## Analysis of Combining Ability and Heritability of Walnut Quality

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**Abstract:** Twenty-four hybrid combinations were formulated by NCII design using hybrids (*J. sigillata* × *J. regia*) Yunxin 7926(P<sub>11</sub>), 8064(P<sub>12</sub>), 8034(P<sub>13</sub>), 7914 (P<sub>14</sub>) as female parents and 6 superior trees of *J. sigillata* from Sichuan as male parents. The combining ability and heritability of walnut characters were studied, including diameter, weight, thickness and rate of kernel. The result showed that both additive variation and non-additive variation were significant in the four characters, but the additive variation was principle. Different parents had different excellent genes and P<sub>24</sub>, P<sub>23</sub>, P<sub>22</sub> and P<sub>14</sub> were the best parents to improve walnut diameter and weight, followed by P<sub>21</sub>, P<sub>13</sub> and P11. In contrast, P<sub>25</sub> and P<sub>26</sub> showed poor effect on diameter and weight. Crosses (P<sub>11</sub>×P<sub>23</sub>), (P<sub>11</sub>×P<sub>24</sub>) and (P<sub>12</sub>×P<sub>24</sub>) were good for increasing diameter, while cross (P<sub>11</sub>×P<sub>26</sub>) was the best in increasing rate of kernel. The broad-sense heritability was ordered from high to low as diameter (0.990) > thickness(0.968) > rate of kernel (0.970) > weight(0.968) and the narrow-sense heritability was ordered as diameter (0.844) > thickness(0.821) <sup>3</sup>/<sub>4</sub>weight (0.735) > rate of kernel (0.693).

**Key words:** Walnut quality • Combining ability • Heritability

## INTRODUCTION

Walnut is a kind of precious nut and oil economic species. Thickness kernel rate, diameterand weight are the main quality indicators of economic character. Kernel rate, diameterand weight are the main quality indicators of economic character. The second distribution of light, temperature, water and air caused by topographic feature forms the unique climate type and vegetable flora in China. But in China the varieties of walnut are in a mess, the good and bad are intermingled. So it is meaningful to society, ecology and economy to breed a new variety with excellent qualities by crossing, which can help the peasants in mountain regions break away from poverty, promote the theory research level of forestry breeding [1].

Nowadays, cross-breeding by heterosis has been the main way in crops breeding [2]. It is uncertain to predict and use the heterosis because of the quantity of walnut quality which is not only affected by its own gene, but also is affected by the environment [3]. Cross breeding of *Juglans* between interspecies began in the sixties of the 20th century [4]. According to incompletely statistics, about 35 hybrid varieties were cultivated up to 2002 [5].

There were few reports about genetic development of walnut at home and abroad. Only Handche [6] analyzed heritability of 18 characters of *J. regia* L. by standard deviation method and no any report about combining ability. This paper studied and estimated the combinding ability and heritability of progenies of 24 hybrid combinations by their 4 main characters can reflect walnut's quality, which provides references for selective mating of hybrid parent, the selection of hybrid progeny and the collocation of clones and families in breeding garden.

# MATERIALS AND METHODS

**Materials:** Female parents  $(P_1)$  used in the study were 7926 (*J. sigillata* × *J. regia*), 8064 (*J. sigillata* × *J. regia*), 8034 (*J. regia* × *J. sigillata*) and 7914 (*J. sigillata* × *J. regia*) cultivated by cross breeding of *J. sigillata*  $\times$  and *J. regia* Dode).  $P_{11} \sim P_{14}$  represent 4 female parents respectively, which were introduced from Yunnan province in China in 2001 and were characterized by early bearing and thin shell. Male parent  $(P_2)$  are 6 *J. sigillata* with characteristics of thin shell and high yields,

which were selected from Si Chuan province in China represented by  $P_{21}\sim P_{26}$  respectively. There were 1080 hybrid nuts picked from above 24 hybrid combinations in 2006 in the present study.

Experiment site located in Chengdu city in Sichuan province of China, where the altitude is 480 m, mean annual temperature is 16.5, annual amount of precipitation is 900 mm and annual sunshine hours are 1298.2 h, has a subtropical climate, the wet summer, moderate and moist climate. And the space of seed tree is 5×5m.

Methods: Incomplete diallele cross design (NCII) was used in experiment, data was processed by Excel 2003, genetic parameter was estimated by the method of Gao [7]. Combining ability was estimated by fixed model and variance component and the genetic parameters were estimated by random model. The nuts were picked after they are fully matured, then natural withered. Measure their thickness of abdomen, diameter of abdomen, diameter of seam and height by vernier caliper, calculate the average of above three values as nut diameter, weigh their weight and kernel weight by electronic balance and calculate the rate of kernel. Each combination repeated 3 times, each repetition selected 5 nuts randomly from 3 seed trees. Analyzed their average means, the percentage should be transferred into arcsine by their square root.

## RESULTS AND DISCUSSION

**Measured Characters:** In this paper, the characters had obvious differentiation between single nuts. Diameter: 29.5-46.1 mm, weight: 9.05-18.91 g, thickness: 0.56-1.06 mm, kernel rate: 50.02-68.33%. But there was no obvious difference between repetitions in a combination. four characters of each combination presented in Table 1.

Four characters of hybrid and parents in original place and hybrid nuts showed significant heterosis on the whole, which is consistent with Fan [8] as shown in Table 4. The values of four characters of hybrid nuts were not only higher than their parents' average but also surpassed the dominant parent reflecting heterobeltiosis to some extent, which is in line with Xu [9].

Table (3) showed that the four characters have no significant difference between repetitions, but have an extremely significant between combinations, which indicate that the true genetic difference dose existed. Then analyzed the general combining ability (GCA) and special combining ability (SCA).

#### **Analysis of Combining Ability**

Variance Analysis of Combining Ability: The results of Variance analysis of combining ability based on the average values of the repetition in combinations are as follow (Table 4).

It can clearly be seen from Table 4 that the characters of nut diameter, weight and thickness were affected by parents GCA and SCA, while rate kernel was only affected by the male parent's GCA and SCA. Male parents GCA were extremely significant difference on four characters, while female parent's GCA had difference only on rate kernel. In combinations, all SCA had an extremely significant difference on four characters, which indicated that the selection of parents mating was very important to the quality of nuts. Seen from the results, we can see that both GCA and SCA will affect the characters of nut. From the standpoint of quantitative inheritance, the four characters were affected by additive effect and non-additive effect at the same time.

Although thickness was also affected by GCA and SCA of parents, up to 0.01 levels notable difference on statistics, thickness of both parents was thin and the hybrid nut had thin shell: 0.56-1.06 mm, which was thinner than top-grade (1.1 mm) one and superior tree (1.5 mm) stipulated by national standard (GB7907-87). It reflected thickness had no large variability from commodity value.

GCA of Parents and its Expression: Different parents had a low GCA on thickness of hybrid nut, because both parents were characterized by thin shell. While nut diameter, weight and rate of kernel displayed a significant difference between different parents. GCA ranged from -3.63 to 2.29 on nut diameter and P24 had the highest GCA, P23 took the second place, P<sub>26</sub> at lowest. For nut weight, GCA ranged -2.12 to 1.34, P<sub>24</sub> had the highest GCA, P<sub>23</sub> took the second place, P25 at lowest. For kernel rate, GCA ranged from -2.64 to 1.59, P<sub>21</sub> had the highest GCA, P<sub>26</sub> took the second place, P23 at lowest. GCA of the same parent was different even on different characters. For example, P23 and P24 had superiority on nut weight and diameter, but their GCA was lower on kernel rate. On the contrary, P<sub>26</sub> and P<sub>25</sub>, with small diameter and light weight, their kernel rates displayed a high positive effect, which showed different parents have different excellent genes. The results indicated that not any parent can promote qualities of nut.

Table 1: Average values of the four characters in the crosses

-	Nut dia	neter(mm	)		Nut we	Nut weight(g)				Thidkness(mm)				Rate of kernel(%)			
	P <sub>11</sub>	P <sub>12</sub>	P <sub>13</sub>	P <sub>14</sub>	P <sub>11</sub>	P <sub>12</sub>	P <sub>13</sub>	P <sub>14</sub>	P <sub>11</sub>	P <sub>12</sub>	P <sub>13</sub>	P <sub>14</sub>	P <sub>11</sub>	P <sub>12</sub>	P <sub>13</sub>	P <sub>14</sub>	
${P_{21}}$	37.98	40.36	38.06	40.50	12.72	13.30	12.36	14.43	0.92	0.93	0.91	0.92	60.89	60.77	59.83	60.04	
$P_{22}$	40.17	40.77	39.06	40.76	13.16	13.62	13.10	14.79	0.93	0.94	0.92	0.94	59.14	58.28	60.00	57.09	
$\mathbf{P}_{23}$	41.35	40.06	39.92	41.47	14.41	14.45	13.32	14.37	0.95	0.96	0.94	0.96	56.10	55.77	57.64	55.08	
$P_{24}$	41.73	42.02	39.78	41.61	13.98	13.90	13.76	15.31	0.92	0.96	0.93	0.95	58.12	57.96	58.28	55.33	
$\mathbf{P}_{25}$	36.53	36.99	36.93	38.40	10.21	10.88	10.72	11.31	0.90	0.90	0.90	0.92	59.86	59.41	59.92	60.43	
$\mathbf{P}_{26}$	34.69	34.77	34.93	37.07	12.75	9.95	10.39	12.67	0.88	0.89	0.89	0.91	61.77	59.17	59.86	60.26	

Table 2: Average values of character of parents and hybrids

	Nut diameter(cm)	Nut weight(g)	Thickness(mm)	Rate of kernel (%)
Female	30.8-36.4	9.19-11.74	0.92-0.95	53.1-55.7
Male	33.4-38.1	9.58-12.37	0.93-0.98	52.2-56.3
Progeny	39	12.91	0.92	58.79

Table 3: Variance analysis

		Nut diameter		Nut weight		Thickness		Rate of kernel	
Source	df	Variance	F	Variance	F	Variance	F	Variance	F
Repetition	2	0.32	2.48	0.162	1	0.003	1	0.087	1
Combination	23	1.03	7.91**	0.823	4.21**	0.014	3.37**	0.451	5.09**
Residual error	46	0.13		0.195		0.004	0.088		

Table 4: Variance analysis of combining ability

		F value			
Source	df	Nut diameter	Nut weight	Thickness	Rate of kernel
Female GCA	3	6.93**	6.05**	9.16**	2.77
Male GCA	5	39.59**	20.18**	30.52**	17.33**
SCA	15	12.11**	6.46*	5.16**	7.81**

Table 5: Effect size of general combining ability in parents

	$P_{11}$	$P_{12}$	$P_{13}$	$P_{14}$	$P_{21}$	$P_{22}$	$P_{23}$	$P_{24}$	$P_{25}$	P <sub>26</sub>
Nut diameter	-0.26	0.17	-0.88	0.97	0.23	1.19	1.71	2.29	-1.79	-3.63
Nut weight	-0.04	-0.23	-0.64	0.90	0.29	0.76	1.23	1.33	-2.13	-1.47
Thickness	-0.01	0.01	-0.01	0.01	0	0.01	0.03	0.02	-0.02	-0.03
Rate of kernel	0.52	-0.23	0.46	-0.75	1.59	-0.16	-2.64	-1.37	1.11	1.47

Table 6: Effect of special combining ability in crosses

	Nut diameter Nut weight						Thickness					Rate of kernel				
	$P_{11}$	$\mathbf{P}_{12}$	$\mathbf{P}_{\!13}$	$P_{14}$	$P_{11}$	$\mathbf{P}_{12}$	$\mathbf{P}_{13}$	$P_{14}$	$P_{11}$	$\mathbf{P}_{12}$	$P_{13}$	$P_{14}$	$P_{11}$	$\mathbf{P}_{12}$	$\mathbf{P}_{13}$	$P_{14}$
$\overline{\mathbf{P}_{21}}$	-0.99	0.96	-0.28	0.30	-0.45	0.33	-0.21	0.33	0.01	0.00	0.00	-0.01	-0.01	0.62	-1.01	0.41
$P_{22}$	0.23	1.37	-0.25	-0.40	-0.47	0.18	0.07	0.22	0.01	0.00	-0.01	0.00	-0.01	-0.11	0.91	-0.78
$\mathbf{P}_{23}$	0.90	-0.81	0.10	-0.20	0.31	0.54	-0.18	-0.67	0.00	0.00	0.00	0.00	-0.56	-0.15	1.03	-0.31
$\mathrm{P}_{24}$	0.69	0.10	-0.63	-0.65	-0.22	-0.10	0.16	0.17	-0.01	0.02	-0.01	0.00	0.17	0.77	0.40	-1.34
$\mathbf{P}_{25}$	-0.43	-0.39	0.60	0.21	-0.53	0.33	0.58	-0.38	0.00	-0.01	0.00	0.00	-0.56	-0.26	-0.45	1.28
$\mathbf{P}_{26}$	-0.42	-0.76	0.44	0.72	1.35	-1.26	-0.41	0.33	-0.01	-0.01	0.01	0.01	0.98	-0.86	-0.87	0.75

Table 7: Heritability and contribution rate of genotype variance of characters

		e variance			ution rate (	,			
Characters	$P_1$	$P_2$	$P_1 \times P_2$	V <sub>g</sub>	$ m V_{g1}$	$V_{g2}$	$V_s$	$h_{\!\scriptscriptstyle B}{}^2$ Broad-sense heritability	h <sub>N</sub> <sup>2</sup> Narrow-sense heritability
Nut diameter	0.519	5.067	0.482	92.06	8.56	83.50	7.94	0.990	0.844
Nut weight	0.222	2.018	0.356	86.30	8.55	77.75	13.70	0.968	0.735
Thickness	0.096	0.519	0.057	91.55	14.24	77.31	8.45	0.973	0.821
Rate of kernel	0.068	0.941	0.201	83.40	5.63	77.77	16.60	0.970	0.693

**SCA Effect:** Seen from Table (6), SCA of different combinations had significant difference on the same character except thickness. Take kernel rate as an example, the variability of SCA between combinations was-1.34~1.28. SCA of  $P_{11}\times P_{26}$  is 1.35 on nut weight, while  $P_{12}\times P_{26}$  is -1.26. Even the same combination had obviously different SCA on different characters. For example,  $P_{11}\times P_{23}$  had a high positive effect on nut diameter, but displayed a high negative effect on kernel rate. The variability of SCA on different characters was larger,  $P_{11}\times P_{26}$  had the highest value which was 1.35,  $P_{24}\times P_{14}$  had the lowest value which was -1.34.

GCA and SCA had no relationship by comparing their effect. For example,  $P_{11} \times P_{26}$  had a higher SCA on kernel rate,  $P_{11}$  and  $P_{26}$  had a higher GCA effect, while  $P_{23}$  had the lowest GCA effect although combination  $P_{13} \times P_{23}$  had a higher SCA; although  $P_{11} \times P_{26}$  had the highest SCA on nut weight, the two parents had negative GCA values; there were not any combinations with a higher SCA among  $P_{14}$ ,  $P_{24}$  and  $P_{23}$  which had a higher GCA on nut weight and diameter. The resulted showed that not any GCA effect can totally decide qualities of nut. All the expression of characters was depend on multiple factors. And even the low GCA effect parents can form a high SCA effect combination.

**Estimation of Colony Genetic Parameters:** Estimate the genotype variance of GCA  $(P_1P_2)$  and SCA  $(P_{1\times 2})$  and analyze their contribution rate  $(V_g \ V_s)$  in total variance and the GCA contribution rate  $(V_{gl},\ V_{g2})$  of two parents respectively to know about the effect of two parents and their co-effect on nut quality. The results showed in Table 7

Data in Table (7), four characters were affected by GCA and SCA at the same time, but the contribution rate of GCA is over 80% while contribution rate of SCA is low, which reflects additive effect of gene has a leading role to decide hybrid expression and nut quality is decided by genetic traits of the parents, matching of parents has a lower effect to hybrid. GCA Contribution rate of male

parent is larger than that of female parent, which indicates that the hereditable character of male parent is larger than that of female one and hereditable character of male parent has a direct effect on nut quality.

## CONCLUSION

Effect of Combining Ability and Parent Selection: GCA reflects additive effect of parents. If the parents had a higher additive effect, they would combine out an excellent progeny. It had a low possibility to possess an ideal GCA effect on several characters at the same time. We should select the parents by the main target breeding in cross breeding work. In this paper, P24, P23, P22 and P<sub>14</sub> had a good effect on improving nut diameter and weight,  $P_{21}$ ,  $P_{13}$  and  $P_{11}$  took the second place,  $P_{25}$  and  $P_{26}$ at lowest. For kernel rate, P21 is the best, followed by P26 and  $P_{25}$ ,  $P_{23}$  and  $P_{24}$  were worst. SCA displayed non-additive effect. Although effect value could not decide expression of offspring directly, it had a meaning of guidance to combinations and collocation of pollination trees. It could not select the combinations only with a higher SCA, but to select the higher SCA ones based on parents with higher GCA. (P<sub>11</sub>×P<sub>23</sub>), (P<sub>11</sub>×P<sub>24</sub>) and (P12×P24) could improve diameter of nut. Although  $(P_{13}\times P_{25})$ ,  $(P_{13}\times P_{26})$  and  $(P_{14}\times P_{26})$  had a high SCA, their parents had a lower GCA, the expression of the offspring was not so good. For nut weight,  $(P_{11} \times P_{26})$  had the highest SCA, but the GCA of P<sub>11</sub> and P<sub>26</sub> were negative, so their hybrid nut had no superiority. Although SCA on kernel rate of (P<sub>11</sub>×P<sub>26</sub>) was not the highest, P<sub>11</sub> and P<sub>26</sub> had a higher GCA and the SCA was also higher, the kernel rate of its offspring was the highest among all the combinations.

Heritability of Hybrid Nut: Heritability was the percentage of genetic variance to phenotypic variance, which reflected stability of parents' property and the ability to pass their traits to offspring. Broad sense heritability reflected co-action of additive effect and

non-additive effect, while narrow sense heritability was the measure of additive effect. For the 4 characters in this study, broad sense heritability was over 0.95, which was more than the heritability (≥0.8) of thickness, diameter, weight and kernel rate estimated by Hsndche. It showed that environment had a little effect on characters can guarantee genetic stability by asexual propagation. Consequently conserving and enlarging excellent genotype by grafting on excellent individual tree. Narrow sense heritability was between 0.693~0.844 showed four characters had a higher additive effect, so they had a stable heritability in sexual reproduction. It is better to reselect after the walnut tree flowering and seedling.

Effect of Genetic Background of Parents: Six male parents used in the research were seed trees selected from Sichuan province in China, where was the regional differentiation of two main walnut populations, with complex topography, diverse climates, rich in resources and the male parents have a larger difference of hereditary basis leading the great variability of the hybrid offspring. From Table 7, variation of offspring mainly came from male parents. Although GCA of different female parents was different, the difference is not large. Effect on offspring variation of Female parents was up to an extremely significant difference but much weaker than that of male parents, because of their consistent genetic background. The largest genotype contribution rate of female parents on thickness was only 14.24 seen from Table 7. It also was the main reason female variability of GCA much smaller than that of male ones.

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