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Divergence analysis reveals the presence of large genetic diversity for yield contributing traits segregated among the rice (*Oryza sativa* L.) Genotypes

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Abstract

The presence of genetic diversity is crucial for making genetic gains in any breeding program and its assessment is even more important in rice hybrid breeding to realize maximum heterosis. To evaluate genetic diversity among a set of 84 rice accessions, the experiment was conducted in an augmented block design with eight blocks and three checks. Based on divergence analysis the genotypes were grouped into ten clusters comprising 7, 18, 15, 19, 2, 5, 10, 6, 1, and 1 genotypes, respectively. Clusters V and X were found to be the most distant meaning genetically diverse from each other followed by clusters V and IX. Cluster I showed the highest mean performance for grain yield per plot, whereas cluster X performed best for effective bearing tillers (EBT/ m²). For days to 50% flowering clusters IX and X showed the lowest and highest mean performance respectively. The results from this study show large potential for increasing genetic gains by combining the desired variation that is present in different diverse groups of genotypes. Additionally, cluster means for yield/plot and EBT, and the inter-cluster distances between IX and X, suggest that the genotypes constituted in these clusters may be used as parents in the future hybridization program. Observed genetic diversity among the rice genotypes indicates the potential for exploitation in the cultivar and hybrid development and thus selection of clusters based on means could be helpful for selecting parents to be used in hybridization in order to combine the desirable yield contributing traits and recover the transgressive segregants with high yield potential.

Keywords: Cluster analysis, divergence analysis, genetic diversity, rice, tocher's method

Introduction

Rice is one of the most important cereal crops supporting over half of the planet's population by supplying 21% and 15% of per capita calories and protein consumption respectively (McLean *et al.*, 2002) [1]. In Asia alone, three billion people are dependent on rice for 35–75% of their total calories (Khush, 2005) [2], and that's the reason rice production, supply, and distribution are important for the future food security of half of the global population. The rice demand is increasing and expected to increase further by as much as 26% (~176 million tonnes) between 2010 to 2035 (Khush, 2013) [3], whereas the rate of the rice production increase is slowing down due to the increased severity and intensity of various biotic and abiotic stresses due to climate change. Most of this increased demand must come from genetic improvement of rice varieties and superior hybrids.

To increase the genetic gains to meet the productivity gap, the availability of genetic variability is the most important prerequisite and is critical for the success of any crop improvement program. One of the reasons for limited yield gains in most of the breeding programs is the lack of sufficient genetic diversity in the breeding population (Xie *et al.*, 2012) [4]. Before broadening the genetic diversity, it is important to assess the genetic diversity present in the breeding population. Genetic diversity assessment is a useful tool that helps select genetically divergent parents to obtain superior recombinants in segregating populations to develop high-yielding cultivars. Genetic diversity assessment also can be deployed to group the genotypes into different diverse groups, which can help in the precise selection of potential diverse parents for hybrid development (Julfiquar *et al.*, 1985) [5]. We conducted the present study to evaluate the amount of genetic diversity present in the rice germplasm in the rice breeding program at Punjab Agriculture University, which can assist in selecting potential parents to develop pre-breeding resources for the future inbred and hybrid cultivar development.

Materials and Methods

The experimental material consisted of 84 rice accessions (81 accessions and three checks; Table S1), selected making sure the presence of acceptable grain quality and overall good agronomic performance so that the selected material can be adapted directly in the breeding program with minimal pre-breeding efforts. Three checks PR 115, PR 126 and PR 121 used in the experiment are inbred cultivars released by PAU and are commonly planted by farmers in Punjab. The experiment was conducted in a field (30°56'N latitude, 75°52' longitude) at a student's research farm at Punjab Agriculture University, Ludhiana, during Kharif 2017 in an augmented design that comprised of 8 blocks and 3 checks. Standard cultivation practices recommended for puddled transplanted rice were followed (Brar *et al.*, 2012) [6]. Observations were taken on days to 50% flowering (days), plant height (cm), effective bearing tillers (EBT/m²), grain yield (g/plot), and grain yield (g/plant).

Analysis of variance was performed using the augmented RCBD package in 'R' (Aravind *et al.*, 2019) [7] and adjusted means were used to estimate the pairwise Pearson's correlation between the traits and its graphical representation using 'copilot' and 'psych' packages in R (Revelle & Revelle,

2015; Wei *et al.*, 2017) [8, 9]. The genetic diversity analysis (Mahalanobis D² statistics) (Mahalanobis, 1936) [10] was performed following the strategy explained by (Rao, 1952) [11] using INDOSTAT.

Results and Discussion

Significant differences were observed for all the five traits among the 81-rice accession which suggests the presence of considerable genetic variability for yield and yield attributing traits in the population under study. The presence of large variability for days to 50% flowering, plant height (cm), effective bearing tillers (EBT/m²), and yield/plot (g) among the genotypes also can be seen in Figure 1. Based on genetic diversity analysis and the degree of divergence following tocher's method (Rao, 1952) [11], the genotypes were grouped into ten clusters (Table 1). A maximum of 19 genotypes were grouped in cluster IV followed by cluster II with 18 genotypes and a minimum of 1 genotype is grouped in clusters IX and X each. The genotypes of the mono-genotypic clusters indicate that those genotypes are of very different genetic makeup from the rest of the population and from each other. The remaining clusters I, III, V, VI, VII, and VIII included 7, 15, 2, 5, 10, and 6 genotypes respectively (Table 1).

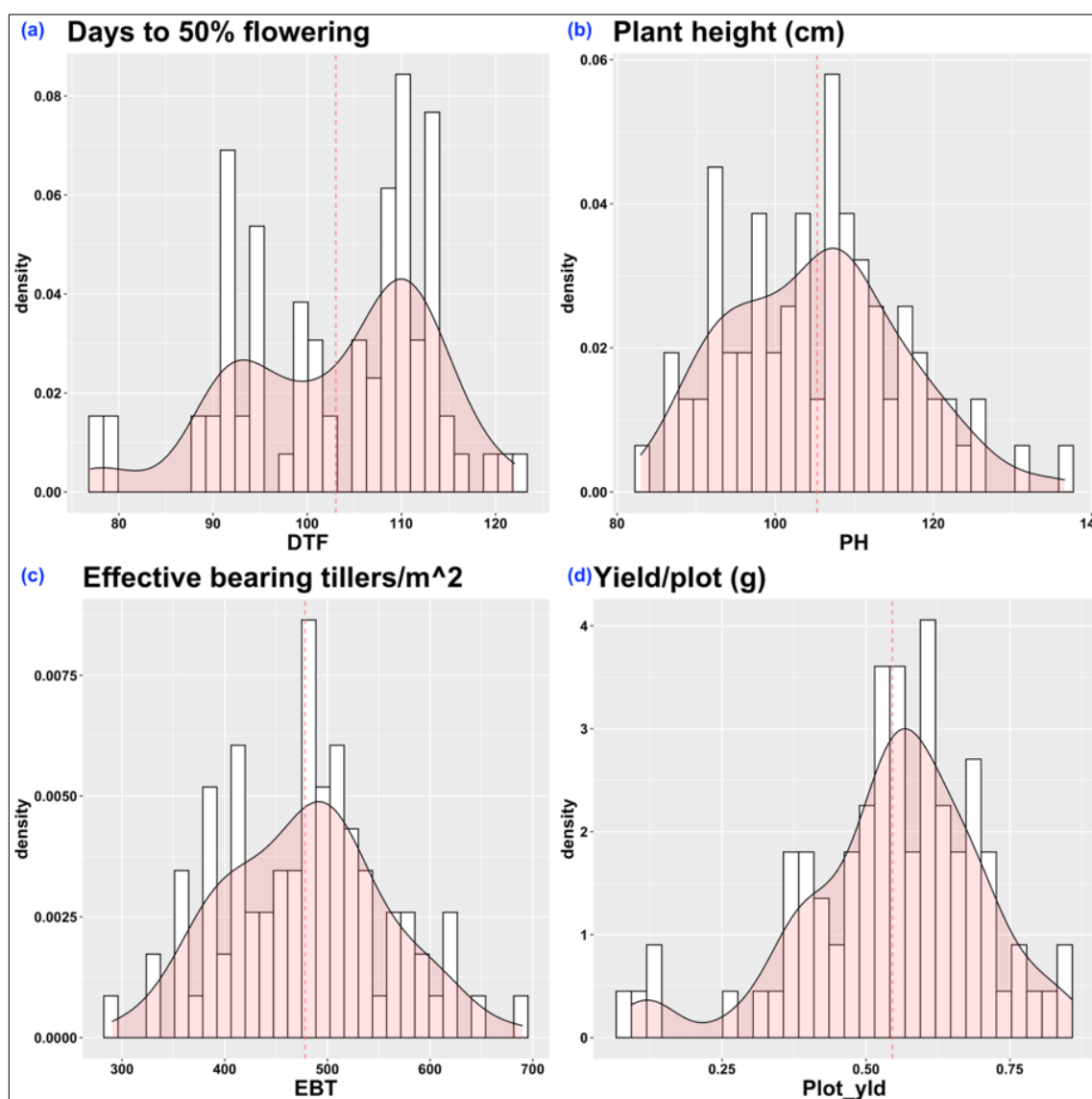


Fig 1: Frequency distribution of (a) days to 50% flowering, (b) plant height (cm), (c) effective bearing tillers (EBT/m²), and (d) yield /plot (g) in 84 rice accessions. The vertical dotted red line indicates the mean performance for the trait.

Table 1: List of genotypes in each cluster obtained by performing divergence analysis following Tocher's method on five agro-morphological traits recorded in 84 rice accessions.

Clusters	Cluster members	Number of cluster member
I	HHZ 15-DT7-SAL 2, BP 11820-5F-KN-10-2, IR 11N313, IR 12L125, IR 11A293, IRMT 4402, PR 123	7
II	HHZ 23-DT16-DT1-DT1, HHZ 4-DT3-Y1-Y1, IR11L236, IR 11A410, Sharbati, IR 11A318, RP 5940-96-7-2-1-1, IR 11A316, IRR1 154, IR 11A534, IR 11A108, IR 71033-4-3, IR 11N187, PANT DHAN 19, HHZ 24-DT11-LI1-LI1, 2k3-322-5-1-1-9-1-1-1-1-1, HHZ 10-DT5-LI1-LI1, IR 75478-282-5-1	18
III	IR 64 Sub 1, BP 10618F-BB8-18-BB4, IR 10F379, IR 11A346, RP 5163-102-3-5-2-2, IR 11N191, IR 11A501, HHZ 11-Y6-Y2-SUB1, PR 121, PR 126, PR 124, R-RHZ-R56, HHZ 2-SUB 2-DT1-DT1, 1, HHZ 21-Y4-Y2-Y1, 2K10-23-451-21-81-5-0-0	15
IV	HHZ 6-DT1-LI1-LI, HUANGHUAZHA, HHZ17-DT6-SAL3-DT1, HHZ 22-Y3-DT1-Y1, HHZ 15 SAL 13-Y3, HHZ 21-DT7-Y1-Y, PR 115, PAU 4320-21-1-3-1, HHZ 3-SAL13-Y1-SAL 1, IR 10M179, HHZ 4-SAL 12-LI1-LI1, IR 12L144, IR 11A106, IR 11N231, HHZ 1-DT3-Y1-Y1, IR 11N169, IR 10N389, HHZ 1-DT7-LI2-LI1, IRR1 105(RC 18),	19
V	HHZ 3-SAL 6-Y1-Y1, HHZ 24-DT11-LI1-LI1	2
VI	IR 04A381, IR 12L159, HHZ 15-SAL 13-Y1, HHZ 14-SAL 13-LI2-DT1, HHZ 3-SAL 13-Y2-DT1	5
VII	PAU 5216-10-1-3-1, HHZ 15-DT7-SAL2, HHZ 16-SAL 13-LI1-LI1, RP 5940-15-13-2-1-1, CB 12599, IRR1 102, RP 5898-101-9-3-1-1, HHZ 1-DT4-LI1-LI1, IR 10A270, RP Bio 5477-NH 686	10
VIII	2k3-322-5-1-1-18-1-1-2-1-1, HHZ 14-SAL 19-Y1, BP 12816F-KN-7-1, Peeli Pusa 1, RP 5949-29-5-1-1-1, CTC NPT-2	6
IX	NVSR-V-2057	1
X	IRRI 123	1

The intra-cluster distance ranged from 0.00 (cluster IX, X) to 1690.975 (cluster VII) which indicates the diversity among the genotype within the cluster (Table 2). Whereas the inter-cluster distance ranged from 2283.4 to 168837.5, depicting the extent of diversity among the clusters (Table 2). The

genotype clusters identified in this study can potentially be used in crop improvement programs to generate a wide range of transgressive segregations for the development of high-yielding rice cultivars (Shekhawat *et al.*, 2015; Ranjith *et al.*, 2018) [12, 13].

Table 2: Intra and inter-cluster distance estimated by following Tocher's method. A total of 84 rice accessions were grouped into ten clusters based on divergence analysis.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	421.4	4461.6	25134.3	11851.3	81150.7	2470.6	2442.6	49475.3	10493.4	16212.6
Cluster II		751.4	9745.9	2669.5	49709.5	11975.8	2283.4	25965.0	25925.7	36149.0
Cluster III			1025.6	3369.8	16934.1	40892.4	16455.9	5057.4	63522.8	80418.6
Cluster IV				655.5	32117.8	23024.2	6371.4	14024.4	40535.8	54450.1
Cluster V					47.5	108008.4	63660.9	4416.1	142647.8	168837.5
Cluster VI						519.7	7090.3	71023.6	3532.4	7184.6
Cluster VII							1690.9	36587.9	17128.7	26677.3
Cluster VIII								798.8	100183.4	121245.7
Cluster IX									0.0	2816.9
Cluster X										0.0

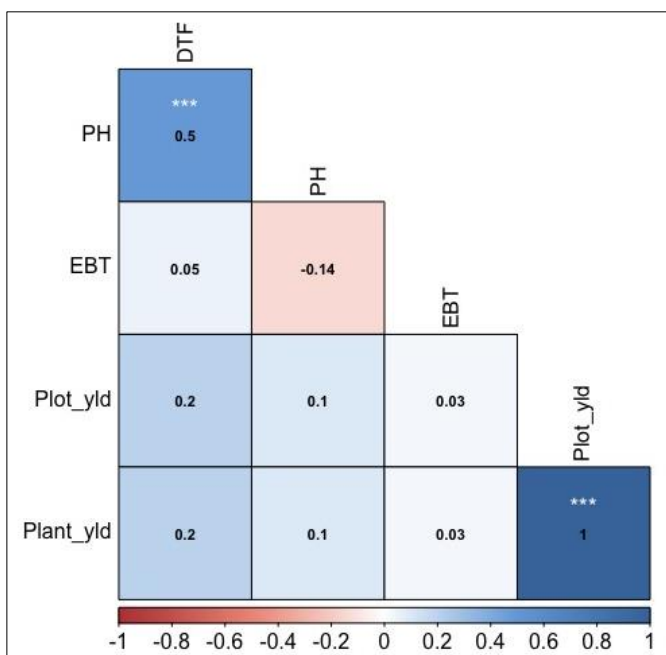
A large variation was observed in cluster means for all the traits studied, but none of the clusters included genotypes with all the desirable traits (Table 3). The cluster I include genotypes HHZ 15-DT7-SAL 2, BP 11820-5F-KN-10-2, IR 11A293, and PR 123 and based on cluster means, performed best for yield (Table 3). Whereas cluster IX (genotypes: NVSR-V-2057), and cluster VII (genotypes: PAU 5216-10-1-3-1, HHZ 15-DT7-SAL2, HHZ 16-SAL 13-LI1-LI1, RP 5940-15-13-2-1-1, CB 12599, IRR1 102, HHZ 1-DT4-LI1-LI1 and RP Bio 5477-NH 686) showed best-preferred mean performance for days to 50% flowering and plant height (Table 3). Cluster X which included only one genotype IRR1 123 showed the highest mean performance for effective bearing tiller (EBT) but undesirable lower yield and tall plant height. These results indicate that desired yield and yield-associated traits are segregated among the clusters or genotypes within the cluster, and it is crucial for improving the yield to combine these traits by means of hybridization for developing inbred or hybrid cultivars (Kulsum *et al.*, 2011) [14]. Another indication of segregation of yield and yield-

associated traits among genotypes and clusters is the absence of any correlation between EBT and yield (Figure 2), meaning the genotypes with higher EBT could be limited by grain number, grain size, or test weight. This also supports the need for combining these traits by hybridization among the selected parents from different clusters to realize higher yield in a resulting population.

From the divergence analysis, it can be concluded that better-performing genotypes in clusters V and X; V and IX separated by high estimated statistical distance could be utilized in a hybridization program for obtaining a wide spectrum of variation among segregants. A wide range of variation was observed among the genotypes and clusters for all the traits studied. Since the range of mean performance for traits among the clusters is large, there is a scope for selecting parents from different clusters to be utilized in hybridization. Selection of clusters based on means could be helpful for selecting parents to be used in hybridization in order to combine the desirable yield contributing traits and recover the transgressive segregants with high yield potential.

Table 3: Cluster means for five agro-morphological traits recorded on 84 rice accessions. The minimum and maximum value under each trait is highlighted in bold.

	Days to 50% flowering	Plant height (cm)	EBT/m ²	Yield/plot (g)	Yield/plant (g)
Cluster I	111.5	107.1	565.4	0.62	0.031
Cluster II	105.9	112.0	503.2	0.56	0.028
Cluster III	105.8	104.4	409.1	0.55	0.028
Cluster IV	99.7	104.4	459.6	0.60	0.030
Cluster V	96.2	108.7	281.3	0.55	0.028
Cluster VI	100.5	103.4	609.6	0.59	0.029
Cluster VII	95.6	96.3	531.8	0.46	0.023
Cluster VIII	106.7	110.3	344.2	0.44	0.022
Cluster IX	77.5	81.1	657.5	0.17	0.008
Cluster X	114.9	97.2	691.6	0.46	0.023

**Fig 2:** Pearson's correlation estimates for days to 50% flowering, plant height (cm), effective bearing tillers (EBT/m²), yield /plot (g) and yield/plant (g). *** $p < 0.001$, DTF Days to 50% flowering, PH plant height (cm), EBT Effective bearing tillers (EBT/m²), Plot_yld Yield/plot (g), Plant_yld yield/plant (g)

Conclusion

In conclusion, the current study found the existence of reasonable variability among rice genotypes, which could be exploited for future breeding. The cluster analysis classified the 84 rice accessions into ten clusters. Genotypes such as HHZ 3-SAL 6-Y1-Y1, HHZ 24-DT11-LI1-LI1, NVSR-V-2057, IRRI 123, HHZ 15-DT7-SAL 2, BP 11820-5F-KN-10-2, IR 11N313, IR 12L125, IR 11A293, IRMT 4402 and PR 123 showed desirable variation for grain yield/plot, EBT/m² and days to 50% flowering suggesting that they may be utilized for increasing genetic gains by combining the desired variation. Hence, the information generated will contribute significantly to rice improvement in Punjab and other related environments in India.

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Supplementary table

Table S1: List of genotypes used in the study along with check and their mean performance for five traits

Entries	Days to 50% flowering (Days)	Height (cm)	EBT/m ²	Plot yd.(g)	Yield/plant (g)
2K10-23-451-21-81-5-0-0	108	93.4	389.4	0.58	0.029
IR 75478-282-5-1	94	116.4	481.8	0.268	0.0134
IRMT 4402	101	106.6	534.6	0.488	0.0244
PAU 5216-10-1-3-1	91	90	508.2	0.306	0.0153
Sharbati	105	123.4	442.2	0.372	0.0186
NVSR-V-2057	77	83	613.8	0.124	0.0062
HHZ17-DT6-SAL3-DT1	91	96.72	415.8	0.597	0.0298
RP Bio 5477-NH 686	79	89.2	452.1	0.141	0.0071
PR 123	109.6	92.3	525.8	0.541	0.0271
CB 12599	77	86.2	495	0.092	0.0046
PR 124	109.2	101.28	397.32	0.549	0.0275
RP 5940-15-13-2-1-1	79	95.4	528	0.118	0.0059
HHZ 11-Y6-Y2-SUB1	95	95	363	0.686	0.0343
HHZ 1-DT4-LI1-LI1	90	91.2	475.2	0.658	0.0329
HHZ 1-DT7-LI2-LI1	90	93.2	455.4	0.704	0.0352
HHZ 3-SAL13-Y1-SAL 1	89	97.4	415.8	0.54	0.027
HHZ 6-DT1-LI1-LI1	95	99	429	0.658	0.0329
IR 64 Sub 1	110	106.2	376.2	0.372	0.0186
IR 71033-4-3	120	111	488.4	0.504	0.0252
HHZ 15-SAL 13-Y1	92	103.6	580.8	0.686	0.0343
HHZ 16-SAL 13-LI1-LI1	93	92	534.6	0.822	0.0411
HHZ 22-Y3-DT1-Y1	91	102.2	462	0.688	0.0344
HHZ 24-DT11-LI1-LI1	93	100.2	514.8	0.526	0.0263
HHZ 2-SUB 2-DT1-DT1	101	105.4	429	0.622	0.0311
IRRI 123	115	97.9	689.7	0.442	0.0221
HHZ 15 SAL 13-Y3	94	93.2	462	0.566	0.0283
HHZ 3-SAL 13-Y2-DT1	92	99.4	620.4	0.602	0.0301
CTC NPT-2	109	131	330	0.508	0.0254
IR 11A106	107	114.2	481.8	0.57	0.0285
IR 11A293	114	107	574.2	0.858	0.0429
IR 11A534	108	110.6	541.2	0.56	0.028
RP 5898-101-9-3-1-1	122	106.8	554.4	0.472	0.0236
HHZ 10-DT5-LI-1-LI1	91	92.6	521.4	0.658	0.0329
HHZ 15-DT7-SAL 2	115	106.6	594	0.774	0.0387
HHZ 23-DT16-DT1-DT1	100	104	514.8	0.692	0.0346
BP 11820-5F-KN-10-2	113	107.2	594	0.558	0.0279
HHZ 14-SAL 13-LI2-DT1	108	98.8	646.8	0.418	0.0209
HHZ 4-SAL 12-LI1-LI1	108.5	107.9	493.35	0.561	0.0281
IR 04A381	107	108.2	620.4	0.506	0.0253
IR 11A501	108	109.4	382.8	0.464	0.0232
IR 12L159	107	102.2	607.2	0.596	0.0298
Peeli Pusa 1	121	108.4	356.4	0.402	0.0201
BP 12816F-KN-7-1	100	103.8	363	0.392	0.0196
IR 11A410	108	115.8	495	0.598	0.0299
IR 11N187	111	117.8	528	0.48	0.024
IR 11N313	114	117.6	580.8	0.54	0.027
IR 12L125	113	110	561	0.546	0.0273
IR 12L144	102	116.6	475.2	0.598	0.0299
PANT DHAN 19	110	136.6	514.8	0.718	0.0359
BP 10618F-BB8-18-BB4	110	108.7	412.5	0.634	0.0317
IR 10F379	103	113.4	382.8	0.438	0.0219
IR 11A108	110	120.6	501.6	0.77	0.0385
IR 11A316	111	112.8	488.4	0.506	0.0253
IR 11A318	114	115.2	468.6	0.59	0.0295
IR 11N169	114	108.8	435.6	0.368	0.0184
IR 11N231	110	116.2	448.8	0.548	0.0274
IR11L236	110	109.6	475.2	0.726	0.0363
IRRI 102	116	98.2	521.4	0.342	0.0171
IRRI 154	110	107.4	488.4	0.394	0.0197
HHZ 21-Y4-Y2-Y1	110	103.2	415.8	0.618	0.0309
IR 10M179	106	110.2	442.2	0.684	0.0342
IRRI 105(RC 18)	89	120.4	415.8	0.358	0.0179

R-RHZ-R56	100	112.4	475.2	0.632	0.0316
2k3-322-5-1-1-18-1-1-1-2-1-1	106	94	382.8	0.666	0.0333
2k3-322-5-1-1-9-1-1-1-1-1-1	106	91.8	561	0.578	0.0289
HUANGHUAZHAN	95	97.7	506.5	0.714	0.0357
PAU 4320-21-1-3-1	100	97.6	501.6	0.804	0.0402
HHZ 14-SAL 19-Y1	91	100.8	386.3	0.595	0.0297
HHZ 21-DT7-Y1-Y1	92	103.8	514.8	0.644	0.0322
HHZ 3-SAL 6-Y1-Y1	97	111.4	330	0.838	0.0419
HHZ 1-DT3-Y1-Y1	113	95.4	462	0.6	0.03
HHZ 4-DT3-Y1-Y1	100.5	103.8	498.3	0.561	0.0281
HHZ 24-DT11-LI1-LI1	94	107.2	290.4	0.41	0.0205
IR 10A270	114	125.6	541.2	0.746	0.0373
IR 10N389	114	121.6	475.2	0.518	0.0259
IR 11A346	113	118	429	0.502	0.0251
IR 11N191	110	107.4	389.4	0.524	0.0262
HHZ 15-DT7-SAL2	101	89.2	561	0.532	0.0266
RP 5163-102-3-5-2-2	110	112.4	396	0.434	0.0217
RP 5940-96-7-2-1-1	99	122.6	508.2	0.604	0.0302
RP 5949-29-5-1-1-1	111	125.4	363	0.388	0.0194
Check 1 (PR115)	94.500	87.700	461.175	0.636	0.032
Check 2 (PR 126)	111.375	87.375	414.975	0.536	0.027
Check 3 (PR 121)	91.750	92.125	410.025	0.681	0.034
Mean	103.015	105.374	478.897	0.547	0.027
Std. Dev.	10.839	11.192	80.426	0.160	0.008
Std. Error	1.183	1.221	8.775	0.017	0.001
C. V. %	10.521	10.622	16.794	29.328	29.586
Lowest	73.875	81.100	279.125	0.168	0.008
Highest	121.875	137.000	691.625	0.876	0.044