *Theor Appl Genet* 60:229-236.

Book chapters:

Peacock WJ, Dennis ES, Gerlach WJ (1981) Molecular aspects of wheat evolution:

repeated DNA sequences. In: Evans LT and Peacock WJ (eds.) Wheat Science - Today and Tomorrow. Cambridge Univ. Press, Cambridge, UK, pp. 41-60.

Books:

Knott DR (1989) The Wheat Rusts - Breeding for Rust Resistance. Springer-Verlag, New York, USA.

Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., K. Tsunewaki personal communication).

Abbreviations

Abbreviations should be explained at first occurrence.

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Gene names and protein names must carefully be discriminated. Gene names and loci should be italicized; protein should be upright. The SI units (<http://physics.nist.gov/Pubs/SP330/contents.html>) should be used throughout.

Nomenclature

Nomenclature of genes and chromosomes should follow the 'Catalogue of gene symbols for wheat' (McIntosh et al.: 10th Int. Wheat Genet. Symp. 2003).

Nucleotide sequences

The DDBJ/EMBL/GenBank accession numbers must be provided for newly reported nucleotide sequences.

Tables

Tables must be numbered consecutively. For Table writing, Microsoft Word is recommended. Prepare a separate file for each table. Refer to the latest eWIS articles for format.

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Figures must be numbered consecutively. Prepare a separate file for each figure.

Outline of the publication process

Authors of accepted manuscripts are informed by e-mail that a temporary URL has been created from which they can obtain their proof in PDF format. Proofreading is the responsibility of the author. Authors should make proof corrections and send them to Editorial Office by e-mail. After online publication, corrections can only be made in exceptional cases when Editorial Office permits the necessity.

The final version of accepted manuscripts will be published in the 'Latest Articles' section of the eWIS web page upon receipt of proof corrections. Editorial Office biannually gathers the accepted manuscripts published in the 'Latest Articles' into a volume. In 'Archive' of eWIS, all manuscripts are open to all wheat researchers.

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