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Analysis on the genetic control of heterosis for guanosine and organic acid contents in tuber of *Pinellia ternata* by using of random amplified polymorphic DNA (RAPD) makers

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To explore advantage traits of *Pinellia ternata* populations for hybridization breeding, effect of parents on contents of guanine nucleoside (hereinafter referred as "guanosine") and total free organic acids (hereinafter referred as "organic acid") of the F_1 groups were analyzed using random amplified polymorphic DNA (RAPD) markers. Results showed that genetic distances (GDs) between 7 of the 9 tested F_1 groups and their female parents were smaller than those between the F_1 groups and their male ones. Heterosis for guanosine and organic acid contents of the F_1 groups were more affected by their female parents than by the male parents, and were significantly correlated with the GDs between the F_1 groups and their female parents; the correlation coefficients were r=0.5258 and -0.6760, respectively. Furthermore, there is a significant correlation between the heterosis for organic acid contents of the F_1 groups and the GDs between their parents; the correlation coefficient was r=0.3212, indicating that GDs between parents can be used to predict the heterosis for organic acid contents of the F_1 groups. But the heterosis for guanosine contents of the F_1 groups does not correlate with the GDs between their parents, indicating that the GD between parents cannot be used to predict the heterosis for guanosine contents of the F_1 group. In addition, relationship between bioaccumulation of the organic acid and guanosine in the tuber of *P. ternata* were discussed.

Key words: *P. ternate*, F_1, genetic distance, guanosine, organic acid.

INTRODUCTION

Pinellia ternata (Thunb.) Berit. (Araceae) is a well known herbal plant in P. R. China, Japan, Taiwan and Southeast Asia. Its tuber, known as *banxia* in Chinese, contains alkaloids, sterols, organic acids, amino acids, inorganic elements, nucleoside and other chemical compounds, and has been widely used in Chinese traditional medicine for its antiemetic, analgesic, and sedative effects (Han et al., 2006). Total free organic acid, one of the main active ingredients for expectorant and antitussive, has been proved to be one of the effective components that have a direct impact on the quality of *banxia* (Wu et al., 2003; Zhang et al., 2001), and has been employed as index compound of *P. ternata* by the Chinese Pharmacopoeia (2010 Edition). Guanine nucleoside, another effective component of *banxia*, can improve cardiocerebral blood circulation, prevent arrhythmia, inhibit neurotransmitter release and regulate acid cyclase activity action (Zhao et al., 2007).

In china, to meet the increasing demand of *banxia*, farmers began to grow *P. ternata* for tuber, and the tuber has been used as reproductive organ as well, resulting in drops of disease-resistant ability and decline in the

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Table 1. Morphological characteristics of the tested *P. ternata* parental populations.

Population (code)	Morphological characteristic		
	Number of bead bud/plant	Shape of lobular	Shape of tuber
Wuling, Chongqing (C)	1	Long ovoid	Ball
Taizhou, Jiangsu (T)	1	Long ovoid	Ball
Xin Jiang, Shanxi (S)	1	Long lanceolate	Rugby
Haimen, Jiangsu (H)	2	Long ovoid	Ball

contents of effective components. It is urgent for us to improve its disease-resistant ability and the content of effective component in tubers of *P. ternata* as well. Heterosis is the phenomenon whereby the hybrid progenies of diverse inbred varieties present an increase in growth rate, yield, fertility, and other changes in desirable agronomic traits (Shull, 1908), and has been exploited in many crops. Utilization of heterosis has significantly contributed to the world agriculture. Genetically, dominance and overdominance hypotheses have been proposed to explain the genetic basis of heterosis (Jones, 1917), which suggested the heterosis originating from allelic and non-allelic interactions, respectively. Both hypotheses suggested that the superior performance of F1 hybrid is closely associated with genetic distance (GD) among the parents. Geographic origin as a criterion was applied to assess GD between parental genotypes in maize (Moll et al., 1965). DNA markers have become useful tools for predicting heterosis in many crops. Positive correlations between GD and heterosis were reported in rice (Zhang et al., 1996), maize (Kiula et al., 2008) and wheat (Lee et al., 1995). To provide information for improvement of the contents of the total free organic acid and the guanine nucleoside in the tube of *P. ternata* by hybridizing, genetic factors involved in the control of the contents of the total free organic acid and guanosine in tuber of *P. ternata* were first reported in this paper.

MATERIALS AND METHODS

Plant

Four parent populations (Table 1) collected from Xinjiang city of Shanxi province (X), Taizhou city of Jiangsu province (T), Wuling city of Chongqing (C), and Haimen city of Jiangsu province (H), respectively, were grown in experimental field of Nanjing Agriculture University (Nanjing), and were crossed with each other to produce 9 hybrids (Table 3). Four parent populations and the nine F_1 groups were grown in experimental field of Nanjing Agriculture University (Nanjing) and used as materials.

Genomic DNA extraction and RAPD amplification

Genomic DNA was extracted from fresh leaves by modified cetyltrimethylammonium bromide (CTAB) method (Doyle et al., 1990). The quality and quantity of purified genomic DNA was checked by spectrophotometer (UV-3300, Meipuda, China) as well as visually on 0.8% agarose gel using ethidium bromide staining, and each sample was diluted to 25 ng/µl with Tris–EDTA (TE) buffer before it was used. Twenty four reported (Bai et al., 2007; Yang et al., 2007) RAPD 10-mer primers were screened to determine their potential for clear polymorphism and reproducibility. RAPD amplification was performed in a volume of 25 µl containing 2 µl of genomic DNA (50 ng), 0.80 µM of RAPD primer (Integrated DNA Technologies, USA), 0.1 mM of each dNTP (Solarbio, China), one unit of Taq DNA polymerase (Solarbio), 2.5 µl of 10X polymerase chain reaction (PCR) buffer (Tris without $MgCl_2$) and 3 mM $MgCl_2$. DNA amplifications were carried out in a DNA thermal cycler (Bioer, China) programmed for 40 cycles as follows: 1st cycle of 120 s at 94°C, 30 s at 36°C and 90 s at 72°C, followed by 40 cycles each of 30 s at 94°C, 30 s at 36°C, and 90 s at 72°C. The final step consists of one cycle of 10 min at 72°C for complete polymerization. Amplified DNA fragments were separated in 1.25% (w/v) agarose (Sigma) gel using 1X TAE (pH 8.0) buffer stained with ethidium bromide. Gels were visualized using a gel documentation system (Peiqing Sci.& Tech., China). The size of the amplicons was estimated by comparing with 1 kb DNA ladder (Bio Enzyme, USA). At least, two independent PCR amplifications were performed for each sample with RAPD primers. Only reproducible bands were considered for analysis.

Determination of the content of total free organic acids

Tubers were harvested in the sprout tumble periods and dried to constant weight under 55°C, after it has been ground and pass through a 60 mesh sieve, 2.5 g sample powder were soaked in 50 ml 95% ethanol and heated to reflux under 80°C for 1 h, and this was repeated 3 times, collected and mix the ethanol extractions. After filtering and evaporating to be free of ethanol, the residue was ultrasonic extracted (500 W, 40 kHz) with 10 ml 0.1 mol/L NaOH for 30 min. Then, the extraction was diluted with distilled water to 25 ml. Finally, the content of the total free organic acids was determined with reference to the method of Wu et al. (2003).

Determination of the content of guanine nucleoside

Sample (0.5 g) powder prepared according to the aforementioned method was ultrasonic extracted (500 W, 40 kHz) with 10 ml distilled water for 30 min and centrifuged under 3500 rpm for 10 min, and this step was repeated 3 times. The supernatant was collected and analyzed on a Anglient high performance liquid chromatography (HPLC) system (1120) with a Eclipse XDB-C18 5 µm column at a flow rate of 1.0 ml min^{-1} and was detected at 254 nm, using a solvent system of H_2O-CH_3CN (97:3) (Zhao et al., 2007).

Evaluation of the heterosis for guanosine and organic acid contents

Mid-parent heterosis (MPH) for guanosine and organic acid was calculated as a percent deviation from the mean of the parents,

Table 2. Screen of the polymorphic primers.

Number of primers	Sequence of primer	Number of polymorphic bands	Number of total amplified bands	Polymorphic loci (%)
OPH 5	AGTCGTCCCC	5	8	62.5
OPN 8	ACCTCAGCTC	10	11	90.9
OPH 12	ACGCGCATGT	5	13	38.4
OPN 14	TCGTGCGGGT	7	8	87.5
OPN 18	GGTGAGGTCA	8	10	80.0
OPN 20	GGGAGACATC	3	7	42.8
S5	TGCGCCCTTC	4	7	57.1
S8	GTCCACACGG	5	7	71.4
S12	CCTTGACGCA	5	6	83.3
S17	AGGGAACGAG	4	8	50.0
S53	GGGGTGACGA	3	9	33.3
S 55	CATCCGTGCT	1	4	25.0
S 85	CTGAGACGGA	2	7	28.6
S 94	GGATGAGACC	5	8	62.5
S164	CCGCCTAGTC	8	11	72.7
S 265	GGCGGATAAG	3	7	42.9

using the equation as follows:

$MPH(\%) = 100 \times (F_1 - MP)/MP$,

where F_1 is the contents of guanosine or organic acid of F_1, MP is the mean contents of guanosine or organic acid of the two parents (Jagosz, 2011). Data in Table 4 were used.

Data analysis

All data, means of three replications were subjected to analysis of variance (ANOVA) using SAS version 8.0 for Windows, and mean comparisons were made using Duncan's Multiple Range Test (DMRT) at 5% level of probability.
GD of pairwise combinations were computed according to Nei and Li (1979):

$GD = 1 - 2Nij/(Ni+Nj)$

where Nij is the number of alleles present in both lines i and j; Ni and Nj are the number of alleles in lines i and j, respectively. Cluster analysis was based on GD values using the unweighted pair group method with arithmetical average (UPGMA) (Sneath and Sokal, 1973). The UPGMA-clustering were conducted and the relationships among parental lines were visualized as dendrograms using the NTSYS-pc package (Rohlf, 1990). Correlation coefficients between GDs and F_1 heterosis were calculated, and significant test was performed using Excel 2003.

RESULTS AND DISCUSSION

RAPD polymorphism survey

Out of the 24 RAPD 10-mer primers screened, only 16 resulted in reproducible bands. These 16 RAPD primers generated 131 amplicons in total, ranging from 350 to 2,500 bp in size. The number of bands in the selected primers varied from 4 (S 55) to 13 (OPH 12), with an average of 8.19 bands per RAPD primer (Table 2).

GDs of parent's populations and their F_1 groups

Figure 1 showed that GDs of the 14 tested trial were between 0.1567 and 0.4444. Under the similarity coefficient of 0.72, 2 populations collected from Wuling city of Chongqing (C) and Taizhou city of Jiangsu province (T) were of the same category, and the other 2 populations those collected from Xin Jiang city of Shanxi province (X) and Haimen city of Jiangsu province (H) were not of the same category, and these results were consistent with the morphological characteristics shown in Table 1. These results indicate that this RAPD marker system can be used for GD analysis.

Furthermore, results of Figure 1 and Table 3 showed that the GDs of those between 7 of the 9 tested F_1 groups and their female parents were smaller than those between the F_1 groups and their male parents indicating that hybrid offsprings were more affected by its female parents than the male parents.

Contents of organic acids and guanosine of the parent populations and their F_1 groups

Results of Table 4 showed that there is a variation in the contents of organic acid between the collected parent populations and their F_1 groups; the contents of organic acid were varied between 0.235 and 0.437%, and the contents of guanosine were varied between 0.0088 and 0.02346%.

Table 3. Honma GDs between the F_1 groups and their parental populations.

F_1 groups	C×T	T×C	C×S	S×C	H×T	S×H	H×S	S×T	T×S
GDs between the F_1 groups and their male parental population	0.25	0.23	0.16	0.25	0.31	0.29	0.31	0.21	0.17
GD between the F_1 groups and their female parental population	0.18	0.18	0.29	0.19	0.17	0.29	0.17	0.17	0.23

Table 4. Correlation coefficient of the contents of organic acids and guanosine in the tuber of F_1 groups and their parental populations (n=3).

Populations/ F_1 groups	Content of organic acids (mg/g DW)	Content of guanosine (mg/g DW)
C	2.62^{h}	0.1480^{g}
H	2.44^{i}	0.2052^{c}
T	4.13^{b}	0.1863^{e}
S	3.87^{c}	0.2107^{b}
C×T	3.26^{e}	0.1896^{d}
C×S	4.37^{a}	0.1037^{h}
H×T	2.35^{j}	0.2346^{a}
H×S	2.96^{f}	0.1676^{f}
T×C	4.08^{b}	0.0972^{i}
T×S	2.80^{g}	0.1907^{d}
S×C	3.87^{c}	0.2067^{c}
S×H	3.56^{d}	0.0880^{j}
S×T	2.86^{g}	0.1859^{e}
Correlation coefficient		-0.4710

Data marked with different letters mean significantly difference ($P < 0.05$).

In addition, the data in Table 4 also showed that there is a negative correlation between the contents of organic acid and those of guanosine. This result indicated that there is a certain mutual antagonism between biological accumulation of organic acid and guanosine in tuber of *P. ternata*.

Effect of GDs between F_1 groups and their female and male parents on heterosis for organic acid and guanosine contents

Results of Table 5 showed that heterosis for organic acid and guanosine contents of the tested 9 F_1 groups were negatively correlated with the GDs between the F_1 groups and their female parents; the correlation coefficients R were 0.5258 and -0.6760, respectively. On the contrary, heterosis for organic acid and guanosine contents of tested F_1 groups does not correlate with the GDs between the F_1 groups and their male parents (Table 6).

These results indicated that: first, the GD between F_1 group and the female parent can be used to predict heterosis for organic acid and guanosine contents; secondly, in accordance with the results in Figure 1, the contents of the organic acids and guanosine content of F_1 groups is much more affected by their female parents than the male parents. In addition, same as those shown in Table 3, the results of Table 6 showed that, the heterosis for organic acids and guanosine contents of the F_1 groups were positively and negatively correlated with GDs between the F_1 groups and their female parents, respectively (Table 6). This result also indicated that there has certain mutual antagonism between the biological accumulation of organic acid and guanosine in the tuber of *P. ternata*, and revealing that it may be difficult for us to get the offspring of high content of organic acid and guanosine by hybridization breeding.

Effect of GDs between female and male parents on heterosis for organic acid and guanosine contents of the F_1 groups

Results in Table 7 showed that the heterosis for organic acid contents of F_1 groups positively correlated with the GDs between their female and male parents; the correlation coefficient is 0.3212. This result reveals that the content of organic acid of offspring can be improved by distant hybridization, and the GD between parents can be used to predict the content of organic acid of the offspring. Contrarily, the heterosis for guanosine contents of F_1 groups just showed a very weak correlation with the GDs between their male and female parents; the corre-

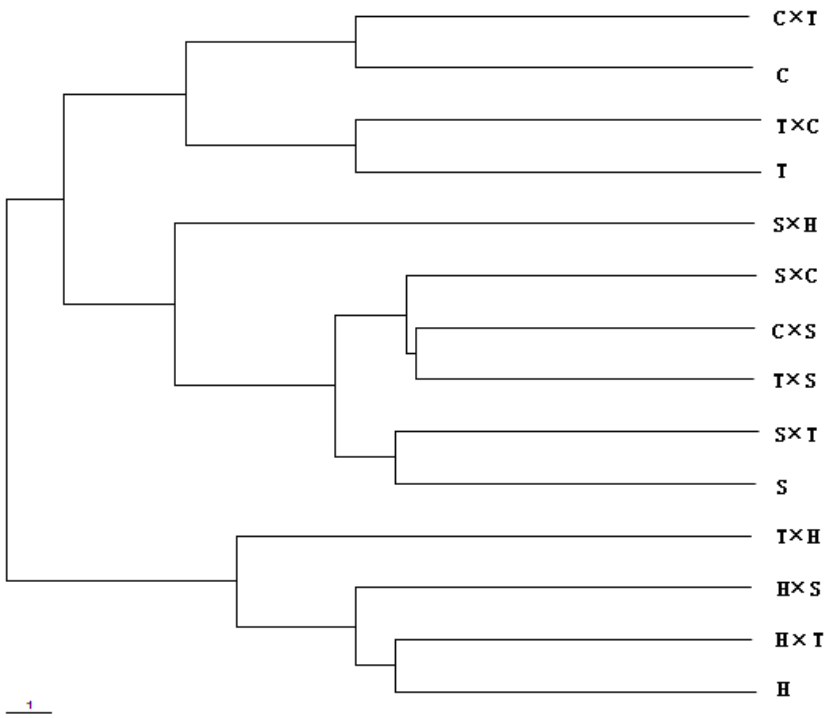

Figure 1. Clustering analysis of the parental populations and F_1 groups based on GDs.

Table 5. Heterosis for organic acid and guanosine contents of the F1 groups.

Parameter	C×T	T×C	C×S	S×C	H×T	S×H	H×S	S×T	T×S
Heterosis for organic acid contents	-3.41	20.89	34.67	19.26	-28.46	12.84	-6.18	-28.50	-30.00
Heterosis for guanosine contents	13.43	-41.85	-42.18	15.25	19.85	-57.68	-19.40	-6.35	-3.93

Table 6. Correlation coefficients of those between the heterosis for organic acid and guanosine contents of the F1 groups and the GDs between the F1 groups and their parental populations.

Parameter	Heterosis for organic acid	Heterosis for guanosine
GDs between F1 groups and their male populations	-0.1509	0.1572
GDs between F1 groups and their female populations	0.5258**	-0.6760**

**Indicates the correlation coefficient has reached the remarkable level of 0.01.

lation coefficient is -0.0975 (Table 7). This result indicated that the content of guanosine is weakly affected by the GDs between their female and male parents, and it is difficult for us to predict the content of guanosine of offspring using the GDs between the male and female parents.

Conclusions

F_1 groups were more affected by female parents than the male parents. GDs between 7 of the 9 tested F_1 groups and their female parents were smaller than those between them and their male parents. Heterosis for guanosine and organic acid contents of the F_1 groups were more affected by female parents than the male parents.

Correlation coefficients of the heterosis for guanosine and organic acid contents of F_1 groups and the GD between the F_1 groups and their female parents were r =0.5258 and -0.6760, respectively, and those of the heterosis for guanosine and organic acid contents and the GD between the F_1 groups and their male parents

Table 7. Correlation coefficients of those between the heterosis for organic acid and guanosine contents of the F_1 groups and the GDs between their parental populations.

Parameter	Heterosis for organic acid	Heterosis for guanosine
Correlation coefficients	0.3212*	-0.0975

*Indicates the correlation coefficient has reached the remarkable level of 0.05.

were r = -0.1509 and 0.1572, respectively. There is a significant correlation between the heterosis for organic acid contents of the F_1 groups and the GDs between their parents, the GD between parental populations can be used to predict the heterosis for organic acid contents of the F_1 group. The heterosis for contents of guanosine of the F_1 groups does not correlate with the GD between their parents, and it is difficult to for us to predict the heterosis for organic acid contents of the F_1 group using the GD between parental populations.

There is a negative correlation between the contents of organic acid and that of the guanosine, and there is a certain mutual antagonism between the biological accumulation of organic acid and guanosine in the tuber of *P. ternata*.

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