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Stability analysis for grain yield and physiological traits in synthetic derived RILs population under different moisture regimes in wheat

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Abstract

Drought stress is one of the major yield limiting factors in wheat among the abiotic stresses. In the present study, different physiological traits at different growth stages and grain yield were examined in the recombinant inbred lines population derived from Synthetic 46 genotype in six test environmental conditions. Grain yield per plot showed significant positive correlation with SPAD1 and SPAD2, but negative correlation with CT2, NDVI1, NDVI2, NDVI3 and NDVI4. Combined analysis of variance suggested genotypic effect as a predominant source of variation followed by GEI and environment effect. AMMI and GGE biplot analysis were used to analyze the effects of GEI on grain yield, and to compute the AMMI stability value and yield stability index which identified G127, G120, G105, G190 and G154 genotypes (RILs) that are highly adapted, stable and high yielding. Hence, the selected RILs according to yield stability index could be used as donors to develop stable high-yielding genotypes and the physiological traits can be best utilized to screen out the lines under different moisture stress regimes.

Key words: Wheat, drought, AMMI analysis, GGE biplot

Introduction

Bread wheat (*Triticum aestivum* L. em Thell) is a major cereal crop that provides staple food to more than 2.5 billion of world population, with a production of 107.18 million tons (13.99 % of global) in an average area of 30.55 million hectares (13.80 % of global) in India (USDA 2020). By the year 2050 the global population will increase to ~9 billion, for that huge population wheat yield have to be increased by 60% (United Nations 2019). To address the challenge, the wheat production must increase by at least 1.6% per year from the current level of 1% (GCARD 2012). However, change in the climatic conditions with the unpredicted rainfall hindered the crop yield (IPCC 2013). The rising in average global temperature and inconsistent rainfall due to climate change results into reoccurrence of drought stress across the globe (Trenberth 2011; Hui-Mean et al. 2018). The impact of drought stress on wheat yield is experienced more in the reproductive developmental stage and its impact increases to many folds if drought is sustained for long time (Daryanto et al. 2016; Fahad et al. 2017; Ding et al. 2018). The physiological traits in wheat reported to be linked with drought tolerance includes normalized difference vegetation index (NDVI) (Lopes and Reynolds 2012; Ramya et al. 2016; Condorelli et al. 2018), total chlorophyll content (Kira et al. 2015; Paul et al. 2016), canopy temperature (CT) (Mason and Singh 2014; Deery et al. 2019) and carbon isotope discrimination (Dixon et al. 2019; Shrestha et al. 2020). Indirect selection of the ideal physiological traits that contribute to yield are better than direct selection for higher yield (Fischer et al. 2018).

It is more precedence if the selected physiological trait has more heritability under a stress environment than yield itself, that evidence have greater possibility of triumph for the development of stress tolerant variety. This implies that estimates of yield attributing physiological traits impartial with grain yield improves the efficiency of selection by reducing the reliance on final grain yield. This allow a window of

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opportunity for the development of more successful crosses in a breeding program by taking advantage of additive gene action (Ataei et al. 2017; Dolferus et al. 2019).

Additive main effects and the multiplicative interaction (AMMI) model helps to study the genotype by environment interaction (GEI) (Gauch 1992). AMMI and GGE (genotype main effect plus genotype x environment interaction) biplot were best suited model for the purpose of development and evaluation of cultivars or genotypes which are stable across the different environmental conditions (Yan 2002; Farshadfar et al. 2011). In AMMI model, the analysis of variance of genotype and environment combines the main effect with principal components analysis (PCA) of the GEI (Gauch and Zobel 1997). The AMMI stability value (ASV) and the yield stability index (YSI) derives from the AMMI model's IPCA1 and IPCA2 (interaction principal components axes 1 and 2, respectively) and mean yield across the environments scores for each genotype incorporate into single criterion (Purchase et al. 2000; Mkumbira et al. 2003). These values commensurate with the stability methods given by Eberhart and Russell (1966) and Shukla (1972). To graphically analyze GEI, GGE biplot is effective way to find identification of high-yielding, stable genotypes, especially in multi-environment trials (Butron et al. 2004; Samonte et al. 2005; Laffont et al. 2007; Ahmadi et al. 2012). The objective of this study was to evaluate and characterize the recombinant inbred lines (RILs) derived from parental cross of Synthetic 46/HD2932 in different moisture regimes for different physiological traits along with grain yield to identify best genotypes with high and stable grain yield.

Materials and methods

Experimental design and materials

A set of 188 RILs developed from a cross between Synthetic 46 and HD2932 were sown in α-lattice design including parents under two sowing conditions, each with two replications constituting ten blocks (each block contains 19 test genotypes). Experiment was designed and carried out at two location (i) experimental farm, Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi and (ii) ICAR-IARI, Regional station, Indore. Trials were conducted in *rabi* 2017 at Delhi under irrigated condition (DIR17); rainfed condition (DRF17), in *rabi* 2018, at Delhi under irrigated condition (DIR18); rainfed condition (DRF18), at Indore under irrigated condition (INDIR18); rainfed condition (INDRF18).

Phenotyping

The physiological traits *viz.,* total chlorophyll content, stay green and CT were recorded at different growth stages of plants of each RIL (genotypes) in each application from middle row of the three lines in the plot.

Total chlorophyll measurements were determined using a portable SPAD-502 sensor Chlorophyll Meter at two different stages *viz.,* SPAD1 (heading stage) and SPAD2 (grain filling stage). Computation were made midway between the margin and midrib on one side of leaf to minimize any effect of a discontinue distribution of chlorophyll in the leaf (Li et al. 2012; Abd El-Halim and Omae 2020). NDVI were recorded with a portable spectroradiometer known as Green-Seeker at six different growth stages of plant *viz*., NDVI1 (vegetative stage; Zadok's 2-3), NDVI2 (booting stage; Zadok's 4), NDVI3 (heading stage; Zadok's 5), NDVI4 (grain filling stage; Zadok's 6-7), NDVI5 (double-dough stage; Zadok's 8) and NDVI6 (maturity stage; Zadok's 9) respectively. CT reading were recorded at vegetative stage (CT1) and heading stage (CT2) of the crop. CT measurements were taken up with help of Infrared Thermometer (Reynold et al. 1998; Ayeneh et al. 2002). The spikes per plot were harvested and threshed at physiological maturity, grains harvested were weighed and expressed as grain yield per plot in grams (GY).

Statistical analysis

Analysis of variance (ANOVA) and best linear unbiased predicted value (BLUP) for all the variable were obtained by the software META-R (Multi Environment Trial Analysis with R) version 6.0. BLUPs values taken out of each six environments was used for analysis. The obtained data were used to enumerate Pearson's correlation coefficients among the different physiological traits and GY using the IBM SPSS statistic version 20 software. To find out GEI effects on grain yield, the recorded data for GY of all different environments were put forward for AMMI and GGE biplot analysis using software Gen Stat 14th edition (VSN International, Ltd, Hemel Hempstead, UK) and GEA-R Version 4.1 respectively.

AMMI model analysis helps to adjust the main or additive effect of genotype and environment, and its PCA inspect the residual interaction component (Farshadfar et al. 2011; Adjebeng-Danquah et al. 2017).

The AMMI model engage the sum of several multiplicative terms rather than only single multiplicative term in estimating the performance of genotypes in different environments (Bernardo et al. 2010). AMMI analysis helps to determine stable performance of the genotypes across different locations using the PCA scores and ASV (Hagos et al. 2013). The ASV is a quantitative stability value based on the AMMI model's IPCA1 and IPCA2 scores of each genotype, and assign genotype in terms of rank (Purchase et al. 2000).

$$ASV = \sqrt{\left[\frac{1PCA1_{Sum\ of\ square}}{1PCA2_{Sum\ of\ square}}(1PCA1_{score})\right]^2 + (1PCA2_{score})^2}$$

$\frac{1PCA1_{Sum\ of\ square}}{1PCA2_{Sum\ of\ square}}$ is the ratio of sum of squares of IPCA1 by IPCA2. More absolute value of IPCA means greater adaptability of genotype for a certain environment. However lower ASV value shows more stability in different environments. Similarly, YSI was calculated using the following formula: YSI = RASV + RY, (Mkumbira et al. 2003). where RASV is the ranking of the ASV and RY is the rank of the genotypes based on yield across environments. Low value of YSI explain the genotype with high mean yield and stability (Olivera et al. 2014).

The GGE biplot is a best model, used to show the graphically depiction of stable genotypes with high-yield across the different environments and also similarities/dissimilarities between environments by evaluating it based on the discriminative ability and representativeness of the GGE view, which is an advantage over the AMMI biplot analysis (Yan and Kang 2002; Yan et al. 2007; Aktas 2016).

Result and discussion

Correlations among traits

The relationship between two factors or variables is well defined by the correlation coefficient. It shows core concept of the relationship among various yield related traits, that is beneficial for the plant breeder to select the varieties having desired attributes (Ghafoor et al. 2013). Pearson's correlation coefficients for different physiological traits with grain yield under rainfed condition are given in Table 1. SPAD at heading and milking stage gives significant positive correlation with GY. Same relationship was earlier seen by Yildirim et al. (2010); Barutcular et al. (2016); Abd El-Halim and Omae (2020) who found positive correlations between SPAD values and grain yield at the heading and mid-milk grain development stage. Environmental stress is associated with chlorophyll loss and this loss in chlorophyll is regarded as a good indicator in moisture stress condition (Hendry and Price 1993; Barutcular et al. 2016). The distinctive relationship of SPAD value with GY shows the relationship of changes of soil moisture with the chlorophyll, and hence it may be used as tool to determine grain yield in moisture deficit condition. The strength of the relationship changed depending on the location and phenological stage (Reynolds 1997). SPAD-validation studies on materials with wide genetic backgrounds are useful to improve selection efficiency

Table 1. Pearson's correlation coeffiants among the different physiological traits and grain yield per plot at DRF is using BLUPs value

	GY	SPAD1	SPAD2	CT1	CT2	NDVI1	NDVI2	NDVI3	NDVI4	NDVI5	NDVI6
GY	1	.268**	.259**	-0.093	-.144*	-.279**	-.306**	-.270**	-.222**	-0.126	0.031
SPAD1		1	.871**	0.028	0.027	-.288**	-.279**	-.278**	-.226**	-.153*	-0.024
SPAD2			1	0.024	0.024	-.274**	-.259**	-.233**	-.143*	-0.116	0.026
CT1				1	.818**	-0.034	-0.016	-0.062	-0.032	-0.076	-0.087
CT2					1	-0.026	-0.001	-0.008	0.012	-0.039	-0.029
NDVI1						1	.807**	.731**	.610**	.490**	.214**
NDVI2							1	.911**	.750**	.608**	.332**
NDVI3								1	.808**	.638**	.383**
NDVI4									1	.825**	.550**
NDVI5										1	.781**
NDVI6											1

in durum wheat breeding. Strong genotype × environment interactions in leaf chlorophyll contents can result in lower selection accuracy in conventional breeding programs. Recently, environmental conditions were shown to affect the relationships between grain yield and chlorophyll retention in populations of spring and winter wheat (Bogard et al. 2011). Therefore, it is necessary to analyze SPAD values of plants in targeted environments to verify the relationship between SPAD values and grain yield. If there is a strong relationship between SPAD chlorophyll at a particular stage and grain yield, then this may serve as an indirect selection tool to differentiate high yielding genotypes. For the SPAD and NDVI traits used in present study, Liebisch et al. (2015) and Yousfi et al. (2016) obtained similar correlation between SPAD and NDVI in durum wheat and in maize found weak and negative correlation respectively.

CT is described as a cheap and effectual indicater to determine high yielding wheat varieties in different moisture regimes (Blum et al. 1989: Olivares-Villegas et al. 2007). Variation in CT among the wheat genotypes is conceivable due to their genetic differences, also supported by Reynolds et al. (1994). A negative correlation was observed between CT2 and GY; genotypes managing low CT had higher grain yield per plot. Moisture stress on association with high temperature stress at heading stage leads to reduction in photosynthetic activity and hindered accumulation of carbohydrates in grain (Sikder and Paul 2010). Significant variation among genotypes for grain yield and negative correlated with CT at different stages was observed by many researchers (Lopes and Reynolds 2010; Harikrishna et al. 2016; Manu et al. 2020) under variable growing conditions. Specifically, CT at reproductive stage is meant to be the crucial stage that affect the GY under drought conditions (Pask et al. 2014). The reduction in CT will affect transpiration (Reynolds et al. 2001) and plant water status (Araus et al. 2003). CT at vegetative stage and reproductive stage was found to be positively associated with each other, and there is negative significant correlation showed by traits SPAD, NDVI and GY as reported by Harikrishna et al. (2016), Ramya et al. (2016) and Manu et al. (2020).

NDVI was significantly negatively correlated with SPAD and GY at different stages as shown in Table 2 (Kyratzis et al. 2017). Aparicio et al. (2002) reported positive interactions between NDVI when analyzed at different growth stages, genotypes and environments. The ideal stages for measuring NDVI vary upon genotypes and the environment (Marti et al. 2007). Lobos et al. (2014) and Gizaw et al. (2016) reported the positive association between the NDVI and GY under different moisture stress and non-significant correlations between grain yield and days to heading. In our study, negative correlation between NDVI at different stages with GY were observed. This may probably occur due to confounding effects of glaucousness and days to heading in the population. The inherent low grain yield of the glaucous wheat was reported in previous studies (Yao et al. 2004; Yang, 2015; Zi et al. 2018). The present results were supported by Kyratzis et al. (2017), who suggested saturation of NDVI at drought is difficult to attain (Montazeaud et al. 2016), and can be used by modifying its calculation *viz*., degree of NDVI reduction after anthesis (Hazratkulova et al. 2012) NDVI ratio before and after anthesis, and cumulative NDVI after anthesis (Li et al. 2011). Overall NDVI is useful tool to select the genotype based on GY under different moisture stress conditions.

Table 2. AMMI analysis of variance for grain yield tested at six environments

Source of variation	df	MS	% varia-biity	% varia-bility accumu-lated
Environment	5	1016737**	21.85	21.85
Genotype	189	38346.99**	31.16	53.02
Interaction	945	11561.11**	46.97	100
IPC1	193	20756.76**	37.02	37.02
IPC2	191	12063.46**	21.29	58.32
IPC3	189	9913.406**	17.31	75.64
IPC4	187	7755.856**	13.40	89.04
IPC5	185	6403.824*	10.95	100
Residuals	1140	5092.752	0	0

** Significant at 0.01 probability
* Significant at 0.05 probability

AMMI, ASV and YSI analysis

Gollobs (1968) test for grain yield, AMMI lattice at six different environments showed high significance ($p<0.01$) for the mean squares of environment, genotypes and GEI which explained 21.85, 31.16 and 46.97 per cent of variability respectively (Table 1). Similar results have been also reported earlier by several workers (Kaya et al. 2002; Mehari et al. 2015; Harikrishna et al. 2016; Manu et al. 2020). The large variation explained by the genotype and GEI indicates the diverse nature of the genotype in the population and across the different environments. The grand mean of GY in between environments varied from 306.8 gm/plot to 463.4 gm/plot. At both locations in year 2018, GY for rainfed is lower than irrigated condition (Table 3). whereas grand mean of GY for genotype varied from 227.1 gm/plot (G34) to 567 gm/plot (HD2932). Best ten genotypes in their respective environments are given in Table 3.

The magnitude of sum of square obtained from the GEI was 1.50 times higher than that of genotypes, that shows the significant differences in genotypic reaction across environments (Yan and Hunt 2002; Mohammadi et al. 2009). In AMMI model the IPCA1 and IPCA2 scores were significant for the GEI and considered to be the indicator of stability. ASV values obtained from analysis depict the stable nature of genotype, less value indicates more stable genotypes and vice versa (Purchase et al. 2000). Among the best 20 genotypes, two genotypes had low ASV values G126 (0.42) and G144 (1.09) with grain yield of 468.6 gm/plot and 472.2 gm/plot respectively (Supplementary Table S1). The best five genotypes across all the environments with low ASV values are G14 (0.22), G60 (0.26), G65 (0.28), G126 (0.42), G92 (0.43). The result was in accordance with present findings of Bavandpori et al. (2015) Melkamu et al. (2015) and Manu et al. (2020), who assigned the ASV values to each genotype to get grain yield stability of bread wheat varieties. The YSI values assist to combine both yield and stability into a single index, to overcome the use of yield stability as the sole criterion to select genotypes. Thus, genotypes with minimum YSI value is beneficial, based on the YSI (Supplementary Table S1), the best genotypes *viz.,* G126, G144, G57, G127, G141, G14, G82, G105, G95, G45, G120 and G104 were found to have high grain yield performance. Thus, they can be selected to advanced yield trials for development of wide-ranging adaptable variety. Although genotypes G127, G120 and G105 have high ASV score and high yield but low YSI, these can be recommended for particular environments where they performed well. The approaches were earlier utilized by Farshadfar et al. (2011); Tekdal and Kendal (2018) to distinguish stable genotypes in multi-environment trials of wheat crop.

GGE biplot analysis

The GGE biplot is used to identify the best performing genotype of each environment and group of environments to assess the stability of the genotypes. The striking feature of GGE biplots is the 'which-won-where' analysis, where GEI, specific genotype adaptation and mega-environment differentiation constitutionally represented as graphically based on their coalition with the site score (Yan 2002; Yan and Tinker 2006; Oral et al. 2018; Thungo et al. 2020).

The most responsive genotypes were at vertex being assigned at the farthest distance from the origin of biplot. Genotypes (best or poor performance) in one or all environments falling within the sectors were considered responsive (Yan and Tinker 2006). The biplot showed the continuance crossover of GE, additionally mega-environment for GY. In biplot hexagon has nine genotypes *viz.,* G35, G97, G169, G34, G179, G175, G58, G107 and HD2932 (G190) at the vertices. The HD2932 (G190) respond well in DRF18 and INDIR18, while G35 and G97 being the best in DRF17 and DIR17. The biplot is splits into seven constructively sectors by the equality lines, out of which three retained all the environments (Fig. 1).

The graph of so-called "ideal" genotype shows the ranking of genotypes based on GY (Fig. 2). The characteristic of ideal genotype is to perform well with high stability across environments (Yan and Tinker 2006), and show longest vector length and no GEI, as represented by an arrow pointing to it (Fig. 2). A genotype closer to ideal genotype is considered as

Table 3. Mean yield performance in different environments and first ten AMMI selections per environment

Environments	Mean	1	2	3	4	5	6	7	8	9	10
INDRF18	306.8	G12	G127	G25	G137	G114	G135	HD2932	G144	G16	G81
DIR18	463.4	G167	G28	G135	HD2932	G3	G31	G131	G58	G1	G130
INDIR18	419	G97	HD2932	G30	G16	G67	G15	G127	G144	G173	G154
DRF18	379.3	HD2932	G28	G167	G31	G135	G131	G3	G26	G125	G154
DRF17	404.6	G97	G35	HD2932	G154	G63	G31	G67	G159	G76	G128
DIR17	397.4	G35	HD2932	G97	G63	G31	G154	G76	G26	G131	G40

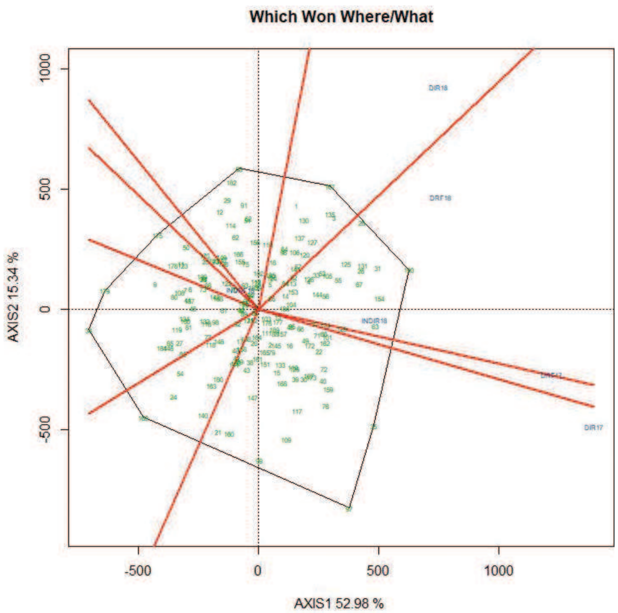

Fig. 1. Polygon view of GGE biplot showing "which won where" pattern for genotypes and environments based on grain yield data

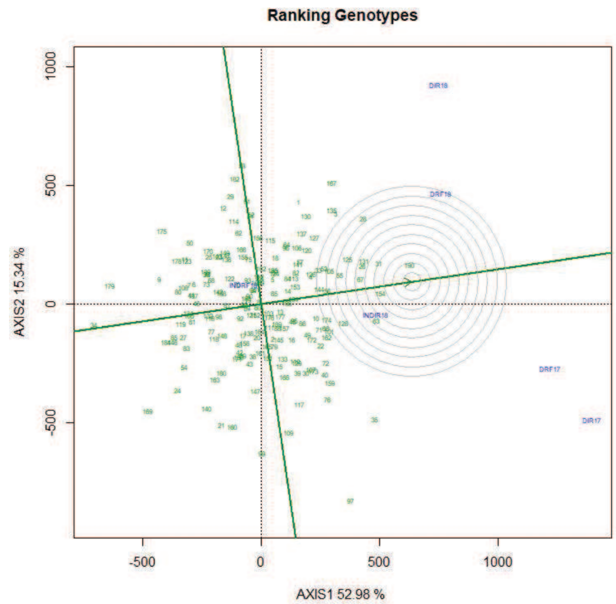

Fig. 2. GGE biplot based on genotype-focused scaling for comparison of the genotype with ideal genotype

more desirable. Thus, plotting the ideal genotype as the main point drawn the concentric circle helped in envisaging the distance between each genotype with the ideal one (Yan and Tinker 2006). Hence, based on the genotypes ranking for both mean yield and stability performance across the six environments HD2932 (G190) followed by G154, G31, G67, G26, G131 and G125 are closest to ideal genotype, thus considered as best genotype out of 190 RILs including parents. On the basis of mean performance (grain yield), AMMI and GGE biplot analysis it is noticeable that G127, G120, G105, G190 and G154 considered to be stable, adapted and high yielding genotype in all suited environments. The researcher can utilize these genotypes to further study stable performance under different moisture regimes. Furthermore, these genotypes can be used for QTLs/genes identification for same physiological traits associated with drought tolerance, in addition to that also used as donors in breeding for drought tolerance as also suggested by Khadka et al. (2020).

Authors Contribution

Conceptualization of research (RK, PKS, GPS, NJ); Designing of the experiments (HK); Contribution of experimental materials (PKS); Execution of field/lab experiments and data collection (RG, HK, DC, SVSP); Analysis of data and interpretation (HK, NJ, RG, RK); Preparation of the manuscript (RG, HK, NJ, RK).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. Grand mean grain yield, AMMI stability value and yield stability index along with their ranking for the 190 bread wheat genotypes tested across six environments

Genotype	IPCA1	IPCA2	GM	GMrank	ASV	ASVrank	YSI	YSIrank
G126	0.26053	0.24565	468.6	19	0.42	4	23	1
G144	-0.52914	-0.84771	472.2	17	1.09	11	28	2
G57	0.6337	-0.73915	449.3	33	1.11	12	45	3
G127	1.3822	0.20931	476.4	12	1.82	33	45	3
G141	0.03887	0.7983	441.8	39	0.80	7	46	4
G14	0.00436	-0.22072	432.8	46	0.22	1	47	5
G82	-0.14348	0.84971	438.7	41	0.87	8	49	6
G105	-1.27952	1.12059	474.3	13	2.01	38	51	7
G95	-1.14731	-0.14963	435.7	44	1.51	20	64	8
G45	-0.5182	-1.47356	438.6	42	1.62	24	66	9
G120	-0.14236	1.83337	450.6	31	1.84	35	66	9
G104	-1.03387	0.0003	428.5	50	1.35	18	68	10
G65	0.04733	0.27572	414.4	73	0.28	3	76	11
G67	-2.33441	0.30391	508.0	3	3.07	74	77	12
G55	-1.93476	1.62951	483.9	10	3.01	72	82	13
G186	-0.42463	-1.546	425.3	58	1.64	25	83	14
G56	-1.97764	0.91045	467.5	22	2.74	62	84	15
G137	1.93221	1.14381	467.7	21	2.77	65	86	16
G185	0.76532	0.72357	415.3	70	1.24	17	87	17
G13	0.33448	-2.12359	428.3	51	2.17	44	95	18
G135	1.10566	3.25158	498.8	8	3.56	88	96	19
G8	0.84686	-0.39582	401.5	85	1.18	14	99	20
G71	-2.21702	-1.09993	458.8	27	3.10	75	102	21
G53	-1.38503	2.93261	468.6	20	3.45	83	103	22
G60	0.01142	-0.26018	391.1	102	0.26	2	104	23
G59	1.22759	0.16382	406.9	81	1.61	23	104	23
SYN46	-1.2755	0.55152	408.0	77	1.76	30	107	24
G177	-1.35236	-0.02681	408.4	76	1.77	31	107	24
G22	-2.50894	-1.26435	462.4	24	3.52	85	109	25
G103	0.29306	-1.87865	409.7	74	1.92	36	110	26
G102	1.31746	0.00634	403.3	83	1.72	28	111	27
G111	-0.12186	-2.00543	409.6	75	2.01	38	113	28
G152	0.08546	-0.8718	389.6	105	0.88	9	114	29
G153	-1.36526	2.03826	426.3	56	2.71	60	116	30
G33	-1.47986	3.02291	459.3	26	3.59	90	116	30
G112	0.76892	1.2527	396.3	95	1.61	23	118	31
G84	-1.06545	1.9736	416.3	67	2.42	51	118	31
G16	-0.51907	-3.31739	441.5	40	3.39	80	120	32
G3	-0.54956	4.20164	482.2	11	4.26	110	121	33
G115	1.70504	1.13573	415.8	69	2.50	53	122	34

G90	0.48307	-0.01832	381.7	117	0.63	6	123	35
G18	0.07037	2.21815	407.7	78	2.22	46	124	36
G32	0.92814	-0.00692	384.1	111	1.21	15	126	37
G113	-1.50117	1.91267	417.6	65	2.74	62	127	38
G4	-2.08782	2.1883	436.3	43	3.50	84	127	38
HD2932	-3.50179	1.97942	567.0	1	4.99	129	130	39
G1	1.84493	3.10424	454.0	29	3.93	102	131	40
G131	-2.84402	3.23716	507.0	4	4.93	127	131	40
G96	0.11654	3.01149	423.7	59	3.02	73	132	41
G106	-0.68131	3.28058	428.2	52	3.40	81	133	42
G89	0.71746	-0.98063	382.9	115	1.36	19	134	43
G124	-0.46665	2.32253	402.8	84	2.40	50	134	43
G158	-0.36177	-0.73221	374.2	127	0.87	8	135	44
G138	-0.71092	0.50406	376.3	125	1.06	10	135	44
G181	1.01666	-0.86582	383.3	114	1.59	22	136	45
G92	-0.1078	-0.40907	365.0	133	0.43	5	138	46
G183	2.20633	-0.10207	414.9	71	2.89	67	138	46
G128	-3.64601	-0.32229	473.1	15	4.78	123	138	46
G121	0.63157	-1.63272	388.7	106	1.83	34	140	47
G176	-1.36366	1.18881	394.3	97	2.14	43	140	47
G101	-3.36161	0.10747	460.6	25	4.40	115	140	47
G154	-4.32604	1.10285	516.4	2	5.77	140	142	48
G2	-1.72287	-0.76183	396.6	94	2.38	49	143	49
G26	-3.33045	2.93951	496.3	9	5.26	134	143	49
G94	-0.21102	1.48752	376.8	124	1.51	20	144	50
G44	1.05909	-0.88973	381.6	118	1.65	26	144	50
G86	-2.67841	1.26458	429.6	48	3.73	96	144	50
G130	0.0178	4.13934	445.6	35	4.14	109	144	50
G79	-0.52732	-3.07984	415.9	68	3.16	77	145	51
G110	-2.36112	-2.03291	427.7	53	3.70	94	147	52
G37	-0.16606	1.73876	381.4	119	1.75	29	148	53
G28	-2.18808	5.14843	505.0	5	5.89	143	148	53
G5	-0.6465	2.60901	401.1	87	2.74	62	149	54
G30	-2.04228	-3.61756	449.9	32	4.50	117	149	54
G20	0.80944	-2.80886	407.0	80	3.00	71	151	55
G150	-0.87016	-1.06711	362.3	134	1.56	21	155	56
G174	-3.6031	1.13941	451.3	30	4.85	125	155	56
G164	-1.48922	0.328	379.3	120	1.98	37	157	57
G64	-0.00687	3.77616	423.2	60	3.78	98	158	58
G48	0.12056	-1.78459	372.3	128	1.79	32	160	59
G87	-0.77864	2.82899	400.3	88	3.01	72	160	59
G145	-2.13868	-0.99021	398.6	91	2.97	70	161	60
G93	2.35145	-0.58646	401.3	86	3.13	76	162	61

G15	-0.68596	-3.99584	426.4	55	4.10	107	162	61
G125	-3.45281	4.4283	473.5	14	6.33	150	164	62
G42	-0.90034	-1.30589	360.8	135	1.76	30	165	63
G43	-1.10903	-1.53557	377.3	123	2.11	42	165	63
G161	-1.84311	0.54715	383.6	113	2.47	52	165	64
G129	-2.63881	-2.24371	425.9	57	4.12	108	165	64
G75	1.7128	1.59265	390.3	103	2.75	63	166	65
G38	-0.64382	-2.49788	384.4	110	2.64	58	168	66
G165	-1.44651	-2.172	391.1	102	2.88	66	168	67
G63	-5.37457	2.1624	504.5	6	7.36	162	168	67
G133	-1.66118	-3.07537	414.7	72	3.77	97	169	68
G66	-3.93899	1.36143	448.4	34	5.33	135	169	68
G173	-3.14588	-3.22365	444.2	37	5.23	133	170	69
G151	-1.263	-2.88251	398.1	92	3.32	79	171	70
G61	0.30949	1.64239	348.9	145	1.69	27	172	71
G77	0.73738	-0.76126	335.6	159	1.23	16	175	72
G155	1.81134	1.28686	382.1	116	2.70	59	175	72
G31	-5.09307	5.12031	499.2	7	8.40	168	175	72
G159	-4.63086	-1.35564	456.3	28	6.21	149	177	73
G78	-0.45859	2.82828	384.0	112	2.89	67	179	74
G88	-2.44949	0.63129	391.4	101	3.27	78	179	74
G39	-3.49908	-0.87306	421.8	62	4.66	119	181	75
G162	-4.42863	1.69977	444.9	36	6.04	145	181	75
G19	1.64365	-2.66317	392.7	100	3.42	82	182	76
G122	0.51672	1.97423	349.2	144	2.09	41	185	77
G157	-2.73092	1.20651	399.7	89	3.77	97	186	78
G168	-1.97234	-3.907	417.1	66	4.68	120	186	78
G167	-1.04692	7.27354	464.1	23	7.40	163	186	78
G142	1.38979	0.9788	346.7	149	2.06	39	188	79
G171	-1.26577	-1.49601	352.4	141	2.23	47	188	79
G166	2.41149	1.01836	385.1	109	3.32	79	188	79
G97	-7.12918	-8.69017	473.0	16	12.75	172	188	79
G35	-9.50906	1.28155	470.7	18	12.51	171	189	80
G98	1.39987	-1.29634	351.6	142	2.24	48	190	81
G72	-4.949	0.39484	443.2	38	6.49	152	190	81
G69	0.08729	-2.97068	378.7	121	2.97	70	191	82
G117	-3.52911	-3.09151	427.4	54	5.56	137	191	82
G70	-0.04505	2.57583	354.6	139	2.58	55	194	83
G54	-0.15161	-1.09778	289.3	182	1.12	13	195	84
G10	-4.0385	3.11798	431.7	47	6.14	148	195	84
G148	0.91581	-2.31186	354.3	140	2.60	56	196	85
G132	1.45405	-0.84126	335.7	158	2.08	40	198	86
G49	-3.96315	0.86941	419.8	64	5.26	134	198	87

G116	1.38578	-1.21387	336.7	157	2.18	45	202	88
G172	-4.40449	1.15829	421.3	63	5.88	142	205	89
G52	2.94464	2.14565	398.7	90	4.41	116	206	90
G40	-5.55586	-0.17499	434.1	45	7.27	161	206	90
G188	2.02568	-1.27541	356.5	138	2.94	69	207	91
G107	-4.6534	-0.691	423.0	61	6.13	147	208	92
G119	1.39761	-0.025	296.7	181	1.83	34	215	93
G73	1.27651	1.9523	325.5	162	2.57	54	216	94
G17	1.24679	-4.0345	388.6	107	4.35	112	219	95
G76	-6.78532	0.55026	429.4	49	8.89	170	219	95
G180	-0.98301	-2.39765	335.0	160	2.72	61	221	96
G68	2.19169	0.51307	342.9	154	2.91	68	222	97
G91	4.11133	2.69668	407.4	79	6.02	144	223	98
G47	2.28251	2.11188	365.5	132	3.66	92	224	99
G136	2.38877	1.95514	365.6	131	3.69	93	224	99
G46	2.03606	-0.42206	322.1	166	2.70	59	225	100
G62	2.4759	2.25156	377.8	122	3.94	103	225	100
G51	2.19795	3.70109	390.2	104	4.69	121	225	100
G156	0.58688	4.64293	385.6	108	4.71	122	230	101
G118	1.56843	-2.93092	350.2	143	3.58	89	232	102
G134	1.99762	-0.77131	312.6	172	2.72	61	233	103
G27	1.63805	-1.48735	304.4	178	2.61	57	235	104
G109	-4.67434	-2.61784	404.9	82	6.65	154	236	105
G114	4.65406	0.64298	397.0	93	6.12	146	239	106
G41	2.06288	1.33922	317.0	170	3.01	72	242	107
G143	2.08457	2.5204	347.4	147	3.71	95	242	107
G23	2.84063	1.69736	359.7	137	4.09	106	243	108
G147	-2.37646	-3.06572	371.7	129	4.37	114	243	108
G146	1.46001	-1.9923	286.2	183	2.76	64	247	109
G149	3.67554	0.1429	376.8	124	4.81	124	248	110
G187	2.67544	-0.50014	324.7	164	3.54	86	250	111
G36	2.96351	0.2593	344.5	151	3.89	101	252	112
G74	2.92987	0.96604	347.0	148	3.95	104	252	112
G29	5.06767	2.7337	394.6	96	7.17	160	256	113
G139	3.31668	0.10098	347.5	146	4.34	111	257	114
G58	4.22227	4.94334	393.6	98	7.41	164	262	115
G100	2.80085	-1.22858	325.2	163	3.86	100	263	116
G170	3.28526	0.70206	345.6	150	4.36	113	263	116
G108	2.67864	0.59031	307.5	177	3.55	87	264	117
G12	5.91092	0.17262	392.9	99	7.74	165	264	117
G83	1.71853	-2.85802	312.0	174	3.64	91	265	118
G163	1.44108	-4.67739	360.1	136	5.04	130	266	119
G182	3.94954	3.7208	379.3	120	6.37	151	271	120

G140	-1.50516	-3.25877	312.5	173	3.81	99	272	121
G7	3.04527	0.00127	321.3	167	3.98	105	272	121
G6	3.71644	-0.78997	338.3	155	4.93	126	281	122
G25	5.17849	-1.26121	375.6	126	6.89	155	281	122
G99	-4.53257	-3.70041	369.2	130	6.99	157	287	123
G123	3.17641	1.90332	315.1	171	4.57	118	289	124
G160	-2.85414	-3.95396	338.2	156	5.44	136	292	125
G21	-1.08569	-5.63883	344.1	152	5.81	141	293	126
G50	3.86099	1.01973	323.0	165	5.15	131	296	127
G11	4.23607	0.70371	325.9	161	5.59	138	299	128
G80	3.88858	-0.90782	317.9	169	5.17	132	301	129
G81	3.90527	-4.09271	343.9	153	6.55	153	306	130
G184	3.00762	-3.02404	296.8	180	4.96	128	308	131
G169	2.37436	-4.69304	279.5	184	5.63	139	323	132
G178	5.25064	-1.77767	323.0	165	7.10	159	324	133
G85	3.97626	-4.7017	318.3	168	7.01	158	326	134
G24	1.54162	-6.33577	302.6	179	6.65	154	333	135
G179	5.32512	-0.17269	230.5	185	6.97	156	341	136
G175	5.83066	2.14303	308.6	175	7.92	166	341	137
G9	5.85275	-3.05649	308.2	176	8.24	167	343	138
G34	6.25115	-3.17901	227.1	186	8.77	169	355	139