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Full Length Article

Effects of Medium Addition on Ovule Enlargement of Watermelon Non-Pollinated Ovary

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Abstract

In order to investigate the effect of the concentration of basic medium, Thidiazuron (TDZ), Naphthaleneacetic Acid(NAA), 6-Benzylaminopurine (BA), and kinetin (KT) on gynogenetic development of non-pollinated ovary, the explants of watermelon with four different genotypes of F1 generation explants were chosen as the material of orthogonal test. The results showed that the basic medium and TDZ concentration have significant effect on ovule enlargement rate and they are the main factors of inducing the isolated gynogenetic development of watermelon. When choosing M2 basic medium and its concentration of TDZ from 0.4 mg·L^{-1} to 0.8 mg·L^{-1}, the induction rate of ovule enlargement is highest, reaching 66.83%. Subsequently, the induction effect of ovule enlargement was the best when the concentration of NAA was 0.5 mg·L^{-1}. When the concentration of NAA was 0.5mg·L^{-1}, the highest rate of ovule enlargement reached 49.33%.The concentration of BA was different due to the difference in explants, the induction effects of ZhongkeNo.6 and XinongNo.8 are the best. The concentration of BA was 1.5 mg·L^{-1}, and ZhongkeNo.6 and XinongNo.8 ovule enlargement rate reached 59.56 and 41.95%. While the concentration reached 1.5 mg·L^{-1}, the rate of ovule enlargement in ZhengkangNo.2 and Zhengkangjufeng was 55.50and 52.90%, respectively. No addition or addition of low concentration of KT is beneficial to the enlargement of ovules. The test results significantly improved the watermelon non-pollinated ovary ovule enlargement rate, which provides strong technical support for success of watermelon non-pollinated ovary culture.

Keywords: Watermelon; Non-pollination ovary; Double haploid; Medium addition; Ovule enlargement; Embryoid

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai] has obvious heterosis, but its genetic background is narrow and it is difficult to obtain target characters. Conventional breeding takes many years of artificial self-selection to obtain the homozygous material, which leads to slow the selection speed of hybrid generation and unable to meet the needs of market. Haploids are doubled by natural or artificially induced to obtain double haploid plants, which can quickly and directly screen the target traits and widen the germplasm resources, thus becoming an efficient method for accelerating the breeding process and obtaining new varieties. However, the frequency of spontaneous haplotypes produced by watermelons is extremely low (Dong *et al.,* 2016) and it cannot meet the needs of breeding work. Therefore, artificial induction to obtain haploid plants is an important means to accelerate the breeding process of watermelon hybrids.

The non-pollinated ovary *in vitro* culture technology belongs to the way of *in vitro* gynogenesis. It is the process of separating the non-pollinated ovary from the maternal body and inducing the development of the female nucleus under the sterile artificial environment so that it can grow and form haploid embryos and plants. It is the process of separating the non-pollinatedovary from the mother, inducing the development of gynogenetic nuclei in a sterile artificial environment, and allowing it to grow and develop into haploid embryos and plants (Li and Zhu, 2002). In the 1950s, many experts and scholars conducted preliminary research on various crops *in vitro* gynogenesis culture. The main research subjects were eucalyptus, hollyhock, green onion, poppy, onion and so on. Although not successful, they were later studied. Related research has accumulated a lot of valuable experience. Although not successful, it had accumulated a lot of valuable experience for subsequent research in related areas. By the middle of the 1960s, research on the *in vitro* culture of non-pollinated ovary plants had made some progress and achievements. For example, the researchers obtained diploid and tetraploid rice plants in *in vitro* culture studies of non-pollinated and unfertilized ovules in plants. San Noeum (1976, 1979) obtained haploid plants in *in vitro* gynogenesis culture of barley in the late 1970s, which indicates that the technology of *in vitro* gynogenesis culture had achieved initial success. Since then, many researchers around the world begun to conduct more extensive and in-depth studies on *in vitro* gynogenesis culture technology to various plants.

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This technology had been extensively studied in Gramineae, Solanaceae, Liliaceae and Cucurbitaceae crops, and considerable achievements had been made. Some regenerated embryo plants had been successfully obtained (Tian and Yang, 1989). However, after the 1990s, the frequency of the application of the technology of the *in vitro* gynogenesis inducing haploids reduced with the rise and rapid development of the method of gene recombination. In the past 20 years, it has been found that the excellent traits obtained by genetic recombination methods are difficult to apply, and people are skeptical about the safety of genetically modified foods. Therefore, haploid breeding techniques, especially the technology of *in vitro* gynogenesis inducing haploids been paid more attention again and had made enormous progress (Doi *et al.*, 2011). At present, although the study on the *in vitro* gynogenesis culture of Cucurbitaceae have obtained haploid plants, which include cucumber (Diao *et al.*, 2009; Tantasawat *et al*., 2015), pumpkin (Sun *et al.*, 2009; Min *et al.*, 2016), squash (Shalaby, 2006, 2007; Xie *et al.*, 2006a, 2006b), watermelon (Li, 2014; Rong *et al.*, 2015) and melon (Chen *et al.*, 2011; Malik *et al.*, 2011; Min *et al.*, 2016). However, there is still a low repetition rate during the culture process, and most of them have been induced to differentiate into plants through callus induction. There are fewer ways to directly induce embryoid bodies. Therefore, the study on the technique of directly induce embryoid bodies still has a lot of room to improve.

The objectives of present experimental research were to study the effects of the microelement, trace elements, organic components and four kinds of exogenous hormones [Naphthaleneacetic Acid (NAA), 6-Benzylaminopurine (BA), Thidiazuron (TDZ) and kinetin (KT)] on the rate of ovary non-pollination in the basal medium, which can provide important technical support for the establishment of breeding double haploid.

Materials and Methods

Experimental Material

In this study, four kinds of the generation of F1 watermelon are chosen as experimental materials, in which Zhengkang No.2, Zhengkangjufeng, and ZhongkeNo.6 are obtained from the Zhengzhou Fruit Research Institute of the Chinese Academy of Agricultural Sciences (Zhengzhou, Henan Province, China), and XinongNo.8 is acquired from the Company Yangling Agricultural High-technology Development (Shaanxi Province, Xi'an, China). Table 1 shows the trade name and fruit characteristics of the experimental materials. On October 2, 2016, these varieties were planted at Hainan Experimental Base (Hainan Province, Sanya, China), and the technology of drip irrigation under film was used to realize the management of fertilizer and water. Every 667 m^2 of ground fertilized 3.0 m^3 organic manure and 50 kg compound fertilizer (N:P:K＝15:15:15).

Experimental Methods

Pretreatment of explants: The material was sterilized with 75% alcohol for 30 s, then peeled and cut into 1 mm slices. Then the treated material was rinsed with sterile water for 3–4 times, and sterile filter paper was used to dry the surface of the ovary sliced, and then inoculated to the induced medium. Each treatment was repeated three times.

Screening of culture medium: The basic medium formula is shown in Table 2. The design of medium included basic medium and the addition of four exogenous hormones (TDZ, NAA, BA, KT), and a blank column was designed as an error term. The orthogonal experiment of L_{25} (5^6) was performed with 6-factors and 5-levels, and 30 $g \cdot L^{-1}$ sucrose and 6 $g \cdot L^{-1}$ agar were added in the inducible medium that the pH was between 5.82 and 5.86 (Table 3).

Culture conditions: The culture was incubated at 35℃ for 5 days at dark, and then incubated at 25℃ dark for 10 days, and 5 days under a light that 3000 Lx for 16 h every day. Finally, the ovule enlargement rate on each induction medium was counted.

Statistics and processing: These variables were therefore square root transformed before performing analysis of variance using the SPSS software version 22.0 and Excel 2017. The data were subjected to analysis of variance (ANOVA) and mean values were separated based on Duncan's multiple range test.

Ovule enlargement rate = Number of with ovule enlargement/Number of ovary slices inoculated×100%.

Results

Effects of Different Factors on Ovule Enlargement of Non-pollinated Watermelon Ovary

Software of excel was used to visually analyze the ovule enlargement data of non-pollinated ovaries. There was a great difference between the 5 factors (basic medium, BA, TDZ, NAA, KT) of explants (Table 4) i.e., Zhengkang No.2(R) was 59.08, 35.21, 7.72, 24.42, 20.55, respectively. It can be seen that the 5 factor had an induction effect on the ovule enlargement, which included the basic medium > TDZ>BA>KT>NAA. The inducing rates of the ovules of the other three explants were Zhengkangjufeng: TDZ> basic medium> NAA> BA> KT, ZhongkeNo.6: basic medium> BA> TDZ> NAA> KT; XinongNo.8: basic medium> TDZ> NAA >BA>KT. Therefore, the sensitivity of explants of different genotypes of ovule enlargement induced by exogenous hormone was not the same. The optimal combinations of factors that favor the ovule enlargement of each test material were initially selected (Table 5).

The analysis of variance of ovule enlargement rate of the four test materials by SPSS 22.0 software showed that the difference of basic medium, TDZ concentration, and BA concentration reached extremely significant levels.

Table 1: The material and fruit's characteristics

Cultivars	Descriptions
ZhengkangNo.2	Large fruit, elliptic, bright red flesh, the fruit developing period is about 26 days.
Zhengkangjufeng	Large fruit, elliptic, red flesh, the fruit developing period is about 33 days.
ZhongkeNo.6	middle fruit, round, bright red flesh, the fruit developing period is about 28 days.
XinongNo.8	Large fruit, elliptic, red flesh, the fruit developing period is about 35 days.

Table 2: Concentration micro and macro elements including vitamins in medium (mg/L)

Component	Basic medium				
	MS	M1	M2	M3	M4
$(NH4)_2SO_4$	0	100	500	500	150
NH_4NO_3	1650	0	0	0	0
KNO_3	1900	750	2000	600	750
$CaCl_2·2H_2O$	440	165	165	165	100
$MgSO_4·7H_2O$	370	75	75	190	300
KH_2PO_4	170	170	420	100	100
$MnSO_4·4H_2O$	22.3	10.5	15	10.5	20
$ZnSO_4·7H_2O$	8.6	8.6	10	8.6	15
H_3BO_3	6.2	7.2	7.2	7.2	15
KI	0.83	0.83	1.5	0.83	0.5
$Na_2MoO_4·2H_2O$	0.25	0	0	0	0
$CuSO_4·5H_2O$	0.025	0	0	0	0
$CoCl_2·6H_20$	0.025	0	0	0	0
$FeSO_4·7H_2O$	27.8	27.8	27.8	27.8	27.8
$Na_2EDTA·2H_2O$	37.3	37.3	37.3	37.3	37.3
Inositol	100	130	150	130	50
Nicotinic acid	0.5	2	5	2	0.5
vitamin B1	0.1	0.5	1	0.5	2
vitamin B6	0.5	1	2	1	4
Glycine	2.0	2	5	2	5

For ZhongkeNo.6, the difference in ovule induction between the basic medium, TDZ concentration, NAA concentration and BA concentration was extremely significant. However, the effect of KT concentration on the ovule enlargement rate of ZhongkeNo.6 was not significant. For XinongNo.8 and Zhengkangjufeng materials, the 5 factors have a very significant effect on the ovule enlargement. For Zhengkang No.2 material, the difference in basal medium, TDZ concentration, BA concentration and KT concentration on ovule enlargement was significantly different, but the difference was not significant effect of NAA concentration on ovule enlargement rate (Table 4).

Effect of Basic Medium on Inducing Ovule Enlargement

Visual analysis and analysis of variance showed that the basic medium had a significant effect on the ovule enlargement rate of the 4 test materials, and was the main factor affecting the effect of ovule enlargement.

The experimental data of the 5 basic culture medium were compared using Duncan's new multiple range method (Table 6). The rate of ovule enlargement on the same genotypic material was also different on the different basic medium. The ovule enlargement rate of material XinongNo.8 on M2 basic medium was significantly higher than that of other medium ($P<0.05$). There was no significant difference in the ovule enlargement rate on MS, M1, and M4 medium, and the ovule enlargement rate was the lowest on M3 medium, and the difference reached a significant level ($P<0.05$). On the other hand, the ovules enlargement rate of M2 on materials ZhongkeNo.6 was the highest and significantly higher than that of other culture media ($P<0.05$). The difference between MS, M3 and M4 medium was not significant, and the ovule enlargement was the lowest on M1 medium. The enlargement rate of material Zhengkangjufeng on M2, M3, and M4 medium was higher and the induction effect was not significantly different. The ovule enlargement rate of material Zhengkangjufeng on MS and M1 medium was lower. For material ZhengkangNo.2, the ovule enlargement rate on M4 medium was the highest and significantly higher than that on other culture medium ($P<0.05$), and the ovule enlargement rate on MS medium was the lowest.

Table 3: Composition of inducing medium

Medium number	Basic medium	TDZ ($mg·L^{-1}$)	NAA ($mg·L^{-1}$)	BA ($mg·L^{-1}$)	KT ($mg·L^{-1}$)
L1	MS	0	0	0	0
L2	MS	0.02	0.5	0.5	0.5
L3	MS	0.04	1.0	1.0	1.0
L4	MS	0.06	1.5	1.5	1.5
L5	MS	0.08	2.0	2.0	2.0
L6	M1	0	0.5	1.5	2.0
L7	M1	0.02	1.0	2.0	0
L8	M1	0.04	1.5	0	0.5
L9	M1	0.06	2.0	0.5	1.0
L10	M1	0.08	0	1.0	1.5
L11	M2	0	1.0	0.5	1.5
L12	M2	0.02	1.5	1.0	2.0
L13	M2	0.04	2.0	1.5	0
L14	M2	0.06	0	2.0	0.5
L15	M2	0.08	0.5	0	1.0
L16	M3	0	1.5	2.0	1.0
L17	M3	0.02	2.0	0	1.5
L18	M3	0.04	0	0.5	2.0
L19	M3	0.06	0.5	1.0	0
L20	M3	0.08	1.0	1.5	0.5
L21	M4	0	2.0	1.0	0.5
L22	M4	0.02	0	1.5	1.0
L23	M4	0.04	0.5	2.0	1.5
L24	M4	0.06	1.0	0	2.0
L25	M4	0.08	1.5	0.5	0

On the same basic medium, the ovule enlargement rate of different genotype materials is also different. In general, M2 medium has the best induction effect and are suitable as the basic medium for inducing ovule enlargement.

Effects of Exogenous Hormones on the Induction of Ovule Expansion

Effect of TDZ concentration on ovule enlargement: By visual analysis and Duncan's new multiple range method analysis, it was found that TDZ concentration had a significant effect on the ovule induction in the four explants,

Table 4: 4 kinds of test materials in the induction medium ovule enlargement rate analysis table

Material code		Basic medium	TDZ	NAA	BA	KT
XINONGNO.8	R	34.01	33.77	30.32	21.28	19.69
	F	19.957**	24.438**	20.723**	9.394**	9.703**
ZHONGKENO.6	R	71.58	20.56	20.10	30.03	15.22
	F	36.636**	3.217**	2.862**	7.335**	1.857
ZHENGKANGJUFENG	R	29.97	42.96	28.97	20.15	17.81
	F	12.971**	22.903**	11.128**	5.779**	4.757**
ZHENGKANGNO.2	R	59.08	35.21	7.72	24.42	20.55
	F	139.692**	41.575**	2.016	24.994**	15.284**

Note: R indicates the difference the maximum and minimum values of $\bar{X}$ value. F indicates the significance sample mean.** represent significant at the P<0.05 and P<0.01 probability levels

Table 5: The optimal level combination of factors to induce ovule enlargement

Test material code	Optimal level combination
XINONGNO.8	M2+0.04 mg·L^{-1}TDZ+1.5 mg·L^{-1}NAA+0.5 mg·L^{-1}BA
ZHONGKENO.6	M2+0.08 mg·L^{-1}TDZ+0.5 mg·L^{-1}NAA+0.5 mg·L^{-1}BA+0.5 KT
ZHENGKANGJUFENG	M2+0.08 mg·L^{-1}TDZ+0.5 mg·L^{-1}NAA+1.5 mg·L^{-1}BA
ZHENGKANGNO.2	M4+0.06 mg·L^{-1}TDZ+0.5 mg·L^{-1}NAA+2 mg·L^{-1} BA+1.0 mg·L^{-1}KT

Table 6: Comparison of Duncan's new bipolar difference method for the five basic media

Basic medium	Ovule enlargement rate (%)			
	XINONGNO.8	ZHONGKENO.6	ZHENGKANGJUFENG	ZHENGKANGNO.2
MS	31.98b	37.99b	31.66bc	19.12d
M1	31.46b	10.66c	23.04c	27.90d
M2	50.72a	82.24a	53.01a	68.31b
M3	16.72c	25.78bc	40.84ab	39.15c
M4	33.68b	37.54b	48.81a	78.20a

Note: The letters in the same column represent the difference significant between the values at the 0.05 probability level

and was a major factor affecting the induction of ovule enlargement (Table 7). For material XinongNo.8, in the inducing medium containing 0.04 mg·L^{-1} and 0.08 mg·L^{-1} TDZ, the expansion rate of ovules was significantly higher than other concentrations, reaching a maximum of 49.23%, while the induction of 0.02 mg·L^{-1} and 0.06 mg·L^{-1} TDZ, and the effect was significantly lower than that of the medium, and the induction effect of the medium supplemented with TDZ was significantly higher than that of the medium without TDZ.

When the concentration of TDZ is not more than 0.04 mg·L^{-1}, the induction enlargement rate of ovules increases with the increase of TDZ concentration. For materials ZhongkeNo.6 and Zhengkangjufeng, when the medium is added with 0.08 mg/L TDZ, the ovule enlargement rate is the highest, reaching 46.62 and 66.83% respectively. For the material ZhengkangNo.2, the induced ovule enlargement rate increases with the increase of TDZ concentration, when the concentration is 0.06 mg·L^{-1}, the ovule enlargement rate is up to 58.91%, and when the TDZ concentration exceeds 0.06 mg·L^{-1}, the induction effect was reduced but did not reach a significant difference. The test results showed that when the concentration of TDZ was 0.04 and 0.08 mg·L^{-1}, the induction of ovule enlargement was better.

Effect of NAA on inducing ovule enlargement: The visual analysis and analysis of variance of the ovule enlargement rate of the 4 test materials showed that the concentration of NAA had a great influence on the ovule enlargement rate of XinongNo.8, ZhongkeNo.6, and Zhengkangjufeng, but had no significant effect on the ovule enlargement rate of ZhengkangNo.2.Using Duncan's new multiple range method to analyze the NAA concentration effects of induction media in XinongNo.8, ZhongkeNo.6, and Zhengkangjufeng (Table 8). With the increase of NAA concentration, XinongNo.8 ovule enlargement rate gradually increased. The difference in ovule enlargement rate among 1.0, 1.5 and 2.0 mg·L^{-1} of NAA concentration was not significant. When NAA was 0 mg·L^{-1}, the ovule enlargement rate was the lowest, and significantly lower than the other concentrations of ovule enlargement rate.

For ZhongkeNo.6 and XinongNo.8, when the NAA concentration was 0.5 mg·L^{-1}, the ovule enlargement rate was the highest, but there was no significant difference with the other three concentrations of NAA. When the NAA concentration was 0 mg·L^{-1}. The ovule induction rate is the lowest. Therefore, when the concentration of NAA is 0.5 mg·L^{-1}, the ovule induction effect is better.

Effects of BA on inducing ovule enlargement: The visual analysis and variance analysis of the ovule enlargement rate of the four test materials showed that the BA concentration had a significant effect on the ovule induction rate of the four materials and was the main factor affecting the ovule enlargement.

Table 7: TDZ concentration levels of Duncan's new bipolar difference method

TDZ(mg·L^{-1})	Ovule enlargement rate (%)			
	XINONGNO.8	ZHONGKENO.6	ZHENGKANGJUFENG	ZHENGKANGNO.2
0	15.47c	26.06b	31.89bc	23.70b
0.02	27.33b	43.99ab	23.87c	43.75b
0.04	49.23a	38.82ab	39.28b	55.72a
0.06	29.58b	38.72ab	35.50bc	58.91a
0.08	42.96a	46.62a	66.83a	50.58ab

Note: The letters in the same column represent the difference significant between the values at the 0.05 probability level

Table 8: NAA concentration levels of Duncan's new bipolar difference method

NAA(mg·L^{-1})	Ovule enlargement rate (%)		
	XINONGNO.8	ZHONGKENO.6	ZHENGKANGJUFENG
0	13.84c	29.14b	20.36b
0.5	30.00b	49.23a	49.33a
1.0	33.34ab	40.84ab	40.61a
1.5	44.15a	34.96ab	40.30a
2.0	43.23a	40.03ab	46.76a

Note: The letters in the same column represent the difference significant between the values at the 0.05 probability level

Table 9: BA concentration levels of Duncan's new bipolar difference method

BA(mg·L^{-1})	Ovule enlargement rate (%)			
	XINONGNO.8	ZHONGKENO.6	ZHENGKANGJUFENG	ZHENGKANGNO.2
0	35.57ab	37.52b	32.75b	52.22a
0.5	41.95a	59.56a	41.59ab	39.23b
1.0	28.96bc	34.84b	35.06b	31.08b
1.5	37.41ab	29.53b	52.90a	54.64a
2.0	20.67c	32.75b	35.07b	55.50a

Note: The letters in the same column represent the difference significant between the values at the 0.05 probability level

Table 10: KT concentration levels of Duncan's new bipolar difference method

KT(mg·L^{-1})	Ovule enlargement rate		
	XINONGNO.8	ZHENGKANGJUFENG	ZHENGKANGNO.2
0	41.79a	51.23a	50.29ab
0.5	26.39bc	36.70b	46.79bc
1.0	39.30a	42.17ab	58.55a
1.5	22.10c	33.86b	39.03c
2.0	34.98ab	33.42b	38.00c

Note: The letters in the same column represent the difference significant between the values at the 0.05 probability level

Multiple comparisons of the 5 levels of BA concentration in the 4 test materials (Table 9) for the materials XinongNo.8 and ZhongkeNo.6, with the addition of 0.5 mg·L^{-1} BA medium, the ovule enlargement rate was the highest. It reached 41.95 and 59.56% respectively. When the BA concentration was greater than 0.5 mg·L^{-1}, the ovule enlargement rate was significantly reduced. The materials of Zhengkangjufeng and ZhengkangNo.2 had the best effect on inducing ovule enlargement when the BA concentration was 1.5 mg·L^{-1}, and the ovule enlargement ratio reached 52.90 and 54.64%, respectively. Therefore, for different genotypes of materials, the appropriate level of BA concentration will be different.

Effect of KT Concentration on inducing ovule enlargement: Visual analysis and variance analysis of the four test materials found that KT concentration had no significant effect on the ovule enlargement rate of the induced material ZhongkeNo.6, and the induction effect on other test materials reached a very significant level.

The experimental data of the KT concentration in the medium XinongNo.8, Zhengkangjufeng and ZhengkangNo.2 induction medium was analyzed by Duncan's new complex-difference method (Table 10). When the KT concentration was 0 mg·L^{-1}, the effect of inducing ovule enlargement was best. The ovule enlargement rate was also high, but when the KT concentration was greater than 1.0 mg·L^{-1}, the ovule enlargement ratios of the three materials were all decreased, indicating that the addition of hormones with low KT or low KT concentration may promote the induction of ovule enlargement.

Discussion

The changes of microelement, microelement and organic elements in the induced medium had an important influence

on the induction of ovule enlargement (Dong *et al.*, 2016). The changes in the composition of culture medium affect the induction of the Isolated gynecological *in vitro*.

Wang *et al.* (2008) studied 5 different induction medium (medium composition is mainly between microelement, microelement, and organic elements difference) influence on the induction rate of cucumber embryogenesis. The results showed that the highest rate of embryoid induction was IM3 and IM4 medium. Two media nitrates and the ammonium nitrogen ratio was close to 1:1. When IM1 in nitrate nitrogen and ammonium nitrogen ratio reached 7.5:1, embryo induction rate was the lowest. The results showed that the ovule enlargement rate of M2 was higher due to having higher content of nitrate nitrogen. This is inconsistent with the study results. Culture medium change of organic components may also influence the induction effect on ovule (Thomas, 2004). It is reported that adding vitamins and glycine in the medium can improve induction rate to a certain extent (Bal *et al.*, 2003). Our results showed that the increase of microelement content in the medium was beneficial to the enlargement of ovule. Therefore, in order to screen out the basic culture medium suitable for the plant *in vitro* gynogenesis culture, a large number of experiments with different permutations are imperative. The results of *in vitro* culture of unfertilized ovules in cucumber showed that TDZ concentration had a significant effect on embryogenesis frequency and was the most important factor in inducing gynogenetic development of cucumber. The concentration of KT significantly affects embryogenesis and no significant effect of BA concentration (Wang *et al.*, 2015). Li *et al.* (2012) found that when the TDZ concentration was 0.06 and 0.08 $mg \cdot L^{-1}$, the germination rate of the tested cucumber varieties was higher, and 0.05 $mg \cdot L^{-1}$ NAA and 0.4 and 0.6 $mg \cdot L^{-1}$ BA was beneficial for embryoid differentiation of regenerated plants in cucumbers. Diao *et al.* (2009), Malik *et al.* (2011) results on the unfertilized ovary of muskmelon on the *in vitro* culture of unfertilized cucumber and melon ovules, respectively also showed that the appropriate concentration of TDZ promoted the formation of embryos. It is shown known that the concentration of NAA below 0.5 mg/L has no significant effect on the incidence of embryoid in the melon test. However, when the concentration of NAA was 0.5 mg/L, it inhibited the induction of embryoid (Dong *et al.*, 2016). All of the above are consistent with the results of this study. Therefore, it is very necessary to study the appropriate hormones and concentration combinations when inducing ovule enlargement and embryoid production in the early stage of watermelon non-pollinated ovary culture. The isolated culture of non-pollinated ovary and ovule in plants showed genotypic differences in this respect. Our data showed that there were great differences in ovule enlargement rate on the same induction medium for four different genotype materials, and the optimal induction medium for each genotype was different.

Studies have reported that there are great genotypic differences for *in vitro* gynogenesis culture. Liu *et al.* (2008) studied the differences in the *in vitro* induced gynecology rates of different genotype squashes and found that genotypes can significantly affect induction efficiency. The induction of different cucumber varieties also revealed the similar effects (Moqbeli *et al.*, 2013). Taşkın *et al.* (2013) performed radiation treatment on the pollen of different watermelon varieties have great differences in the induction rate of double haploids. Therefore, for different genotypes, appropriate induction medium should be selected to increase the ovule enlargement rate and achieve the purpose of obtaining embryoid bodies.

Conclusion

Basic medium and TDZ concentration had significant effect on ovule enlargement rate and they are the main factors for inducing the isolated gynogenetic development of watermelon. When choosing M2 basic medium and its concentration of TDZ from 0.4 to 0.8 $mg \cdot L^{-1}$, the induction rate of ovule enlargement was highest, reaching 66.83%. Subsequently, the induction effect of ovule enlargement was the best when the concentration of NAA was 0.5 $mg \cdot L^{-1}$. When the concentration of NAA was 0.5 $mg \cdot L^{-1}$, the highest rate of ovule enlargement reached 49.33%. The concentration of BA was different due to the difference in explants, the induction effects of ZhongkeNo.6 and XinongNo.8 are the best. The concentration of BA was 1.5 $mg \cdot L^{-1}$, and ZhongkeNo.6 and XinongNo.8 ovule enlargement rate reached 59.56 and 41.95%. While the concentration reached 1.5 $mg \cdot L^{-1}$, the rate of ovule enlargement in ZhengkangNo.2 and Zhengkangjufeng was 55.50 and 52.90%, respectively. No addition or addition of low concentration of KT is beneficial to the enlargement of ovules.

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