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RAPD Markers and Heterotic Effect of Walnut Quality in Sichuan of China

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Abstract: Genetic diversity and genetic distance of walnut samples were studied based on RAPD, which hybridized from Yunxin variety, Sichuan variety and their hybrid (Yunxin variety ♀×Sichuan variety). Then combine the dominant ratio of walnut quality to discuss how to predict heterosis by genetic distance based on RAPD. The results showed that different cross combination has different genetic similarity index and genetic distance. Genetic distance of parents has a negative relationship with thickness but has a positive relationship with kernel rate. Related coefficient between genetic distance and heterosis of thickness was significant ($P<0.05$). While the related coefficient between genetic distance and heterosis of diameter and weight was very small. Analysis of variance showed that thickness variation was small between progeny and maternal parent, their difference was not obvious, which showed that character of thickness has a stable genetic ability. In a word, it is valuable to estimate the heterosis of main characters by RAPD genetic distance.

Key words: Walnut · RAPD marker · Genetic distance · Heterosis

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. The second distribution of light, temperature, water and air caused by topographic feature forms the unique climate type and vegetable flora in Sichuan. South of Qinba mountain, east of Tibetan plateau and north of the Yunnan-Guizhou Plateau are important climate boundary of Sichuan, also the important variation line of floristics. Sichuan is the main distribution area of walnut also the variation line of J. regia L. and J. sigillata Dode. North of Dadu River is the main variation area of J. regia L. While J. sigillata Dode mainly distributes in South of Dadu River. But in Sichuan the varieties are in a mess, the good and bad are intermingled, restrict the healthy development of walnut industry [1]. Therefore it is meaningful to society, ecology and economy to breed new varieties with excellent qualities and suits for Sichuan ecological environment by crossing, which can help the peasants in mountain regions break away from poor, promote the theory research level of forestry breeding.

Nowadays, heterosis has been the main way in crops breeding. 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. With the appearance and usage of molecule marker technology, study parents' relative ship and predict the heterosis of hybrids has become a new research field. Different reports revealed diverse opinions on the correlation of genetic distance and heterosis. Lee [2] and Smith [3] indicated that high correlation can be used to predict the heterosis. While Yuan Lixing and Zhao Qingyong [4] estimated low correlation that not predicted the heterosis. Zhang Fengwei [6] suggested that the degree between genetic

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distance and heterosis is different with different characters. Zhang [7] and Xiao [8] found their correlation was different with the materials studied. Up to now, there is no any report about their relationship on walnut. The aim of this study is to analyze the correlation between RAPD analysis and hetrotic values of walnut quality and the possibility to predict the heterosis based on RAPD markers, which offered theory proof for selection of parents match, heterosis prediction and molecule marker-assistant breeding.

MATERIALS AND METHODS

Female parents are 3 hybrid lines from Xinjiang eraly-bearing (J. regia Dode) and YunnanYangbi walnut (J. sigillata L). Male parets are 6 excellent trees (J. sigillata) selected in Chaotian of Guangyuan in Sichuan, 60 cross combinations were disigned by NCII, 12 combinations flower and fruit for 3 years continuously and their parents were selected to analyze their quality and RAPD study. 26 primers (produced in Sai Bai Sheng Genitic Company) were selected to amplify profiles.

Measurement of Walnut Quality: Walnuts were picked at mature stage and dried by natural withering. Five walnuts were randomly selected by measuring the thickness of abdominal shell, belly diameter, gap diameter and height, then their average values were calculated as diameter of walnut. Dried nut and kernel were weighed and kernel rate was calculated.

DNA Extraction and RAPD Reaction Conditions: DNA extraction method suitable for RAPD analysis has been established by improving the CTAB method [9]. Primers produced distinct, reproducible, polymorphic profiles were used to PCR amplification. The reaction was carried out in a volume of 20 μl and was prepared as follows: (20 ng/μl) 1μl of genomic DNA, (5U/μl) 0.3 μl *Taq* Enzyme (Sangon, Inc., Shanghai), (25 mM/l) 1.2 μl $MgCl_2$, 2.0 μl 10X reaction buffer, (2.0 mM/l) 1.8 μl dNTPs and (2 μM/l) 0.3 μl of each primer. Each reaction solution was overlaid with one drop of mineral oil to prevent evaporation. PCR-amplification reactions were performed in a thermocycler (Eppendorf Authorized Thermal Cycler PCR) programmed as follows □An initial denaturizing at 94°C for 5 min followed by 40 cycles of 45s at 94°C, 45s at 36°C, 90s at 72°C and finally extended at 72°C for 10 min. PCR products were analyzed using 1.2% agarose gel electrophoresis and visualized with 0.5 μg/μl ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 2000 bp. Each amplified reaction was carried out three times to ensure result consistency.

Statistical Analysis: RAPD fragments were scored as present (1) or absent. RAPD analyses were analyzed using the Nei genetic similarity index based on the equation: Similarity = 2Nab/(Na+ Nb) where Nab = number of scored amplified fragments with the same molecular size shared between a and b, Na and Nb = number of scored amplified fragments in a and b, respectively. Correlation between walnut quality and heterosis was estimated using SPSS 13.0 software. Heterosis= (F_1value-average value of parents)/ average value of parents×100%.

RESULTS AND ANALYSIS

RAPD Amplified Results: 26 primers were selected to amplify walnut samples from 200 random primers, which could produce distinct, reproducible, polymorphic profiles (Table 1). A total of 250 bands were amplified by 26 primers among 28 samples; one primer amplified 6~16 profiles; the average bands amplified by a primer was 9.62; the size was 200□2100bp. Among them, 181 were polymorphic bands, up to 72.4%. Fig. 1 was presented the amplification result of primer N5 of cross combination A_{39}♀×JG_3♂ and Fig.2 was presented the result of primer C6 on cross combination $9D_{28}$♀×LS♂.

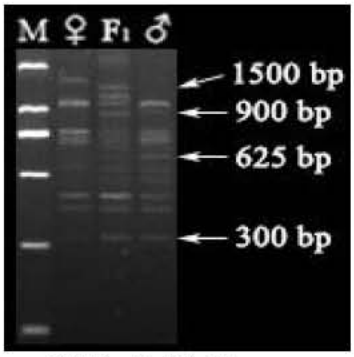

Fig. 1: Primer N5□A_{39}♀×JG_3♂□

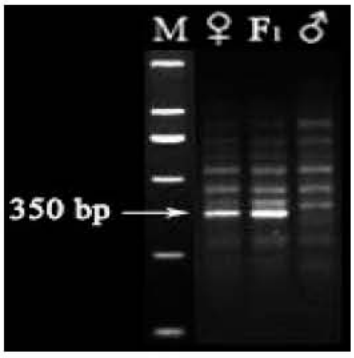

Fig. 2: Primer C6□ D_{28} ♀×LS♂□

Table 1: Bands amplified by 26 primers

Primer	Nb	NP	P%	Primer	Nb	NP	P%
AA10	9	7	77.78	P10	10	6	60.00
B11	6	4	66.67	Q11	13	7	53.85
C6	10	7	70.00	R13	16	15	93.75
C16	8	7	87.50	S4	11	10	90.91
E17	8	4	50.00	T16	8	4	50.00
F12	8	4	50.00	T19	12	12	100.00
F13	7	2	28.57	W13	9	5	55.56
I20	16	14	87.50	W18	6	5	83.33
M4	11	5	45.45	X17	10	8	80.00
M9	7	6	85.71	X20	9	6	66.67
N5	14	11	78.57	Y13	12	11	91.67
O11	7	4	57.14	Z15	10	8	80.00
P4	6	3	50.00	Z19	7	6	85.71

Nb= No. of amplified bands, NP=No. of polymorphic bands, P%=Polymorphic loci (%)

Table 2: Genetic similarity coefficients and genetic distances between parental lines

	Ntb				
Cc	Paternal	Maternal	Nmb	Sc	Gd
C_{20}×JG_4	199	181	161	0.8474	0.1526
A_{50}×JG_4	199	205	176	0.8713	0.1287
D_{28}×JG_4	199	196	171	0.8658	0.1342
C_{35}×JG_3	193	189	164	0.8586	0.1414
C_{46}×AM	172	188	150	0.8357	0.1643
A_{36}×JG_2	188	189	165	0.8753	0.1247
A_{39}×JG_3	193	187	158	0.8316	0.1684
A_{50}×LS	184	205	163	0.8399	0.1601
D_{28}×LS	184	196	162	0.8549	0.1451
D_{34}×LY	183	199	161	0.8429	0.1571
D_{16}×LS	184	199	166	0.8691	0.1309
D_{45}×LS	184	200	164	0.8564	0.1436

Cc= Cross combination, Ntb= No. of total bands from parents, Nmb= No. of mutual bands from parents, Sc=Similarity coefficient, Gd=Genetic distance

Table 3: Heterosis of 4 quality traits and their correlation coefficients with genetic distances

Cc	Nd	Nw	T	Rk
C_{20}×JG_4	21.230	54.540	-3.510	15.560
A_{50}×JG_4	9.540	40.820	1.820	7.670
D_{28}×JG_4	3.140	42.450	-0.460	13.670
C_{35}×JG_3	6.750	16.830	-10.530	12.530
C_{46}×AM	4.600	9.530	-24.070	12.300
A_{36}×JG_2	13.590	12.050	5.830	3.460
A_{39}×JG_3	-5.060	-3.310	-22.270	15.200
A_{50}×LS	-1.370	13.710	-9.260	7.480
D_{28}×LS	14.820	56.680	10.800	6.930
D_{34}×LY	34.260	71.250	6.000	11.600
D_{16}×LS	8.020	12.130	0.970	9.960
D_{45}×LS	0.780	7.460	-1.480	9.580
r	-0.103	-0.084	-0.659*	0.523

Cc= Cross combination, Nd= Nut diameter, Nw= Nut weight, T= Thickness, Rk= Rate of kernel

Note: *Correlation was significant at the 0.05 level

Table 4: Variance analysis of walnut traits

	Nd		Nw		T		Rk%	
Cc	Maternal	F_1	Maternal	F_1	Maternal	F_1	Maternal	F_1
$C_{20}\times JG_4$	3.779	4.285	12.606	17.624	1.16	1.10	49.64	57.30
$A_{50}\times JG_4$	3.692	3.824	10.452	14.542	1.08	1.12	54.51	56.01
$D_{28}\times JG_4$	3.528	3.516	8.775	13.516	1.05	1.08	51.83	57.61
$C_{35}\times JG_3$	3.465	3.803	12.657	16.351	1.21	1.02	48.78	57.58
$C_{46}\times AM$	3.199	3.414	9.251	12.56	1.06	0.94	52.07	58.96
$A_{36}\times JG_2$	3.259	3.741	9.907	12.612	1.09	1.18	53.59	54.51
$A_{39}\times JG_3$	3.597	3.445	9.444	11.979	1.04	0.82	54.13	62.03
$A_{50}\times LS$	3.692	3.486	10.452	13.256	1.08	0.98	54.51	56.18
$D_{28}\times LS$	3.528	3.964	8.775	16.952	1.05	1.18	51.83	54.46
$D_{34}\times LY$	3.121	4.338	12.232	18.926	0.90	1.06	51.64	57.27
$D_{16}\times LS$	3.255	3.582	9.049	12.286	0.98	1.04	52.44	56.34
$D_{45}\times LS$	3.267	3.348	9.268	11.892	0.95	1.00	52.65	56.26
F Test	5.963*	24.980**	0.080	36.809**				

Cc= Cross combination, Nd= Nut diameter /cm, Nw= Nut weight /g, T= Thickness /mm, Rk%= Rate of kernel /%

Note: * Means the differences are significant at 0.05 leve1; Means the differences are extremely significant at 0.01 leve1

Genetic Distance and Genetic Index of Parents: Recorded the amplification results of 26 primers and ruled out the vague and low repeatability. Calculate the genetic index and genetic distance of 12 cross combinations by Nei equation (Table 2). Seen from Table 2, parents of Combination $A_{39}\times JG_3$ had the smallest genetic index which is 0.8316 and with the biggest distance which is 0.1684. Genetic breeding theory and practice shows that the key to use heterosis is to find the most excellent combination. The genetic distance is bigger, heterosis is stronger. Consequently, among the 12 combinations, $A_{39}\times JG_3$ should be observed primarily, because it had the biggest genetic distance.

Coefficients between genetic distance of parents and walnut quality of 12 cross combinations were calculated as shown in Table 3.We can see from Table 3, absolute value of related coefficient between genetic distance of parents and 4 walnut qualities was 0.084□0.659. Genetic distance of parents has a negative relationship with thickness but has a positive relationship with kernel rate. Related coefficient between genetic distance and heterosis of thickness was significant ($P<0.05$). While the related coefficient between genetic distance and heterosis of diameter and weight was very small. The results showed that character of thickness is a valuable indicator to select hybrid parents. But the character of size will change on progeny.

Analysis of Genetic Characters of Walnut: Analysis of variance was used for diameter, weight, thickness and kernel rate of progeny and maternal parent by using software SPSS 13.0. See from Tab.4, diameter has a significant difference ($P<0.05$) between maternal parents and progenies. And there is an extreme difference ($P<0.01$) between maternal parents and progenies on weight and kernel rate. While on thickness, there was no obvious difference between maternal parents and progenies. All of these results support the above conclusion about the related relationship of genetic distance and heterosis.

DISCUSSION

The base of heterosis is heterozygosity of the hybrid genes. The individuals have farther genetic relationship and bigger genetic distance, the heterosis is stronger [10]. Boppenmaier etc. [11] think that in certain extent, genetic distance of parents is bigger, heterosis is stronger. In this study, among 12 combinations, Female parent A_{39} and male parent JG_3 has the biggest distance which is 0.1684, be higher than the other combinations and their combination had stronger heterosis. Relationship of genetic distance and heterosis is a complicate problem, involving the interaction of two different genetic background parents. In selection of hybrid parents, it is better to select the combination with big genetic distance.

Some of the correlation ship between RAPD genetic distance and different walnut qualities are close, some of which are very small. In this paper, related coefficient between genetic distance of parents and thickness heterosis was significant. So thickness is the most important index to select parents. The results of variance analysis also showed that thickness had a small variation

between progenies and maternal parent. In real practice, especially on J□sigllata Dode, some thickness of walnut are more than 10mm and some of them are less than 1mm. Even if J. regia L. and J. sigillata Dode. mix for a long time, they still have not been assimilated. In species introduction and cultivation, no matter how to change the ecological environment and cultivation techniques, thickness character still could not be changed. From theory to practice, all indicate that thickness has a stable genetic ability.

Correlation coefficient between Genetic distance and heterosis of diameter and weight, which shows that parents with big size walnut cross could produce progenies with small size walnut. Parents with small size walnut cross also could produce progenies with big size walnut. But ecological environment, management and nutrition level will affect diameter, weight, kernel rate these characters.

Stability and variation of genetic characters are not only decided by parents genetic and variation ability but also affected by background of parents, their allele type and type of gene effect and their interactions, genotype and environment interaction effect and so on. In Sichuan, the climate featured by "One mountain at Four Seasons, different weather within 10 km" caused by low latitude and high altitude complex and diverse topography with three-dimensional climate and significant regional differences in climate characteristics. And Sichuan is the transitional area of J. regia L.and J. sigillata Dode. Different samples could lead different results because walnut individuals have obvious difference. This may be the main reason different researchers got different results. But we can say that it has a certain reference value to estimate walnut quality heterosis based on RAPD genetic distance.

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