# Stability analysis for grain yield and micronutrients in bread wheat genotypes

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#### Abstract

6 This study was carried out in order to determine stability of some traits plant height, days to 7 heading, 1000-grain weight, grain Zinc and Iron concentrations and grain yield of fifty bread 8 wheat genotypes. The experiment was conducted at three environmental conditions during 2015-9 2016 using randomized block design with two replicates. For all the traits investigated in this study, component of variation due to environment was larger than the component of variation 10 due to genotype and G x E interaction. Different traits like plant height, days to heading, 11 thousand grain weight, grain iron and zinc concentrations and grain yield showed range from 12 92.8 to 107.1 cm, from 91 to 101 days, from 32.0 to 46.1 g, from 37.5 to 45.7 ppm, from 30.2 to 13 41.9 ppm and from 2.1 to 3.3 kg, respectively over three environments. Two stability parameters 14 were used to develop and evaluation of stable genotypes. The study of genotypic stability 15 showed that the adaptation ability of the 8 genotypes (403, 413, 416, 428, 430, 435, 440 and 449) 16 for grain Fe concentration and 2 genotypes (410 and 431) for grain Zn concentration are 17 relatively high and they are more stable than the other genotypes. Also, genotype number 440 for 18 grain Fe concentration, genotypes 410 and 431 for grain Zn concentration and genotypes 420 and 19 425 for grain yield had high mean value compared with mean value of check genotype 401 as 20 well as high stability. 21

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23 Key words: Grain Fe, Grain Zinc, Grain yield, Genotype x environment interaction, Stability

### 25 Introduction

For humans, cereals are the main source of micronutrient minerals. Biofortification, which aims to improve micronutrient concentrations and bioavailability in plant based foods through genetic enhancement, is a cost effective way of solving the micronutrient deficiency problem (Bouis 2002; Nestel et al. 2006). Knowledge of the difference in the trait among the available germplasm is required for breeding of cereal crops with improved micronutrient concentration (Liu et al. 2006; Morgounov et al. 2007).

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For zinc (Zn) and iron (Fe) concentrations, a significant genotype x environment ( $G \times E$ ) interactions have been observed in wild and improved wheat cultivars (Oury et al., 2006; Ortiz-Monasterio et al., 2007; Trethowan, 2007; Gomez-Beccara et al., 2010a). Particularly, in case of grain Zn concentration, environmental conditions complicate the breeding, specially the soil composition (Trethowan, 2007). Thus, despite advances in breeding for uptake efficiency or mobilization to the grain, grain Zn concentration is restricted by Zn availability in the soil (Ortiz-Monasterio et al., 2007; Ortiz-Monasterio et al., 2011, Garg et al., 2014).

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The high Zn lines developed at CIMMYT, Mexico, and evaluated in a multilocation trial in India's Eastern Gangetic Plains (EGP), revealed that wheat grain Zn concentrations were highly unstable (Joshi et al., 2010) as the performance of the elite lines varied across locations and years. Cause for greater  $G \times E$  interaction for grain Zn concentration may be its quantitative inheritance, as reported in maize (Long et al., 2004), rice (Gregorio et al., 2000) and wheat

46 (Trethowan, 2007). One more study tested biofortified wheat lines at multiple locations in South 47 Asia and revealed high heritability and high genetic correlation between locations for grain Zn, 48 suggesting that  $G \times E$  may not be a serious issue in breeding high Zn wheat genotypes (Velu et 49 al., 2012, Velu et al., 2013).

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For breeders, stability of micronutrients is important in terms of changing ranks of genotypes across environments and affects selection efficiency (Mut et al., 2010). A genotype is therefore considered to be stable if its contribution to the  $G \times E$  interaction is low. Several stability measures including univariate and multivariate ones have been developed to assess the stability and adaptability of varieties. The most widely used is the joint regression including regression coefficient (b<sub>i</sub>) (Finlay and Wilkinson, 1963) and variance of deviations from regression (S<sup>2</sup><sub>di</sub>) (Eberhart and Russell, 1966).

59 Thus, in present investigation, 50 bread wheat genotypes developed by CIMMYT, 60 Mexico were used to evaluate their stability in plant height, days to heading, 1000-grain weight, 61 grain Zinc and Iron concentrations and grain yield across three environments in NWPZ 62 (Northern Western Plains Zone).

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## 64 Materials and Methods

## 66 *Plant material*

Fifty lines of bread wheat (*Triticum aestivum* var. aestivum) including one check cultivar HD 3086 (401) were grown at three sites in NWPZ (Ludhiana, Bathinda, Gurdaspur) during 2015-16 crop season. Each line was sown in two replicate plots of 5 metre long with six rows spaced at a distance of 20 cm. Recommended package of practices was followed to raise a good crop. Observations were recorded on plant height (cm), days to heading (days), 1000-grain weight (gm), grain yield (kg/plot), grain Zn concentration (ppm) and grain Fe concentration (ppm).

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## 75 Grain analysis

The concentration of elements Fe and Zn in wheat grains was determined using a benchtop, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK), previously standardized for high throughput screening of Zn and Fe in whole wheat grain (Paltridge et al. 2012).

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## 81 *Statistical analysis:*

Combined analysis of variance on data from trials in three environments was computed 82 83 according to the method given by Comstock and Moll (1963). Two stability parameters were applied to assess stability performance of genotypes and to identify superior genotypes; b<sub>i</sub>, the 84 linear regression of the phenotypic values on environmental index (Finlay and Wilkinson, 1963) 85 and  $S^{2}_{di}$ , the deviation mean square from regression (Eberhart and Russell, 1966). Analysis was 86 performed using the statistical software OPSTAT for ANOVA and for stability statistics. To 87 predict stability, a genotype was considered stable for grain Zn and Fe concentrations if it 88 appeared stable in two stability analysis. Genotypes that proved to be stable for both stability 89 90 parametres were then selected as the best.

#### 92 **Results and Discussion**

93 This study aimed to define environmental adaptation and stability features and the 94 relationships between stability parameters using 50 bread wheat genotypes that were grown in 95 the ecological conditions of three locations of NWPZ.

The combined analysis of variance for plant height, days to heading, 1000-grain weight, 96 97 grain Zn and Fe concentration and grain yield across environments is given in Table 1. The difference between environments and genotypes and all interactions for most of the traits 98 99 investigated were statistically significant (p<0.01). Analysis of variance showed significant  $G \times$ E interaction. For all the traits investigated in this study, components of variation due to 100 genotype and G x E interaction were smaller than the component of variation due to 101 environment. These results are similar with the results of earlier studies (Garg et al., 2014, Mut et 102 al., 2010; Robert, 2002; Rharrabti et al., 2003). 103

Values of the mean, regression coefficient ( $b_i$ ) and deviation from regression ( $S_{di}^2$ ) are given in Table 2. The mean values of total 47 genotypes (from 32.3 to 41.9 ppm) for grain Zn concentration had better performance than check HD 3086 (genotype 1, 31.2 ppm). Twenty two genotypes had better performance for grain Fe concentration (from 41.2 to 45.7ppm) in terms of mean values than genotype 401 (41.1 ppm). For grain yield per plot, four genotypes had better performance (from 3.3 to 3.1 kg) as check (3.1 kg).

In general, genotypes with high yield, regression coefficient (bi) close to 1, and nonsignificant deviation from the regression line are considered as the most desirable (Eberhart and Russell, 1966; Becker and Leon, 1988; Kurt Polat et al., 2016). Value of regression coefficient less than 1 indicates that the genotype can adapt to poor environmental conditions, whereas a *bi* value greater than 1 indicates that the plant can adapt to favourable environmental conditions (Yildirim *et al.*, 1979, Akgun and Altındag, 2011).

The value of  $b_i$  of five genotypes (413, 424, 408, 448, 437) for plant height; eight genotypes (425, 444, 414, 448, 450, 439, 421, 445) for days to heading; four genotypes (426, 409, 445, 439) for 1000-grain weight; three genotypes (426, 424, 412) for grain Zn concentration and ten genotypes (435, 416, 441, 436, 449, 403, 439, 440, 448, 428) grain Fe concentration was unit. These genotypes showed a good stability for corresponding traits.

The value of b<sub>i</sub> of six genotypes (421, 445, 410, 426, 422, 420) for grain yield per plot 121 were also unit. Based on the methods of Finlay and Wilkinson (1963), these genotypes can adapt 122 well to all environmental conditions even if the conditions improve or worsen. It is further 123 understood that their yields remain stable. Additionally, four genotypes (420, 425, 435, 437) 124 which had better or same performance with check (genotype 1) for yield, also showed b<sub>i</sub> as unit 125 or near to unit (from 0.9 to 1.3) indicated that grain yield of these genotypes is expected to 126 increase if the conditions improve and to remain stable if the conditions deteriorate. Some 127 geotypes were able to adapt to favourable conditions, and their yields were stable only under 128 favourable conditions as their  $b_i$  values more than unity  $(b_i>1)$ . Three of these genotypes i.e. 129 (402, 438, 444) were able to adapt well to favourable conditions, and their yields are expected to 130 increase as the conditions improve. 131

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Additionally, genotypes 407, 413 and 450 did not remain stable for grain yield under favorable or unfavorable conditions as their  $b_i$  values less than unity ( $b_i<1$ ). Similarly, eight genotypes (414, 442, 436, 418, 419, 450, 407, 420) for grain Zn concentration and eight genotypes (442, 414, 433, 432, 419, 437, 417, 407) for grain Fe concentration had  $b_i$  values more than unity ( $b_i>1$ ) and were able to adapt to favorable conditions. In case of  $b_i$  values less than unity ( $b_i$ <1), eight genotypes (405, 406, 408, 425, 415, 413, 447, 428) for grain Zn concentration and seven genotypes (446, 425, 445, 405, 450, 447, 443) for grain Fe concentration included in this category.

141  $S_{di}^{2}$  serves as another stability parameter. For stable genotypes, this value should be low and close to zero (Kurt Polat et al., 2016, Eberhart and Russell, 1966; Yagdi, 2002; Amin et al., 142 2005; Aycicek and Yildirim, 2006; Hassan et al., 2013). In the present study, the twenty six 143 genotypes (from -3.3 to 0.0) for plant height, ten genotypes (from -0.4 to -0.2) for days to 144 heading, nine genotypes (from -0.9 to -0.1) for 1000-grain weight, eighteen genotypes (from -2.2 145 to 0.0) for grain Zn concentration, twenty four genotypes (from -2.1 to -0.3) for grain Fe 146 147 concentration and thirty five genotypes (0.0) for grain yield had greatest stability according to this criterion all with values less than or equal to 0 (Table 2). 148

Results revealed that high yielding genotypes can also be stable. Genotypes 437, 420, 425 and 435 had better performance than check HD 3086 and desired performance for grain yield per plot in term of high mean, unit  $b_i$  and least deviation from regression ( $S^2_{di}$ ), indicating the role of linear portion of G x E interaction in the performance of these genotype.

In view of the stability and adaptation parameters values determined in this study, it can 153 154 be concluded on basis of two stability analysis that adaptation ability of two genotypes (410 and 431) for grain Zn concentration, eight genotypes (403, 413, 416, 428, 430, 435, 440 and 449) for 155 grain Fe concentration and seventeen genotypes (406, 408, 410, 414, 420, 421, 422, 424, 425, 156 426, 427, 428, 430, 442, 443, 445 and 447) for grain yield are relatively higher and they are more 157 stable than the other genotypes. Genotypes number 410 and 431 for grain Zn concentration, 158 genotype number 440 for grain Fe concentration and genotypes numbers 420 and 425 for grain 159 160 yield, also had high mean values compared with mean value of check genotype number 401. Genotypes numbers 410 and 427 are stable for both grain Zn concentration and grain yield. 161 Similarly genotypes numbers 428 and 430 were stable for both grain Fe concentration and grain 162 163 yield. Any genotype which is highly stable for three traits i.e. grain Zn and Fe concentration and grain yield, not found in this study. As compared to genotypes which are stable for grain Zn 164 concentration and Fe concentration more genotypes showed stability for grain yield over three 165 environments. 166

167 Robert and Dennis (1996) have explained that the breeder must keep in mind that the 168 evaluation of stability depends on the sets of genotypes and environments studied. In stability 169 analysis, various statistics should be applied to characterize the genotypes for responsiveness to 170 environments as much as possible and to be sure of the G × E interaction effects.

Our results suggest that almost all traits measured, changed substantially with 171 environments (Table 2). Therefore, production of a cultivar with improved grain Zinc and Fe 172 concentrations and grain yield may need a growing environment that favors expression of this 173 genetic potential. This directs to the production of high yielding biofortified grains. Thus, some 174 genotypes were stable for some traits and unstable for another, suggesting that the genetic factors 175 involved in the G x E differed between traits (Grausgruber et al., 2000; Rharrabti et al., 2003a; 176 Baric et al., 2004, Mut et al., 2010). The cultivation of more unstable cultivars should be 177 recommended only for specific regions where they can attain a high performance with regard to 178 179 quality traits independent of seasonal effects.

180 Genotypes selected according to stability of grain micronutrients and grain yield in
 181 present study verified the possibility of combining both stable and high performances. Though,
 182 breeders must be aware of the difficulties in selection. The important goal for breeders is to find

genotypes with stable traits, not only to provide good raw material for end users, but also toprovide parents in the future breeding programmes.

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*Table 1.* Combined Analysis of Variance for Stability (Eberhert and Russel Model) of 50 genotypes across three environments

Source of variation	d.f.	M S												
variation		Plant Height	Days to heading	Thousand grain weight	Fe	Zn	GY							
Variety	49	29.98**	24.10**	35.21**	11.95	19.79**	0.15**							
Environment	2	1,374.70**	491.30**	71.95**	955.12**	457.56**	18.21**							
Var. X Envion.	98	12.57**	3.38**	9.66**	9.55**	8.68**	0.06*							
Env+Var X Env	100	39.82	13.14	10.90	28.46	17.66	0.43							
Env (Linear)	1	2,749.39**	982.60**	143.90**	1,910.24**	915.13**	36.42**							
Env X Var(Lin)	49	11.09	1.81	7.79	13.44**	9.68	0.06							
Pooled														
Deviation	50	13.77**	4.86**	11.29**	5.54**	7.53**	0.06**							
Pooled Error	147	6.56	0.75	1.75	4.27	4.45	0.04							

Figures with \* and \*\* are significant at 5% and 1% level of significant, respectively

298 299		days to heading (DTH), 1000-grain weight (TGW), grain Zinc concentration (Zn), grain Iron concentration (Fe) and grain yield (GY) for each genotype (G) tested over three environments																
		PH	uuuon	DTH TGW Zn							esteu (		Fe	GY				
G	М	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	М	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	М	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	М	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	М	b <sub>i</sub>	S <sup>2</sup> <sub>di</sub>	М	<b>b</b> <sub>i</sub>	S <sup>2</sup> <sub>di</sub>
401	101	0.6	64.6	96	0.8	-0.4	38.0	0.0	6.8	31.2	1.3	12.8	41.1	0.5	-1.9	3.1	1.2	0.1
402	103	0.6	-2.7	93	0.8	2.0	34.0	-0.1	33.3	30.8	1.4	9.6	39.5	1.6	17.3	2.8	1.6	0.0
403	101	1.2	-1.0	101	1.3	-0.2	33.0	2.2	2.9	30.2	0.9	2.6	37.5	1.0	-1.4	2.9	1.4	0.0
404	101	1.3	-2.4	91	0.8	1.5	42.0	1.4	5.5	38.0	0.6	7.9	45.7	1.2	-1.7	2.6	1.3	0.0
405	100	1.3	4.3	101	1.1	-0.3	36.9	2.5	2.8	41.9	-1.0	0.7	41.6	0.2	-1.2	2.1	0.7	0.0
406	99	0.9	-2.4	99	0.9	7.2	43.9	-0.1	17.1	32.7	-0.5	0.0	44.6	0.8	-2.0	2.9	1.1	0.0
407	105	0.6	8.4	94	0.8	0.6	40.1	-1.3	14.7	33.6	2.3	-0.1	43.3	2.4	1.3	2.9	0.5	0.0
408	107	1.0	19.2	95	0.6	11.7	41.0	-0.3	7.2	37.3	-0.4	2.3	40.8	1.1	21.8	2.9	1.1	0.0
409	93	1.4	12.2	100	0.5	2.1	34.5	1.0	19.7	39.7	0.4	12.8	42.5	1.6	-1.8	2.7	0.7	0.0
410	107	1.5	31.9	98	1.1	3.2	40.2	0.5	17.2	38.0	1.2	-1.8	41.1	0.6	3.6	2.7	1.0	0.0
411	100	0.8	1.1	98	1.1	4.2	39.3	1.6	5.1	34.9	1.3	1.1	39.8	0.5	23.7	2.6	0.7	0.2
412	100	1.2	-3.3	98	0.6	1.1	37.6	-1.8	39.7	33.1	1.0	1.5	39.5	1.6	1.9	2.6	0.6	0.0
413	101	1.0	29.6	95	0.8	0.4	32.0	1.6	15.6	32.3	0.3	4.1	37.9	0.9	-1.2	2.5	0.5	0.1
414	97	1.2	-0.8	98	1.0	8.1	34.0	0.3	2.1	32.9	1.8	1.2	37.7	1.7	3.4	2.8	0.9	0.0
415	102	1.3	-2.7	92	0.6	3.6	43.4	-0.9	-0.1	33.8	0.2	9.7	42.2	0.5	12.5	2.8	0.7	0.0
416	101	0.7	24.0	96	1.2	3.6	37.5	2.4	42.4	35.1	1.2	10.6	38.2	1.0	-2.0	2.5	1.2	0.0
417	97	1.9	12.8	98	1.2	4.3	41.5	2.4	1.8	33.6	0.8	7.1	43.0	2.0	-1.8	2.9	1.2	0.0
418	95	1.1	-2.0	96	0.9	5.1	35.5	-1.1	29.9	34.6	1.9	20.5	39.4	1.6	-0.3	2.5	0.6	0.0
419	97	1.4	-3.1	101	1.1	-0.3	34.7	2.0	3.7	37.7	2.1	-0.8	40.6	1.7	-0.7	2.8	1.2	0.0
420	99	0.7	-0.3	101	0.7	-0.3	43.7	3.0	13.3	36.8	2.6	2.4	41.9	1.1	2.9	3.2	1.0	0.1
421	98	1.4	-2.3	100	1.0	1.7	39.7	3.4	1.2	34.2	0.5	4.6	42.9	0.5	-2.1	2.8	1.0	0.0
422	101	1.5	-0.2	100	0.9	5.1	40.6	3.3	-0.8	33.4	0.9	1.5	39.8	1.4	3.3	2.8	1.0	0.3
423	102	0.2	-1.0	93	0.5	4.3	39.2	0.5	-0.5	33.5	0.4	9.4	38.5	0.6	-1.5	2.7	1.2	0.0
424	106	1.0	-0.6	94	0.7	20.1	40.1	3.5	42.3	38.0	1.0	29.9	41.2	1.2	1.3	2.8	1.1	0.0
425	102	1.7	-2.2	98	1.0	-0.3	42.6	1.4	8.8	36.0	0.0	2.4	43.8	-0.3	4.3	3.2	0.9	0.1
426	93	1.4	1.0	93	0.4	1.9	35.5	1.0	-0.3	32.9	1.0	9.4	39.2	0.8	-1.9	2.9	1.0	0.0
427	100	2.1	1.6	100	1.2	1.4	39.3	1.4	-0.1	34.6	1.3	-2.1	42.4	1.3	39.0	2.5	0.9	0.0
428	100	0.4	33.2	96	0.4	1.9	36.6	-2.1	1.5	34.1	0.3	-1.3	38.9	1.0	-2.1	2.9	1.1	0.0
429	99	0.8	-3.1	101	1.5	-0.3	38.9	0.4	2.3	33.3	0.4	9.1	42.1	1.4	-0.8	2.8	1.2	0.1
430	101	-0.1	51.5	98	1.3	1.7	40.5	3.4	-0.8	33.4	1.7	-2.2	38.5	0.9	-1.8	2.9	0.9	0.1
431	101	0.6	-2.3	101	1.3	-0.4	42.7	2.4	23.3	35.8	0.8	-2.1	38.7	0.6	-2.1	3.0	1.3	0.0
432	99	0.9	-1.2	97	1.1	8.3	39.6	2.4	-0.6	36.1	0.7	27.2	40.2	1.7	5.4	2.8	1.2	0.0
433	104	0.8	-3.2	100	1.1	5.4	42.7	0.8	1.1	33.5	1.4	30.3	40.7	1.7	0.7	2.8	0.8	0.0
434	103	0.9	62.1	101	1.4	0.6	33.7	1.7	8.5	36.8	1.4	11.2	39.9	0.8	-1.9	2.5	0.6	0.3
435	103	0.0	6.6	94	0.9	10.2	40.4	3.1	3.8	37.6	0.8	18.9	39.6	1.0	-1.0	3.1	1.3	0.0

Table 2. Mean (M), regression coefficient (b<sub>i</sub>) and deviation from regression (S<sup>2</sup><sub>di</sub>) for plant height (PH),
 days to heading (DTH), 1000-grain weight (TGW), grain Zinc concentration (Zn), grain Iron

436	99	0.7	2.0	100	1.9	0.9	36.2	2.2	22.4	37.5	1.8	-2.2	38.8	1.0	0.5	2.8	1.3	0.0
437	99	1.0	3.1	96	0.8	7.2	41.6	2.9	6.3	32.9	0.6	-1.7	41.2	1.8	2.4	3.3	1.2	0.1
438	103	1.3	-1.5	98	1.3	1.7	38.7	-0.3	2.0	35.6	1.4	1.2	42.3	0.9	7.6	2.8	1.5	0.0
439	100	0.6	-1.6	96	1.0	1.2	35.7	1.0	22.8	37.5	1.6	-0.3	43.4	1.0	6.7	2.4	0.8	0.3
440	98	1.7	-1.9	97	1.7	-0.2	41.8	3.5	6.6	41.8	1.6	10.1	41.6	1.0	-2.1	2.9	1.3	0.0
441	107	1.1	0.0	99	1.2	4.3	35.4	1.5	3.6	34.0	0.7	-0.4	37.6	1.0	2.7	2.8	1.2	0.0
442	102	1.5	7.4	93	1.1	0.2	45.5	1.2	19.7	36.5	1.8	5.6	42.2	1.7	-2.1	2.5	0.9	0.0
443	99	0.8	-3.2	99	1.1	42.4	37.1	-4.0	1.5	32.4	1.5	-2.1	39.6	0.4	6.7	2.7	0.9	0.1
444	101	0.8	-3.0	94	1.0	-0.3	46.1	-0.8	-0.9	36.0	1.4	-2.2	42.7	0.8	-2.1	2.9	1.6	0.0
445	100	0.8	-1.7	98	1.0	16.6	44.1	1.0	7.6	39.1	0.5	-1.3	42.1	-0.2	18.4	2.8	1.0	0.0
446	97	0.9	107.4	100	1.3	1.9	42.2	-0.3	14.1	34.6	1.2	9.9	42.7	-0.7	15.4	3.0	0.6	0.1
447	98	1.1	39.9	95	1.1	24.4	38.2	-0.6	3.1	36.9	0.3	-0.4	43.1	0.4	2.9	2.9	1.1	0.0
448	97	1.0	5.1	100	1.0	0.7	39.4	-1.0	30.2	35.0	1.1	1.5	37.9	1.0	3.0	3.0	0.8	0.1
449	104	0.7	26.3	98	1.1	3.6	40.2	0.6	-0.6	37.3	0.5	-1.7	39.5	1.0	0.4	3.0	1.3	0.1
450	104	0.5	19.0	100	1.0	0.7	43.1	1.2	11.9	35.6	2.2	-0.9	39.3	0.3	-1.5	2.7	0.4	0.0

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