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Genetic analysis, yield and yield stability of rice (*Oryza sativa* L.) near isogenic lines under irrigated environment

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Abstract

Rice being a staple food crop for more than half of the world's population is very important for global food security. Rice is cultivated on wide range of ecosystems varying in hydrological conditions. Irrigated rice accounts for more than 50% of total area globally and contributes significantly to global food security. Genotype and environment are the main factors influencing grain yield in irrigated rice production. The present study was conducted to study genotype x environment interaction and yield performance using eight near isogenic lines (NILs) developed from IR20/CT9993//IR20 for grain yield and its stability under irrigated environment over different seasons. Graphical genotyping of the eight NILs was performed using microsatellite (SSR) markers. The results showed that NILs #172 and #212 are high yielding but unstable across seasons. NILs #65 and #297 are stable across seasons, while NIL #65 is low yielding, NIL #297 is moderate yielder. NIL #297 had 57% of recurrent parent genome in the introgressed QTL region. NIL #297 may be useful in breeding for rice yield improvement.

Key words: Rice, QTL, NILs, SSR, grain yield and yield stability

Introduction

Rice is a staple food crop for more than half of world's population and grown on 163 million hectares globally with more than 87 percent of rice area in Asia alone (FAO, 2016), which is home for 60% of world population. India has largest area of 44.5 Mha under rice and contributes nearly half of the global rice production (481.9 million tons of milled rice), but with relatively low average productivity (www.worldriceproduction.com).Rice is cultivated in a wide range of ecosystems and climatic conditions ranging from lowland to upland and irrigated to rainfed situations. India is the only country in the world which has much diversity in rice ecosystems (Mahajan et al. 2017), mainly differing in hydrological

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conditions. Rice is an essential part of the daily diet in southern and eastern parts of India, where the farmers have small fragmented land prone to various abiotic and biotic stresses.

The world's population is increasing demographically and is estimated to reach 9 billion by 2050. With population increase, the global rice demand will increase and an additional of 116 million tons of rice will be needed by 2035 to feed growing population (Seck et al. 2012). International Food Policy Research Institute (IFPRI, 2015) predicts a 12 – 14 percent decline in rice production by 2050 compared to 2000 level due to climate change, with South Asia being most affected. The irrigated rice ecosystem which provides 75% of the world's rice production (GRiSP, 2013) has potential to further yield increase and is important for global food security. The 56% of irrigated rice area in Asia (Swain et al. 2005) was the major source of the large increases in productivity leading to the Green Revolution (Fischer et al. 2012). Further increase in rice production requires the development of high yielding genotypes with desirable yield related traits and stability over diverse ecosystems (IRRI, 2006).

Irrigated rice farms tend to be small (0.5 to 2 ha) with rice grown for different seasons - once, twice or even three times per year in the same field (Seck et al. 2012). Further land increase, however, is limited by future environmental issues caused by extensive farming (Milovanovic and Smutka, 2017) and other competing uses. Yield increase through new technologies and production systems are therefore the only viable option (FAO, 2000). Recent advances in genomics, with a range of modern tools and methods will fast-track the conventional breeding. Marker assisted backcross breeding (MABC) is one of the promising approach to introgress one or more locus controlling a trait of interest while retaining the essential characteristics of an elite variety which is already well adapted to the local environment and widely cultivated by the farmers, eliminating the undesirable linkage drag as quick as possible and have superiority in precision and efficiency as compared to that of conventional breeding. The method utilizes (marker-assisted) recurrent selection to quickly pyramid the major genes/QTL in the first few selection cycles and maintain genetic variation contributed by many minor genes to be explored in later cycles (Liang et al. 2016). Backcrossing is also useful to dissect the genetic architecture of quantitative traits because it isolates a gene or chromosomal region in a different genetic background (of the recurrent parent). It is one of the few reliable methods to validate the additive effect of a quantitative trait locus (QTL) or a candidate gene (Hospital, 2005).

There are interactions between genes/QTLs underlying yield-related traits and particular ecological environment. Yield performance of different rice genotypes vary greatly depending on the environment. Most of the Asian farmers are small farm holders with diverse environment. Genotype, environment and the genotype-by-environment interaction (GEI) collectively determine the phenotypic performance of a variety (Falconer and Mackey, 1996). The relative importance of genotype, environment and GEI on grain yield is less studied for irrigated rice (Liang et al. 2015). The stability of yield performance in particular environment, especially similar to farmers' field is one of the most desirable characters of a genotype to be released as a variety, which allows the developed varieties to be adopted in large area in that environment (Liang et al. 2015).

Considering this strategy and challenges, in the present study, the marker assisted backcrossed derived lines (near isogenic lines) from a cross between high yielding IR20 and drought tolerant CT9993 were evaluated for phenology, plant production and yield stability across seasons under irrigated environment.

Materials and methods

Field phenotypic evaluation

Field trials were conducted in the experimental fields of Tamil Nadu Agricultural University at Paddy Breeding Station, Coimbatore, India for three consecutive seasons using a sub set of eight backcrossed derived near isogenic lines (NILs) along with parents. The BC derived lines were developed from long term research in our lab by crossing IR20 (female) with CT9993-5-10-1-M (abbreviated as CT9993) (male) and subsequent back crossing of true F_1 hybrids as male and IR20 as recurrent female. The eight NILs had QTLs for root traits introgressed from CT9993 into IR20 and the scheme of marker-assisted backcross breeding of these eight NILs is described in our earlier paper (Suji et al., 2012). The eight BC_4F_7 NILs were evaluated for yield and its stability in three different seasons, February – June, 2014 (Trial 1), August – December, 2014 (Trial 2) and August – December, 2015 (Trial 3). Twenty-one day old seedlings were transplanted in plots of 2.0 x 0.2 m with a spacing of 0.2x0.1 m between and within rows, respectively. The plots were surface irrigated to field capacity once in every four days. NPK fertilizers were applied at the rate of 100:50:50 kg ha^{-1}.

Data on days to 50% flowering, plant height, number of productive tillers, panicle length, number of grains per panicle and yield per plant were collected following standard evaluation system for rice (IRRI, 1996). All the plants in each plot were harvested at maturity and grain yield (GY) and straw yield (SY) were recorded after sun drying. Total above ground biomass was computed by summing grain and straw yields. Harvest index (HI) was calculated as the ratio of grain weight to total above ground biomass for each germplasm

Genotyping

DNA was extracted from the eight BC_4F_7 lines of IR20/CT9993//IR20 and parents following CTAB procedure (Gawel and Jarret, 1991) and amplified using the markers flanking target QTLs (Table 1). The flanking SSR markers, RM252-RM348 and RM348-RM280 for basal root thickness, penetrated root thickness and root pulling force QTLs, respectively on chromosome 4 and RM257-RM242 for penetrated root thickness QTL on chromosome 9 were used in foreground selection for introgressed QTLs. Additionally, 53 polymorphic markers out of 210 rice microsatellite markers screened between the parents, were also used in genotyping the eight MABC derived lines. The genotypic data generated was analysed using the software, GGT ver. 2.0 (acronym for GraphicalGenoTypes). It uses the data of markers with known map positions on a genetic map, allowing GGT 2.0 to display estimated lengths of genomic compositions as coloured chromosome bar segments.

Statistical analysis

All trials were separately analysed for descriptive statistics using single environment analysis using Plant Breeding Tools (PBTools, 2014) V:1.4 (bbi.irri.org). PBTools was also used to perform multienvironment analysis for one stage and two-stage analysis to estimate variance components and pairwise mean comparison with parent IR20. The three trials considered as three seasons (environments). The GEI was also decomposed into genotype-by-season interaction. The additive main effects and multiplicative interaction (AMMI) model and the genotype main effects and genotype × environment interaction effects (GGE) model (Gauch, 2006) was used in analysing the GEI for the traits. Stability analysis and GEI was presented using GGE biplot.

Results and Discussion

Performance of BC derived lines for phenology and yield traits

Considerable variation in phenology and plant production traits was observed among the

MABC derived lines and parents in all the trials (Table 1). The average grain yield across the lines was 2335, 2376 and 2253 kg ha^{-1} in trial 1, 2 and 3, respectively. Single environment analysis showed significant performance of NILs over parents. For instance, in trial 1, NILs 172, 182, 212, 297 and 354 significantly yielded higher than IR20. In trial 2, NILs 182, 212 and 121 significantly yielded better, while NILs 65, 125, 172, 297 and 354 yielded less than IR20. Similarly, in trial 3, NILs 172 and 212 gave higher yield, while other NILs yielded less than IR20. In all the trials, NIL 212 recorded significantly higher yield over IR20. One stage analysis in multi-environments did not show any NIL significantly out performing IR20 in grain yield. However, NIL172 recorded significantly higher total biomass over IR20. Similar significant high and low performance of NILs across seasons were reported in rice earlier (Liu et al. 2016, Brondani et al. 2002, Xie et al. 2006). Total biomass was very strongly correlated with grain yield with R^2 = 0.84**, 0.90** and 0.86** in trial 1, 2 and 3, respectively (data not shown). The strong positive correlation between biomass and grain yield was reported earlier in rice (Babu et al. 2003).

Relationship among genotypes and trials

Significant variation was observed in trials and Genotype x Environment (GE) interaction. The biplot graph explained 50.8% interaction in PC1, while PC2 explained 28.5% of interaction, totally 79.4% of significant variation, which is sufficient to explain the GEI. This is in accordance with Gauch and Zobel's (1996) recommendation that the first two PCs are usually sufficient. Similarly, Yan and Rajcan (2002) also suggested that most of the interaction occurred in the first few axes. Significance of GE interaction indicates inconsistency of genotypes in response to changing seasons/environments (Ayalneh et al. 2014) and exhibit both additive and crossover types of interaction (Palanog et al. 2014). Similar results were reported earlier in rice (Khatun et al. 2015; Das et al. 2010). Proper interpretation of GE interaction is vital in plant breeding programs which need multi-location testing.

The result of GGE biplot summarizes the interrelationship of different trials (Fig 1). The environment 1 (E1) (trial 1) having acute angle with environment 2 (E2) (trial 2) signifies positive correlation between environments (trials), while the environment (E3) (trial 3) with E2 (trial 2) forms a right angle showing no correlation. This indicated the similarity of the seasons when the trial was conducted. The trial 2 and trial 3 were sown during August 2014 and 2015, respectively, coinciding with Samba season of rice cultivation, while the trial 1 was conducted during Navarai season of cultivation in experimental location.

Stability analysis

Based on mean yield and stability performances (Fig 2), genotype G5 (NIL 212) and G3 (NIL 172) are located near concentric circle meaning the genotypes have higher yield across trials compared to IR20, but are less stable across trials as they have longer vector length. The NIL 212 recorded higher yield in trial 2, while NIL172 had higher yield in trial 1 and trial 3. Shorter the vector length more stable the genotype. The genotype G8 (NIL 65) followed by G6 (NIL 297) showed stability across seasons with comparatively low yields (Fig 3). This is consistent with the earlier report of Palanog et al. (2014) and Khatun et al. (2015), wherein the high yielding varieties have shown more instability across the environments.

Genetic Analysis

Foreground analysis revealed presence of introgressed region in all the eight NILs. Among 53 polymorphic markers genotyped, ten markers are located within the introgressed region (RM252 – RM280) from chromosome 4, which further saturated the introgressed QTL region. The eight NILs have atleast one QTL segment from the donor region. The NIL297 with higher percentage of recurrent parent genome (57%) in the introgressed region and having four segments from donor, performed stably in all the environments and comparatively yielded more than that of the other more stable NIL65 (recurrent parent genome recovery is only 29%). The high yielding NIL 172 has higher proportion of recurrent parent genome (71%) within the introgressed region but was not stable (Fig 4). The effect of introgressed region in the background of the recurrent parent genome seemed to contribute to yield stability in certain NILs. This positive effect on yield and its stability under control condition may be due to alleles for yield related traits from IR20. Earlier reports identified this introgressed region, RM252 – RM348 - RM280 on chromosome 4 linked to grain yield under drought, plant height and total biomass under control (Babu et al. 2003) and metaQTL for grain yield and panicle length under drought and panicle number under control and stress in CT9993/IR62266 double haploid rice lines (Sellamuthu et al. 2011). Hence, the increased grain yield in certain NILs under control may be due to higher biomass and panicle number contributed by IR20. The genetic analysis using IR20 NILs confirmed the additive effect of the donor CT9993 on panicle length (R^2 = 20.7 and 53.95% in trial 1 and 2, respectively) and total biomass (R^2 = 16.3% in trial 2). Understanding the effect of alleles from the same donor in a different recurrent genome and how they interact to generate a new phenotype will help to better manipulate them in breeding for yield improvement in rice.

Conclusion

To summarize, the NILs showed differences in yield and yield stability across trials, which showed the importance of genotype and environment interaction, suggesting possibility to select genotypes with stable performance across seasons. The stable genotype G6 (NIL297), with relatively more proportion of recurrent genome can be further tested in multi-locations to study its suitability as an advance breeding line for rice yield improvement. NIL297 along with NIL212 had thicker and longer nodal roots in rainfed target populations of environments (Suji et al. 2012). Hence, these NILs may be useful for rainfed rice improvement as well.

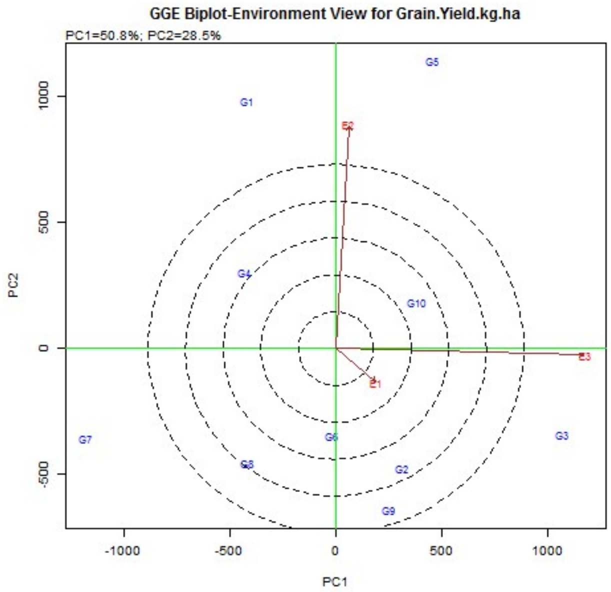

Figure 1: Interrelationship of different irrigated trials conducted using IR20/CT9993//IR20 NILs and parents

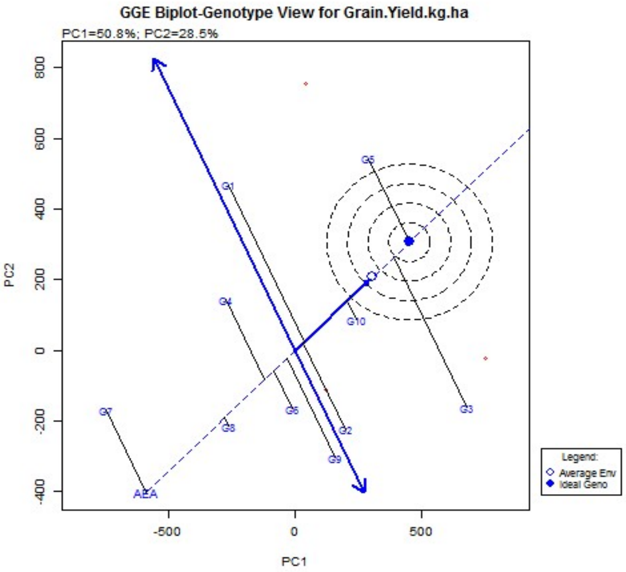

Figure 2: Mean yield and stability performances of eight IR20/CT9993//IR20 NILs and parents under irrigated conditions

Figure 3: Stability and response plot of eight IR20/CT9993//IR20 NILs and parents under irrigated conditions across seasons

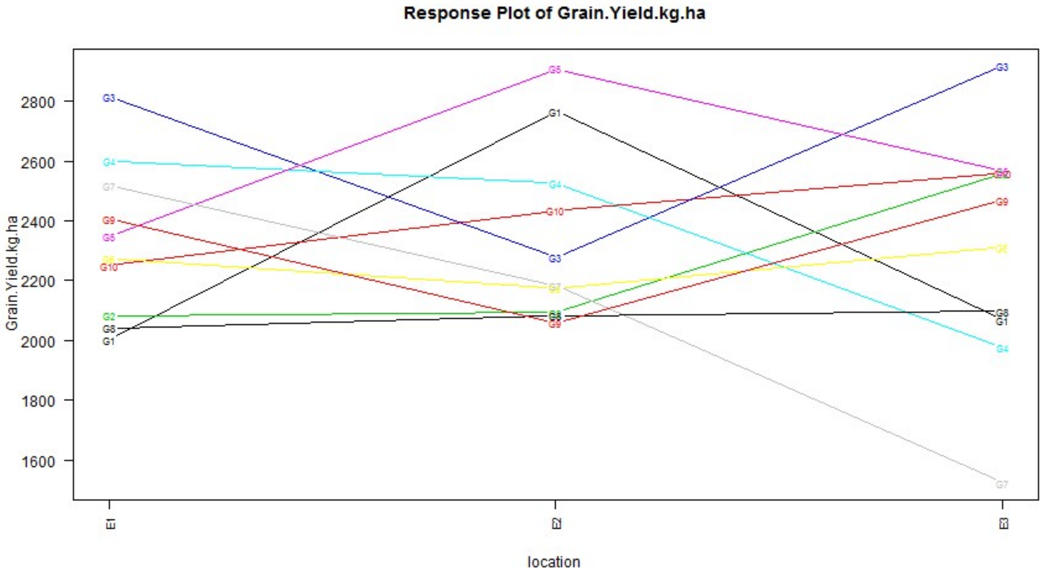

Figure 4: Variation in introgressed genomic region among eight IR20/CT9993//IR20 NILs

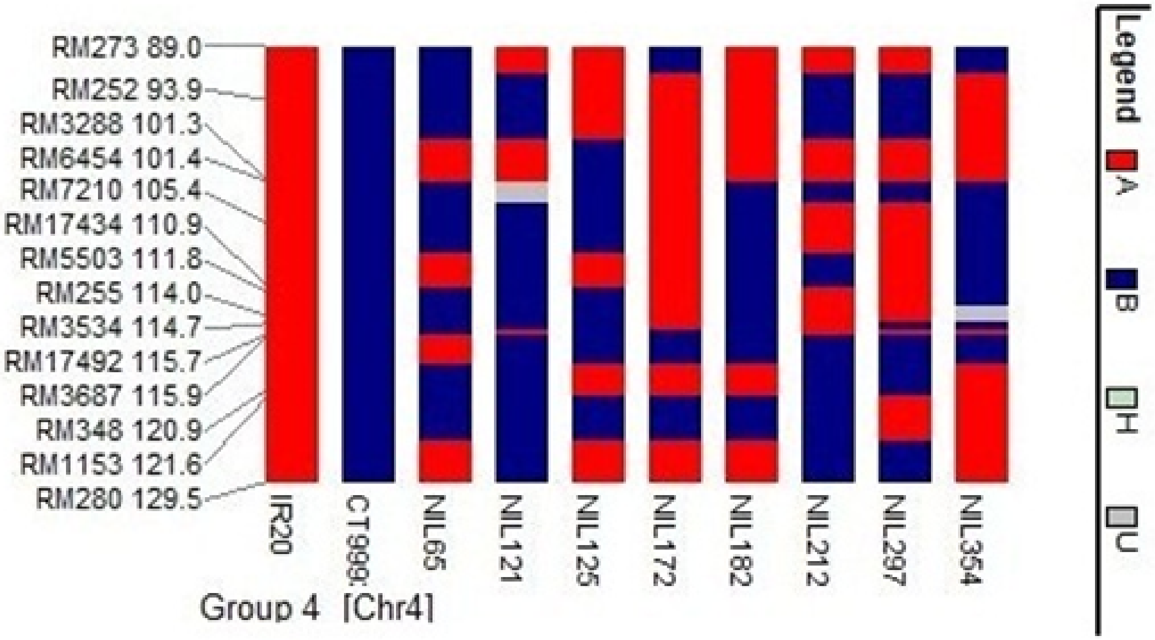

Table 1: Performance of IR20/CT9993//IR20 NILs over seasons under irrigated condition in Coimbatore, India

Traits	**Trial**	**Parents**		**NILs**										
		IR20	**CT9993**	**65**	**121**	**125**	**172**	**182**	**212**	**297**	**354**	**Mean**	**Range**	**SD**
Days to 50 % flowering	**1**	80	95	97	86	88	85	86	94	97	93	90.75	85.0 - 97.0	5.1
	2	100	101	98	94	105	108	103	103	106	107	103.00	94.0 – 108.0	4.8
	3	94	91	89	89	95	98.0	95	90	94	91	92.63	89.0 – 98.0	3.3
Plant Height cm	**1**	87.3	91.3	100.7	105.3	92.7	89.0	87.3	82.3	94.3	86.3	92.25	82.33- 105.33	7.7
	2	90.0	105.3	132.0	95.0	105.7	113.0	113.0	108.3	108.7	94.0	108.71	94.0 – 132.0	11.9
	3	97.0	105.3	87.3	96.3	95.3	116.3	103.3	112.7	102.0	99.7	101.63	87.3 - 116.3	9.4
No of Tillers	**1**	12.0	13.0	12.0	17.0	14.0	10.3	12.7	11.7	16.7	16.3	13.83	10.3 - 17.0	2.6
	2	10.3	12.0	14.0	12.0	8.7	12.3	12.7	9.7	8.0	9.0	10.79	8.0 – 14.0	2.2
	3	16.0	10.3	19.7	11.0	14.3	12.7	11.0	12.0	14.0	10.7	13.17	10.7 - 19.7	3.0
No of Productive Tillers	**1**	12.0	13.0	12.0	17.0	14.0	10.3	12.7	11.7	16.7	16.3	13.83	10.3 - 17.0	2.6
	2	10.3	11.7	11.0	10.7	8.7	12.3	12.7	9.7	8.0	9.0	10.25	8.0 - 12.7	1.7
	3	16.0	10.3	17.0	9.7	13.0	11.3	9.7	11.7	13.0	10.7	12.00	9.7 - 17.0	2.4
Spikelet Fertility %	**1**	90.5	86.2	79.1	87.2	91.9	88.8	90.3	93.1	91.4	84.6	88.31	79.1 - 93.1	4.6
	2	60.1	87.4	73.0	87.9	89.9	75.3	82.5	83.6	87.5	75.8	81.95	73.0 - 89.9	6.5
	3	59.0	77.2	80.1	83.0	76.0	70.4	76.1	67.3	68.8	63.9	73.21	63.9 - 83.0	6.6
Panicle Length cm	**1**	21.0	23.0	19.2	21.3	24.0	22.8	25.5	25.5	24.8	23.3	23.31	19.2 - 20.2	2.2
	2	23.8	23.0	22.9	24.2	23.9	25.7	26.3	27.2	22.6	26.8	24.97	22.6 - 27.2	1.8
	3	24.9	23.9	20.3	24.6	20.3	23.3	23.7	24.3	22.8	23.1	22.79	20.3 -24.6	1.6
Grain Yield kg ha^{-1}	**1**	2250	2408	2042	2000	2083	2817	2600	2350	2275	2517	2335.4	2000 - 2817	292.9
	2	2433	2058	2083	2767	2093	2277	2525	2909	2175	2183	2376.5	2083 - 2909	318.9
	3	2561	2470	2098	2068	2560	2919	1976	2568	2311	1523	2252.9	1523 - 2919	432.3
Straw Yield kg ha^{-1}	**1**	7083	7667	8667	9300	8933	7833	6667	6700	8825	8500	8178.1	6666 - 9300	1012.6
	2	3750	3875	3250	4767	3608	4958	5117	4600	3350	3417	4133.3	3250 – 5116	797.3
	3	6769	8292	8729	7996	9696	8846	7836	8769	7767	6261	8237.7	6261 - 9696	1025.9
Total Biomass kg ha^{-1}	**1**	9333	10075	10708	11300	11017	10650	9267	9050	11100	11017	10513.5	9050 – 11300	863.7
	2	6183	5933	5333	7533	5702	7235	7642	7509	5525	5600	6509.9	5333 - 7648	1048.0
	3	9330	10762	10827	10064	12256	11765	9812	11337	10079	7786	10490.6	7785 - 12256	1399.3
Harvest Index	**1**	0.24	0.24	0.19	0.18	0.19	0.26	0.28	0.26	0.20	0.23	0.22	0.18 - 0.28	0.04
	2	0.39	0.35	0.39	0.37	0.37	0.31	0.33	0.39	0.39	0.39	0.37	0.31 - 0.39	0.03
	3	0.27	0.23	0.19	0.21	0.21	0.25	0.20	0.23	0.23	0.20	0.21	0.19 - 0.25	0.02

Trial 1: Feb., – June 2014, Trial 2: Aug., – Dec., 2014 and Trial 3: Aug., - Dec., 2015

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