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## **Evaluating Boron Efficiency in Heat Tolerant Wheat Germplasm**

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#### **Abstract**

Heat tolerance is a desirable trait for wheat in the sub-tropics and in traditional wheat areas under threat from global warming, where the crop may also be exposed to boron (B) deficiency. The 13<sup>th</sup> High Temperature Wheat Yield Trial (13HTWYT) from CIMMYT was evaluated with Fang 60, a 1977-bred variety as a B-efficient check, in a sand culture with (+B) and without (-B) added B in the nutrient solution in Chiang Mai, Thailand where temperature for the coolest month and during the time of heading both averaged 22.3°C. The wheat genotypes were affected differently in their grain set and grain yield by B deficiency (G x B, p<0.001). Responses to B of the 13HTWYT expressed as grain yield in -B relative to +B correlated positively with the grain set index and the relative number of grains/spike, but not with the relative number spikes, while profuse tillering in -B was associated with lower yield. In -B grain yield also increased with the number of grains/spike. When B was not limiting, the grain yield increased with days to heading, number of tillers/plant, spikes/plant, spikelets/spike, grains/spike and grains/spikelet. Based on their grain set in -B, 39% of the 13HTWYT were considered B-inefficient, with grain set in fewer than half of competent florets, while 26% were B-efficient in the same order as Fang 60, 16% moderately efficient and 18% moderately inefficient. Boron efficiency is available in the 13HTWYT, suggesting that joint tolerance for B efficiency and heat stress can be selected if required. © 2019 Friends Science Publishers

Keywords: Boron; Grain set; Heat tolerance; Wheat; Yield formation

## Introduction

The world's wheat is grown largely outside the tropics, with temperature for optimum growth at about 25°C (Briggle, 1980) and in the sub-tropics the crop is grown during the cool months, from October to February in the northern hemisphere. In Asia, where farmers face rising wage and water scarcity, wheat provides a promising dry season alternative to water-hungry rice and labour-intensive vegetables. However, tolerance to high temperature is essential for avoiding yield loss due to heat stress in much of the area that is subjected to average temperature for the coolest month of > 17.5°C (Fischer et al., 2014), designated the wheat mega-environment 5 (ME5) by CIMMYT (the International Maize and Wheat Improvement Centre). Interest in heat tolerant wheat in India, which accounts for most of the global wheat in ME5, has been stimulated by observation of 34% yield loss due to heat stress in the country's major wheat cultivars and the concern about climate change (Mishra et al., 2014). In addition to ME5, several other wheat mega-environments are also coming under threat of rising temperature (Braun and Payne, 2012).

Some of the first heat tolerant wheat genotypes were identified in the Hot Climate Wheat Screening Nursery, an exchange of wheat germplasm among breeders in hot environment as well as materials generated at CIMMYT for seven seasons in the 1980's (Mann, 1994). Since then wheat breeding for stress-prone environments has been more precisely targeted by the application of physiological understanding of tolerance to high temperature combined with economical and easy to use phenotyping methods such as the measurement of canopy temperature, notably for tolerance to heat and drought (Reynolds *et al.*, 2012).

In addition to the problem of heat stress, however, wheat yield in high temperature environments has also been reported to be depressed by boron (B) deficiency, including the Brazilian cerrados (Ciba-Geigy da Silva and de Andrade, 1983), south-western China (Yang, 1992), north-western states of India and along the Indo-Nepal border (Singh *et al.*, 1976; Tandon and Naqvi, 1992), to Nepal (Misra *et al.*, 1992; Subedi *et al.*, 1997) and Bangladesh (Reuter, 1987; Rerkasem, 1996). However, genotypic variation in responses to B is especially broad among crop species, including wheat (Rerkasem and Jamjod, 1997a).

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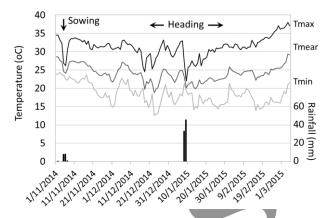
Boron-efficient wheat genotypes may yield normally in the same soil in which B-inefficient genotypes suffer severe yield losses due to B deficiency, as has been found in CIMMYT's international wheat germplasm so far evaluated, although B-inefficient genotypes were in the majority (Rerkasem and Jamjod, 1997b; Rerkasem *et al.*, 2004). This paper reports on an evaluation of a relatively recent heat tolerant wheat germplasm from CIMMYT for B response and yield potential in a high temperature environment in comparison with Fang 60, a local B-efficient check.

## **Materials and Methods**

The 13th High Temperature Wheat Yield Trial (13HTWYT) distributed by CIMMYT in 2014 with 49 entries and Fang 60 as local check was grown in a sand culture experiment at Chiang Mai University (18.81°N; 98.95°E). Each of the 50 entries was grown with 2 B treatments in duplicated drainable pots (Ø 30 cm, 30 cm deep) containing quartz sand with no detectible B, with 5 plants/pot. The pots were watered twice daily with 1 litre/pot of complete nutrient solution containing 10 µM  $H_3BO_3$ , 10  $\mu$ M Fe-EDTA, 1  $\mu$ M MnSO<sub>4</sub>, 0.5  $\mu$ M ZnSO<sub>4</sub>, 0.2 μM CuSO<sub>4</sub>, 0.1 μM CoSO<sub>4</sub>, 0.1 μM Na<sub>2</sub>MoO<sub>4</sub>, 250 μM K<sub>2</sub>SO<sub>4</sub>, 1000 μM CaCl<sub>2</sub>, 250 μM MgSO<sub>4</sub>, 500 μM KH<sub>2</sub>PO<sub>4</sub>, and 5 mM KNO<sub>3</sub> (+B), or the same but without B added to nutrient solution (-B) (Rerkasem et al., 2004). Days to heading were determined when 50% of the spikes had emerged. At maturity plants were harvested to determine seed vield, number of tillers/plant, spikes/plant and weight of individual seed for the whole pot (5 plants); number of spikelets/spike, number of grains/spike and grain set index (percentage of grain in the first two basal florets of 10 central spikelets) were determined on 10 spikes from each pot (main stem plus first tiller). Measures of performance of a genotype in B deficiency relative to B sufficiency were expressed as the -B value divided by the corresponding +B value.

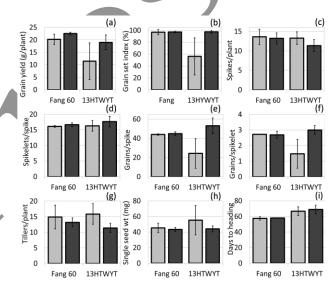
## Results

January was the coolest month during the experimental period, with monthly average temperature  $22.3^{\circ}$ C, mean minimum  $16.8^{\circ}$ C and maximum  $29.1^{\circ}$ C; 19 mm of rain fall in early November 2014 and 76 mm in early January 2015 (Fig. 1). Temperature from sowing to heading averaged  $24.8^{\circ}$ C, mean minimum  $19.9^{\circ}$ C and maximum  $31.2^{\circ}$ C, with temperatures during heading (21 December to 28 January) coincided exactly with the temperatures during the coolest month. Genotypic variation within the 13HTWYT was indicated by significant effects of genotypes and genotype by boron interaction on grain yield and some yield components (Fig. 2 and Table 2). Highly significant genotypic variation in the response to B was observed in grain yield, grain set index, and the number of tillers/plant, grains/spikelet, and grains/spike (p<0.001), but not in the number of spikes/plant



**Fig. 1:** Temperature and rainfall in Chiang Mai during the experimental period, with sowing and heading time for the sand culture experiment

Source: Chiang Mai Meteorological Centre



**Fig. 2:** Grain yield (a), grain set index (b), yield components (c-h), and days to heading of the heat tolerant wheat germplasm 13HTWYT and boron efficient check Fang 60 in a sand culture with (+B, ■) and without (-B, ■) added B (with standard deviation bars)

and weight of individual seed. Days to heading differed significantly among the 13HTWYT genotypes, which generally took longer (averaging 68±6 days) than the 58±1 days for Fang 60, but with no apparent effect of B. In +B, the number of grains/spikelets and grains/spike of the 13HTWYT genotypes averaged higher than Fang 60. The weight of individual seeds was generally higher in -B, although in +B it was not significantly different among the genotypes. The variation in the B response in the 13HTWYT was indicated by exceptionally large standard deviations in -B, with coefficient of variation for grain yield, number of grains/spike, grains/spikelet and grain set index ranging from 53 to 65%.

**Table 1:** Relationship between grain yield and days to heading, yield components and grain set index of a heat tolerant wheat germplasm (13HTWYT) in sand culture with (+B) and without (-B) added boron in the nutrient solution

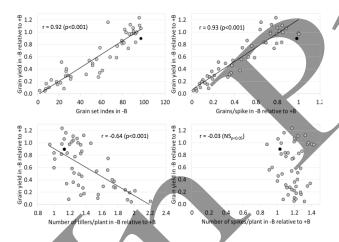
Boron treatment	+B	-B	+B	-B		
	Correlation coefficient (r) <sup>a</sup>					
	With grain yield		With grain set index			
Grain yield (g pot <sup>-1</sup> )	-	-	0.05(NS <sub>0.05</sub> )	0.90***		
Days to heading	0.35(p<0.05)	$0.10(NS_{0.05})$	-0.28(NS <sub>0.05</sub> )	$0.11(NS_{0.05})$		
Tillers plant <sup>-1</sup>	0.55(p < 0.001)	-0.52(p<0.001)	$-0.00(NS_{0.05})$	-0.63***		
Spikes plant <sup>-1</sup>	0.56(p < 0.001)	$0.03(NS_{0.05})$	$0.00(NS_{0.05})$	-0.02(NS <sub>0.05</sub> )		
Spikelets spike <sup>-1</sup>	0.34(p<0.05)	$0.15(NS_{0.05})$	$-0.09(NS_{0.05})$	$0.07(NS_{0.05})$		
Grains spike-1	0.41(p<0.01)	0.93(p<0.001)	$0.04(NS_{0.05})$	0.96(p<0.001)		
Grains spikelet <sup>-1</sup>	0.34(p<0.05)	0.93(p<0.001)	$0.18(NS_{0.05})$	0.96(p<0.001)		
Seed weight (mg seed-1)	$-0.12(NS_{0.05})$	$-0.05(NS_{0.05})$	-0.06(NS <sub>0.05</sub> )	-0.16(NS <sub>0.05</sub> )		
Grain set index (%)	$0.05(NS_{0.05})$	0.90(p<0.001)	-	-		

<sup>&</sup>lt;sup>a</sup>Significance of linear association by analysis of variance in brackets

**Table 2:** Significant effects of genotype (G), boron (B), and GxB, by analysis of variance

Effect	Grain yield	Tillers/plant	Spikes/plant	Spikelets/spike	Grains/spike
G	***(3.6)	***(3.4)	***(2.3)	***(1.1)	***(6.3)
В	***(0.7)	***(0.7)	***(0.5)	***(0.2)	***(1.3)
GxB	***(5.1)	*(4.8)	$NS_{0.05}$	*(1.6)	***(9.0)
Effect	Grain set index	Grains/spikelet	Seed weight	Days to heading	
G	***(10.2)	***(0.33)	$NS_{0.05}$	***(7.2)	
В	***(2.0)	***(0.07)	***(4.9)	<b>**</b> (1.4)	
GxB	***(14.0)	***(0.47)	$NS_{0.05}$	NS <sub>0.05</sub>	

(\* p<0.05; \*\* p<0.01; \*\*\* p<0.001), with LSD<sub>0.05</sub> in brackets



**Fig. 3:** Association between grain yield in -B relative to +B and grain set index in -B (upper left), the number of grains/spike (upper right), tillers/plant (lower left) and spikes/plant (lower right), in -B relative to +B of a heat tolerant wheat germplasm (13HTWYT) with Fang 60 (●) as the B-efficient check. (Each data point is mean from 2 replications)

Grain yield response to B of the 13HYWYT wheat genotypes, expressed as the yield in -B relative to +B ranged from 0.05 to 1.21 (the relative yield in the B-efficient check Fang 60 was 0.90), were closely and positively associated with their grain set index (r=0.92, p<0.001) and the relative (-B/+B) number of grains/spike (r=0.93, p<0.001), negatively with the relative number of tillers/plant (r=-0.64,

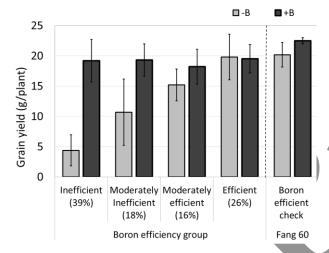
p<0.001), but not with the number of spikes/plant (r=-0.03, NS<sub>p<0.05</sub>) (Fig. 3). The B status had different effects on the relationship between grain yield and days to heading and yield components (Table 1). When B was not limiting grain yield increased with days to heading and most of the yield components. In +B grain yield was positively correlated with days to heading (r=0.35, p<0.05), spikes/plant (r=0.56, p<0.001), spikelets/spike (r=0.34, p<0.05), grains/spike (r=0.41, p<0.01) and grains/spikelet (r=0.34, p<0.05), but not with grain set index (r=0.05, NS<sub>0.05</sub>) and weight of individual seeds (r=-0.12, NS<sub>0.05</sub>). When B was limiting in -B grain yield correlated strongly and positively with the grain set index (r=0.90, p<0.001), number of grains/spike (r=0.93, p<0.001), and grains/spikelet (r=0.93, p<0.001).

The 13HTWYT genotypes were classed into 4 B efficiency groups based on their grain set index in -B, in which the B-efficient check Fang 60 set grain normally (Fig. 4). The B-inefficient group, comprising 39% of the germplasm, set grain in less than half of competent florets in -B (grain set index < 50%), and produced grain yield averaging 23% of the yield in +B. The B-efficient group, accounted for 26% of the germplasm, had >85% grain set index in -B and indistinguishable grain yield in -B and +B, as in the B-efficient check Fang 60. The moderately Befficient group (grain set index 71-85% in –B) accounted for 16% of the germplasm and in -B produced grain yield approaching that in +B. Moderately B-inefficient group (grain set index 51-70% in -B) accounted for 18% of the germplasm and in –B produced 55% of the grain yield in +B. Pedigrees of the B-efficient wheat genotypes from the 13HTWYT are listed in Table 3.

Table 3: Boron efficient genotypes in the 13HTWYT, with grain set index >85% in sand culture without added boron (-B)

	Genotype			Grain set index in -Ba	
1	PRL/2*PASTOR//WHEAR/SOKOLL		97.0	(107±12)	
2	QUAIU #1/SUP152		95.8	(111±1)	
3	FRANCOLIN #1		95.5	(121±37)	
4	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX/S'	TAR/3/HE1/3*CNO79//2*SERI	93.3	(128±48)	
5	FRET2*2/BRAMBLING//KIRITATI/2*TRCH/3/FRET2/TUKURU//FRET2			(84±0)	
6	KIRITATI//HUW234+LR34/PRINIA/3/FRANCOLIN #1/4/BAJ #1			(98±18)	
7	BAJ #1		90.8	(98±18)	
8	CROC_1/AE.SQUARROSA (213)//PGO/10/ATTILA*2/9/KT/BAGE//F	FN/U/3/BZA/4/TRM/5/ALDAN/6/	90.0	$(102\pm11)$	
	SERI/7/VEE#10/8/OPATA/11/ATTILA*2/PBW65				
9	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/BECARD		89.8	$(107\pm 9)$	
10	KIRITATI//HUW234+LR34/PRINIA/3/CHONTE/5/PRL/2*PASTOR/4/CHOIX/S	TAR/3/HE1/3*CNO79//2*SERI	88.3	(99±33)	
11	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/KIRITATI/2*TRCH		87.0	(112±1)	
12	FRANCOLIN #1*2//ND643/2*WBLL1		86.3	$(97\pm14)$	
13	FRANCOLIN #1/CHONTE//FRNCLN		85.3	(74±8)	
	Boron efficient check, Fang 60 (PIIFDIIPI/MZ/3/MXP)		97.0	(90±7)	

<sup>&</sup>lt;sup>a</sup> Grain yield in -B as % of the yield in +B ± standard deviation in brackets



**Fig. 4:** Average grain yield (± standard deviation) of wheat genotypes from the 13HTWYT classed by grain set index in –B (grain set index <50%, inefficient; 51-70%, moderately inefficient; 71-85%, moderately efficient; and efficient, >85%), compared with B-efficient check Fang 60

## **Discussion**

The strong genotype by B interaction effects on grain yield, grain set index, number of tillers/plant, grains/spike and grains/spikelet, and the lack the interaction for days to heading, number spikes and spikelets and weight of individual grain mean that the relevance of these parameters to yield in the 13HTWYT need to be considered separately, when B was not limiting (+B) and when it did (-B). Significant correlation between grain yield and days to heading, number of tillers, spikes, spikelets and grains indicated that these parameters contributed positively to grain yield when B was not limiting. The 13HTWYT genotypes were clearly differentiated from Fang 60 by heading much later and having greater number of grains/spike and grains/spikelet (as the result of more competent florets in each spikelet) in +B. The positive correlation between grain

yield and days to heading that ranged from 59 to 79 days, found here contrasts with the wheat genotypes of the 1980's to 1990's that tended to have negative correlation between yield and days to heading when grown under high temperature. For example, grain yield of 84 wheat genotypes grown under heat stress was shown to correlate negatively (r=-0.55, p<0.01) with days to heading ranged from 29 to 64 days, with an average of 54 days (Ortiz Ferrara *et al.*, 1994).

Accelerated development towards reproduction before the plant had developed sufficient leaf area and source capacity has been suggested as one of the ways in which wheat yield is limited by high temperature (Rawson, 1988). The advantages of longer time for vegetative development before flowering in the 13HTWYT, however, did not result in higher grain yield than Fang 60 (PIIFDIIPI/MZ/3/MXP) which was bred in Pakistan in 1977, selected in Thailand and released in 1987 (Rerkasem, 1996). Although the number of tillers/plant and spikes/plant were strongly correlated with grain yield in +B, the 13HTWYT however generally had fewer spikes than Fang 60. The 13HTWYT was superior to Fang 60 by having more spikelets/spike, grains/spikelet and grains/spike, when B was not limiting. All these suggested some improvement in heat tolerance, as previously high temperature had been noted to reduce tillering, size of the spike, grain size and test weight, and thus adversely affecting biomass and grain yield (Rajaram, 1988).

The adverse effect of high temperature on wheat yield is likely to depend on the seasonal temperature pattern. For example, wheat in the Indo-Gangetic Plain is subjected to high temperature from flowering to maturity, whereas in Southeast Asia the wheat crop is exposed to high temperatures from sowing to maturity (Rajaram, 1988). Some heat tolerance studies of wheat have focused on the heat stress on reproduction and grain filling (Farooq *et al.*, 2011). This study has shed light on wheat adaptation to high temperature for the entire growing season, with grain yield of the heat tolerant wheat germplasm of the 13HTWYT responding positively to longer period of development to

heading, tillering ability and number and size of the spikes (more spikelets with more competent florets), when water and nutrients are not limiting.

Boron efficiency is an agronomic term for describing ability, without any mechanism being inferred, of plant genotypes to grow and yield well in soil in which B is limiting for other genotypes (Rerkasem and Jamjod, 1997b), which has been extended from the idea proposed and applied to zinc and nutrients in general (Graham, 1984). By this definition, wheat genotypes have been identified as B-inefficient (e.g., SW41, BL1022) and B-efficient (e.g., Sonora 64, NL460) in the field in Nepal, south-western China and Campinas in Brazil, as well as several locations in Thailand (Rerkasem, 1992; Subedi et al., 1997). The mechanism for B efficiency in Fang 60, confirmed in field tests in Nepal and Thailand and in several sand culture trials (Anantawiroon et al., 1997; Rerkasem and Jamjod, 1997b, Rerkasem et al., 2004) has been demonstrated by tracing its ability to deliver limited <sup>10</sup>B supply B from the roots to the developing anthers and pollen in the growing spike, while grain set in B-inefficient SW41 with the same limited external source of B failed because it was unable to do the same (Nachiangmai et al., 2004). Thus Fang 60 was able to maintain sufficient level of B in its anthers in low B soil in which anther B concentration in Binefficient genotypes was depressed by B deficiency (Rerkasem and Jamjod, 2004).

The 13HTWYT was predominantly B-inefficient, with grain yield in –B being determined by the adverse effect of B deficiency on grain set. Tillering was enhanced by B deficiency, just as lateral branching was stimulated by B deficiency that depressed apical growth in mung bean (Rerkasem et al., 1990) and soybean (Rerkasem et al., 1997c). The degree to which tillering was increased by B deficiency in the 13HTWYT genotypes was inversely related to the level of their B efficiency, as indicated by the negative correlation between number of tillers and grain set index in -B. However, profuse tillering in -B was in fact detrimental to grain yield which declined with increasing number of tillers, even while grain yield in +B increased with increasing number of tillers. The international wheat germplasm from CIMMYT previously evaluated (the 4HTWYT, 2<sup>nd</sup> and 3<sup>rd</sup> International Breeding Nursery, 5th and 6th Hot Climate Wheat Screening Nursery, 17<sup>th</sup> Elite Selection Wheat Yield Trial, 18<sup>th</sup> Semi-arid Areas Wheat Screening Nursery and the 33<sup>rd</sup> International Bread Wheat Screening Nursery) were largely B-inefficient, but some B-efficient genotypes were also identified. Sixty genotypes from a total of 759 (8%) set grain normally along with Fang 60 under the condition in which 60% of the genotypes set grain in less than half of their competent florets (Rerkasem and Jamjod, 1997b; Rerkasem et al., 2004). With grain set index in -B >85% in 13 of 49 genotypes, the 13HTWYT (distributed in 2014) was more B-efficient than the 4HTWYT (distributed in 1999) in which only 2 genotypes having grain set in -B in the same range as Fang 60.

## Conclusion

Boron efficiency is a trait already available in the heat tolerant international wheat germplasm and can be easily selected for, if so desired. Where B is not limiting, the yield potential of the heat tolerant 13HTWYT might be increased simply by increasing the stand density by increasing seed rate to compensate for their limited tillering and number of spikes/plant.

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