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Evaluating Genetic Parameters and Combining Ability of Starch Viscosity Parameters in Rice Cultivars (*Oryza sativa* L.)

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A B S T R A C T
This study was undertaken to assess the combining ability of 6 rice varieties, for viscosity parameters and determining gene action controlling Rapid Visco Analyser (RVA) characters. F2 progenies derived from a 6×6 half diallel mating design with their parents were grown in a randomized complete block design with three replications at the research farm of Rice Research Institute of Iran (RRII) in 2015. The diallel analysis by Griffing's method indicated the involvement of additive and
non-additive gene actions controlling RVA traits. For traits PV and FV RI18447-2 and IR50 were the best combiners for increasing and decreasing, respectively. Deylamani and IR50 were the best combiners for increasing and decreasing BV, respectively. Beside, due to more portion of non-additive gene action in controlling trait SV, The Gilaneh × RI18430-46, and Deylamani × RI18430-46 crosses were the best for increasing and decreasing SV, respectively. The high estimates of broad sense heritability and narrow sense heritability for BV and FV, indicated the importance of additive effects in expression of these traits. Therefore, selection base breeding methods will be useful to improve these traits and selection in the early generations could be done to fix the favourable genes. Low estimate of narrow sense heritability for SV revealed that non-additive gene effects play important role in controlling setback viscosity. So, hybrid base breeding methods will be useful to

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Introduction

Rice (*Oryza sativa* L.) is the most important and extensively cultivated cereal crop stand next to wheat in the global food grain production and is the main source of livelihood for more than 1.50 million rural households (Roy et al., 2013). It provides the main staple food for more people in the world than any other crop (Feng et al., 2013). Rice productivity has greatly improved over the past few decades. However, new varieties need to be developed with much higher yields to cope with increasing population levels, but potential new varieties need to be accepted by farmers, the market, and consumers (Stuart et al., 2016).

Grain quality is one of the most important objectives of most rice breeding programs. In many rice-producing areas of the world, cooking and eating quality always represents a major criterion in evaluating rice grain. Cooking and eating quality in rice is mainly determined by the starch composition (Wang et al., 2010). Among various physicochemical properties, cooking properties are the mainly traits, determining the application quality of rice. Meanwhile, those properties have high heritability, supporting early selection for breeding to efficient to get the desired target trait (Chen et al., 2014). Starch is the major component of rice and described its physiochemical properties, such as gel strength and pasting properties (Singh et al., 2000; Martin and Fitzgerald, 2002; Derycke et al., 2005). The gelatinization and retrogradation properties of starch or flour are the most important properties of raw and cooking rice (Huang and Lai, 2014). Extensive studies have been undertaken to determine the quality of rice and starch-based foods, and new tools have been developed.

Currently, Rapid Visco Analyser (RVA) is seen as a rapid and reproducible analytical tool that can be used to assess the gelatinization and retrogradation properties of rice starch and flour during the heating and cooling cooking cycles (Fitzgerald et al., 2003). Effective information can be obtained from the RVA profile. The formation of a viscosity profile involves the transition from semi-crystalline power suspensions to a gelatinized paste (Zhou et al., 2016). The starch granules of rice play active role; if there is less swelling of starch during gelatinization, the peak viscosity of starch paste will be lower (Tananuwong and Malila, 2011). To understand the viscosity properties of rice starch, the RVA works as a physical index for the estimation and as a precursor of cooking and processing qualities (Martin and Fitzgerald, 2002; Bryant et al., 2012). Among the different RVA profiles, setback and breakdown values play significant role as compared to peak viscosity and others in the estimation of cooking quality of rice starch properties. The characteristic of RVA is closely connected to taste quality of rice, where higher peak viscosity and breakdown and the smaller setback value enhance the grain quality. The rice cooked is soft and glutinous in texture (Sharifi et al., 2009). The amount of amylose content in starch is positively correlated with rate and extent of retrogradation (Swinkels, 1998). Study showed that cooked rice with higher amylose rice starch varieties had lesser stickiness, cooked dry, while, keeping higher setback values or cultivars with lower breakdown value, peak viscosity and taste meter values fall in the category of inferior qualities rice variety (Kurasawa et al., 1972). Therefore, understanding the inheritance, specifically gene action, for rice appearance quality traits is desirable.

Diallel analysis is the first step in most plant breeding programs aimed at improving yield and other related parameters (Griffing, 1956). The diallel cross designs are frequently used in plant breeding research to obtain information about genetic properties of parental lines or estimates of general combining ability (GCA), specific combining ability (SCA) and heritability. The knowledge of combining ability is useful to assess nicking ability among genotypes and at the same time explicate the nature and magnitude of gene actions involved (Haghighi Hasanalideh et al., 2017). It also provides information on the additive and dominance variance which may be useful for suggesting an appropriate breeding strategy to be followed for isolation of pure lines or exploitation of heterosis. Based on combining ability analysis of different characters, higher SCA values refer to dominance gene effects and higher GCA effects indicate a greater role of additive gene effects controlling the characters. If both the GCA and SCA values are not significant, epistatic gene effects may play an important role in the genetic of characters (Sprague and Tatum, 1942). It is usually difficult to obtain sufficient F₁ seeds crops where hand emasculation must be done. Due to easiness of production of large quantity of F2 seeds, many researchers use F2 generation for diallel analysis to estimate combining ability and other genetic parameters, they also reported that F₂ analysis provide reliable and better information than F₁ generation (Hahghighi Hasanalideh et al., 2017).

Jin-Song et al. (2006) reported that the total main genetic variances accounted for over 64% of the total genetic variance for breakdown viscosity, consistency, and setback, indicating that these traits were mainly controlled by the main genetic effects in addition to the genotype environment interaction effects. The estimated total narrow-sense heritability was 67.8%, 79.5%, and 79.5% for breakdown, consistency, and setback respectively. The general heritability accounted for over 75% of the total heritability, indicating that early selection would be effective for those traits.

Jin et al. (2004) analyzed taste meter value and Starch RVA properties by Griffing's diallel method with eight *indica* rice cultivars. They revealed that the additive variation was principal in taste meter value, peak viscosity, trough viscosity, final viscosity, breakdown, setback, consistency, while the non-additive variation was dominant in pasting temperature. the higher the taste meter value and RVA properties of parents, the greater the GCA effect in progeny population. The correlation coefficient between plot means in taste meter value and RVA properties and the GCA effect were significantly positive at the 0.01 level, therefore, it will be possible to be used in the forecast of taste meter value and RVA properties for the hybrid of rice.

Considering the above contents, the present study was undertaken to assess the combining ability of 6 rice varieties, for viscosity parameters and determining gene action controlling RVA characters with the aim of recognition breeding methods for improving quality traits of rice.

Materials and Methods

The plant material consisted of six parent diallel cross excluding reciprocals. The experiment was conducted at Rice Research Institute of Iran (RRII), Rasht, Guilan province, during 2012-2015. Six Iranian and foreign rice varieties (Table 1) were selected as parental lines in this study. These parents had differences in origin, pedigree and some of the quality and morphological traits (Table 1). Parent lines selected among 94 rice genotypes from different regions and origins thorough molecular analyzing for genetic diversity (Allahgholipour et al., 2014). These parental lines crossed in a diallel mating design in 2013. In order to produce F_2 progenies, F_1 populations from a 6×6 half diallel cross selfed in 2014. The plant genetic materials (parents and F₂s) were grown in a randomized complete block design with three replications in research farm of Rice Research Institute of Iran (RRII) in 2015. After processing paddy to white rice, the samples were floured with a UD milling machine at a rate of 100 mesh. The rice flour samples (3g, based on 14% moisture) were mixed with distilled water (25 ml) in an aluminum vessel. The pasting properties were measured by an RVA (RVA-3D model, Newport Scientific, Sydney, Australia). A rice standard profile was used and the heating and cooling cycle was as follows: the starting temperature was 50°C, which was held for 1 min, then the sample was heated through linear increasing of temperature to 95°C in 4 min 48 sec and this was maintained for 7 min 18 sec Finally the sample was cooled to 50°C (linear decreasing in temperature for 11 min 6 sec), which was held for 12 min. The device was set to start at 960 rpm in the first 10 secs, and after, the speed was set at 160 rpm. (American Association of Cereal Chemists, 1995). Then starch RVA parameters including Peak Viscosity (PV), Breakdown Viscosity (BV), Final Viscosity (FV) and Setback Viscosity (SV) were evaluated.

Table 1 Name, origin and pedigree of rice genotypes used in this study

S.N	Genotypes	Pedigree	Origin
1	Gilaneh ^a	Saleh ^a / Abjiboji ^b // Abjiboji	RRII ^c , Iran
2	Daylamani	Local cultivar	Mazandran, Iran
3	IR50	IR50	IRRI ^d , Philippines
4	Line 23	IR75479-199-3-3	IRRI, Philippines
5	RI18447-2	Sepidrood ^a / Gharib ^b	RRII, Iran
6	RI18430-46	Saleh ^a / Hashemi ^b	RRII, Iran

^aImproved rice variety of Iran, ^bLocal rice variety of Iran, ^cRice Research Institute of Iran, ^dInternational Rice Research

Table 2 Analysis of variance for RVA parameters.

Same of mains	df		Mean	Square	
Source of variance	ai	PV	BV	FV	SV
Genotypes	20	1328.16**	514.25**	1524.84**	110.96**
Replications	2	12.29 ^{ns}	0.32 ^{ns}	9.65 ^{ns}	9.10 ^{ns}
Error	40	72.77	29.20	84.96	20.24

^{ns} and ** non-significant and significant at 1% probability level respectively, PV= Peak Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity and SV= Setback Viscosity.

Table 3 Analysis of variance for combining ability

Source of verience	df -	Mean Square			
Source of variance	ui	PV	BV	FV	SV
Replication	2	13.24 ^{ns}	4.14 ^{ns}	20.86 ^{ns}	1.99 ^{ns}
GCA	5	1482.45**	628.32**	1969.9**	85.87**
SCA	15	486.27**	143.36**	616.49**	141.25**
Error	40	90.39	36.43	104.01	18.97
Baker Ratio		0.86	0.89	0.86	0.55

^{ns}, * and ** non-significant and significant at 5 and 1% probability levels respectively, PV= Peak Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity and SV= Setback Viscosity.

Analysis of variance was performed by MSTATC ver 1.42 (ANOVA) and Griffing's (1956) diallel analysis was conducted by Dial 98 software (Ukai, 2006) to estimate the general (GCA) and specific (SCA) combining abilities determined according to Singh and Chaudary (1977). This method was calculated by following model:

$X_{ij} = u + g_i + g_j + s_{ij} + e_{ijk} \label{eq:constraint}$

Where, u=the population mean, g_i =the general combining ability effect of the ith parent, g_j =the general combining ability effect of the jth parent, s_{ij} =the specific combining ability effect of the cross between ith and jth parents such that s_{ij} = s_{ji} and e_{ijk} the environmental effect associated with ijkth observation. The combining ability ratio was calculated according to Baker (1978) as follow:

Baker ratio=
$$\frac{2MS_{GCA}}{2MS_{GCA} + MS_{SCA}}$$

F₂'s genetic parameters determined according to Dhellon and Malhi (1976) and Sharma (2006). Hayman's graph (V_r-W_r graph) is drawn with the help of variances of arrays (V_r) and covariance's (W_r) between parents and their F₂ progenies. The array refers to the crosses in which a particular parent is common. The W_{ri} values are estimated for all the arrays by the formula: W_{ri} = (V_{ri} × V_{OLO})^{0.5} where, V_{ri} is the variance of rth array and V_{OLO} is the variance of parents. The W_{ri} values are plotted against V_r values to draw the limiting parabola. The W_{ri} values are obtained by the formula: W_{ri} = W_r- bV_r + bV_{ri} for drawing regression line, where, V_{ri} is array mean of variances, W_r is array mean of covariance's and b is regression coefficient (Singh et al., 1990; Singh and Narayanan, 1993).

Results

Analysis of variance for RVA parameters revealed highly significant differences among genotypes (parents and hybrids) for all studied traits, showing the presence of genotypic variability among them (Table 2). The diallel analysis by Griffing`s method indicated highly significant differences of GCA and SCA variances for traits PV, BV, FV and SV (Table 3). Baker's ratio (Table 3) showed the amounts of 0.86, 0.89, 0.86 and 0.55 for PV, BV, FV and SV, respectively.

Estimate of GCA for RVA parameters of parents using in this study showed that for PV parameter, parents Gilaneh and IR50 had negative estimation of GCA, whereas other parents had positive estimate of GCA. Parent RI18447-2 had highest GCA and parent IR50 showed lowest amount of GCA. For BV, IR50 and Deylamani had the lowest and highest GCA, respectively. For FV, IR50 and RI18447-2 revealed the minimum and maximum estimation of GCA, respectively. Parents RI18430-46 and RI18447-2 had highest and lowest estimation of GCA, respectively, for SV parameter. The estimates of SCA for RVA parameters (Table 4) indicated that The Gilaneh × Daylamani and Gilaneh × RI18447-2 crosses had the highest and lowest SCA for PV and BV. For FV, the IR50 × Line 23 and Line $23 \times RI18447$ -2 crosses had the lowest and highest SCA, respectively. The Gilaneh × RI18430-46, had the highest SCA for SV, and the Deylamani × RI18430-46 cross had minimum amount of SCA estimation.

Damanta		RVA pa	rameters	
Parents	PV	BV	FV	SV
Gilaneh	-0.27	4.09	2.45	2.72
Daylamani	6.21	10.09	6.13	-0.08
IR50	-21.57	-10.03	-24.27	-2.71
Line 23	5.13	-2.51	5.3	0.16
RI18447-2	9.28	-5	12.48	3.2
RI18430-46	1.21	3.36	-2.08	-3.29
SE(g _i)	3.07	1.95	3.29	1.40

Table 4 General combining ability (GCA) of rice genotypes for RVA parameters.

PV= Peak Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity and SV= Setback Viscosity.

Table 5. Specific combining	ability (SCA) of crosses	s genotypes for RVA parameters.

Creases		RVA pa	rameters	
Crosses	PV	BV	FV	SV
1×2	13.21	10.21	7.26	-5.95
1×3	12.02	3.75	14.44	2.42
1×4	4.9	3.65	-0.88	-5.78
1×5	-19.75	-10.45	-21.09	-1.34
1×6	-10.37	-7.17	0.27	10.65
2×3	-1.16	-4.75	7.43	8.58
2×4	-5.11	-2.91	0.13	5.24
2×5	1.47	-3.95	0.73	-0.74
2×6	-8.41	1.41	-15.55	-7.14
3×4	-17.33	-2.68	-22.83	-5.49
3×5	3.13	3.34	0.27	-2.86
3×6	3.34	0.34	0.69	-2.65
4×5	8.63	3.79	14.54	5.91
4×6	8.92	-1.85	9.04	0.12
5×6	6.52	7.28	5.55	-0.97
SE(s _{ij})	8.43	5.35	9.04	3.86

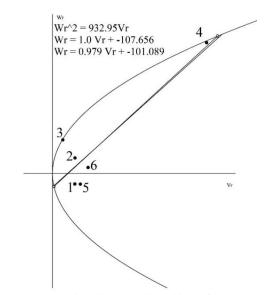
PV= Peak Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity and SV= Setback Viscosity. 1=Gilaneh, 2=Deylamani, 3=IR50, 4=line 23, 5=RI18447-2, 6=RI18430-46.

Constis Demonstration	RVA parameters				
Genetic Parameters	PV	BV	FV	SV	
D	932.95**	378.27**	911.06**	19.29*	
H1	1363.57**	154.3*	1691.93**	135.77**	
H2	870.59**	128.7*	1099.6**	123.92**	
F	1042.78**	155.3 ^{ns}	1070.65**	14.23 ^{ns}	
0.25sqr(H1/D)	1.21**	0.32**	1.36**	1.33 ^{ns}	
kd/(kd+kr)	0.73**	0.66**	0.72**	0.57**	
uv	0.16**	0.21**	0.16**	0.23**	
h ² b	0.94**	0.94**	0.94**	0.86**	
$h^{2}n$	0.44**	0.75**	0.61**	0.18*	

^{ns}, * and ** non-significant and significant at 5% and 1% probability levels respectively, PV= Peak Viscosity, BV= Breakdown Viscosity, FV= Final Viscosity and SV= Setback Viscosity, D=Additive variance, H1=Dominance variance, H2=Dominance variance, F=Relative frequency of dominant and recessive allels, 0.25sqr(H1/D)=Average degree of dominance, (kd/kd+kr)=Proportion of dominance genes, uv= Balance of positive and negative alleles, (h_{h}^{2}) =Broad-sense heritability, (h_{n}^{2}) =Narrow-sense heritability.

Estimates of genetic parameters (Table 6) revealed that the parameters D, H1 and H2 were significant for all RVA parameters. Average degree of dominance (0.25sqr(H1/D)) was significant and lesser than one for BV. While, it was greater than one for PV, FV and SV. Although, the average degree of dominance was not significant for SV. Broad sense heritability (h_b^2) had high estimations for all RVA parameters. Narrow sense heritability (h_n^2) estimates was moderate for FV, high for PV and BV and low for SV.

Hayman's graphs revealed that regression line passed below the origin cutting W_r axis in the negative region for PV, FV and SV (Figure 1, 3 and 4 respectively). showing the presence of over dominance. However, high difference between regression line and regression line with slope of one for SV, suggested the presence of non-allelic interaction. Hayman's graph for BV (Figure 2) revealed the presence of partial dominance of gene effect due to passing regression line above the origin. The dispersion of parents around the regression line for PV and FV (Figure 1 and 3) indicated that parents Gilaneh, Deylamani, IR50, RI18447-2 and RI18430-46 were close to the origin of the coordinate, and accordingly had more than 75% dominant genes, while, parents Line 23 was far from the origin, therefore it had <25% of dominant genes. The dispersion of parents around the regression line for BV (Figure 2) showed that parents IR50 and RI18447-2 were close to the origin of the coordinate, and accordingly have more than 75% of dominant genes parents Gilaneh, Line 23 and RI18430-46 had 50-75% of dominant genes, while parent



Deylamani was far from the origin and therefore had lesser than 25% of dominant genes. Most of the dominant genes for SV (Figure 4) were distributed in parents Gilaneh, and RI18447-2, while, recessive genes were mostly distributed in parent RI18430-46.

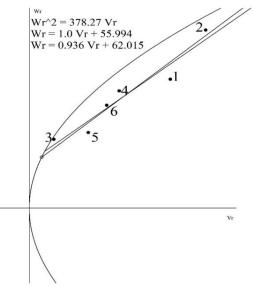


Figure 1 Regression line and dispersion of parents around origin for Peak Viscosity. 1=Gilaneh, 2=Deylamani, 3=IR50, 4=line 23, 5=RI18447-2, 6=RI18430-46.

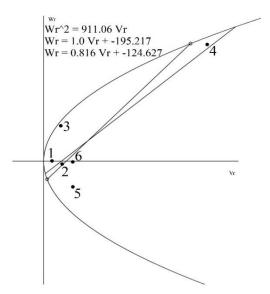
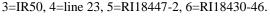


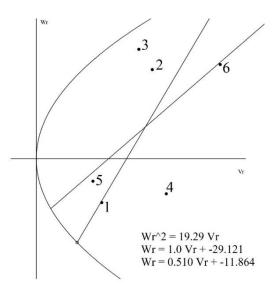
Figure 3 Regression line and dispersion of parents around origin for Final Viscosity. 1=Gilaneh, 2=Deylamani, 3=IR50, 4=line 23, 5=RI18447-2, 6=RI18430-46.

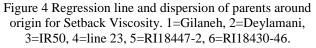
Discussion

The results of combining ability analysis indicated that GCA and SCA variances were significant for all RVA parameters, indicating the involvement of additive and non-additive effects of gene action in their inheritance, however high amounts of Bakers ratio for PV, BV and FV remarked more portion of additive gene effect in controlling these traits. Jin et al. (2004) reported that additive gene effect was principal in PV, FV, BV and SV. Gravois and Webb (1997) reported the presence of additive effects because of significant GCA for peak, hot paste, and cool paste viscosities.

Figure 2 Regression line and dispersion of parents around origin for Breakdown Viscosity. 1=Gilaneh, 2=Deylamani, 3-IP50 4-line 23 5-PI18447 2 6-PI18430 46







Due to high estimates of Baker's ratio and likewise, more portion of additive gene action in controlling traits PV, BV and FV, according to GCA estimates, for PV and FV, parents IR50 and RI18447-2 were the best combiners for increasing and decreasing, respectively. Parents IR50 and Deylamani were the best combiners for increasing and decreasing BV, respectively. Beside, due to more portion of non-additive gene action in controlling trait SV, The Gilaneh × RI18430-46, and Deylamani × RI18430-46 crosses were the best combines for increasing and decreasing SV, respectively.

Estimation of genetic parameters for RVA characters showed that the additive and dominance variances was significant for all RVA parameters, indicating that there was additive and non-additive effect of gene action in genetic controlling of these traits. For PV and FV, significant amount of parameter H2 and deviation from 0.25 of parameter uv (Balance of positive and negative alleles) showed that the distribution of alleles in parents was not uniform. Estimation of H1 parameter was greater than estimation of parameter H2, for all studied traits. So, most of the genes in the parent for these traits are genes with positive effects. Estimates of parameter F were greater than zero for all RVA parameters. Therefore, the ratio of dominant alleles is greater than the recessive alleles for these traits. It confirmed due to greater than 0.5 estimation values of Proportion of dominance genes (kd/(kd+kr)). The average degree of dominance was greater than one for PV, FV and SV, and revealed the existence of over dominance phenomenon in these traits. Whereas, partial dominance phenomenon indicated for BV due to lesser than one estimate of average degree of dominance. The high estimates of broad sense heritability and narrow sense heritability for BV and FV, indicated the importance of additive effects in expression of these traits. Therefore, selection base breeding methods will be useful to improve these traits and selection in the early generations could be done to fix the favorable genes. Low estimate of narrow sense heritability for SV, revealed that non-additive gene effects play important role in controlling this trait. So, hybrid base breeding methods will be useful to improve this trait. These results are in agree with results reported by Jin-Song et al. (2006). Also, Allahgholipour and Rabiei (2017) reported that additive gene effects play important role in controlling PV and BV, whereas non-additive gene effects are the principle players in controlling FV and SV. Allahgholipour et al. (2012) reported greater additive variance in compared to dominance variance for FV, PV, BV and SV, suggesting the uses of selection base breeding methods for improving these traits.

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