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Tissue culture capacities of different explants from China elite common wheat and their correlation analysis

Jie Gao¹, Fengzhi Guo², Yu lian Li¹, Qingqi Fan¹, Peng Meng¹, Shuimei Liang¹,
Chengyan Huang¹, Xiusheng Chu¹ and Genying Li^{1*}

¹Crop Research Institute, Shandong Academy of Agricultural Sciences/Key Laboratory of Wheat Biology and Genetic Improvement On North Yellow and Huai River Valley, Ministry of Agriculture P.R China/National Engineering Laboratory For Wheat and Maize, Jinan 250100.

²Crop Research Institute, Heze Academy of Agricultural Sciences, Heze, Shandong, 274000, China.

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To screen out cultivars with excellent tissue culture capacities for genetic transformation, the callus induction and regeneration response of immature embryos, inflorescences and anthers from 50 China new released cultivars with excellent agronomic performance were evaluated. The results showed that, among the surveyed cultivars, the callus induction frequency of immature embryos and inflorescences were genotype-independent. Whereas, the regeneration frequency of callus induced from immature embryos were significantly different, with less than 60%, from 60 to 80% and more than 90% in 36, eight and six cultivars, respectively. The regeneration frequency of the induced callus from inflorescences differed significantly, with less than 60%, from 40 to 90% and more than 90% in 34, 11 and five cultivars, respectively. However, both the callus induction and green plantlets regeneration frequencies of anthers were significant different among the cultivars, with the induction frequency less than 25%, from 25 to 60%, and more than 75% in 33, 14 and three cultivars, respectively. The green plantlets regeneration frequency of the callus induced from anthers ranged from 42.5 to 88.5% in different cultivars. Tissue culture capacities of immature embryos and inflorescences were highly correlated. Whereas, both of them have low correlations with anther culture capacity.

Key words: Common wheat, inflorescences, immature embryos, isolated anthers, tissue culture capacity.

INTRODUCTION

Common wheat is one of the most important food resources. The sustainable and sufficient wheat supply plays critical roles in food security. The continuous increasing population requires more and more wheat production, conflicting with the declining and deteriorating land and water resources. To solve this problem, the only way is to harvest more from the limited arable land. However, due to the limited available germplasm and the

changeable climates, conventional breeding is facing a bottleneck on how to increase the yield. Genetic engineering makes it possible to introduce the alien genes to wheat chromosome and create new germplasm with excellent agronomic traits, which was vital in varieties breeding. Thus, genetic engineering was considered as one of the most important technologies to break through the bottleneck (Bhalla et al., 2006).

*Corresponding author. E-mail: lgy111@126.com. Tel: (+86)53183178122. Fax: (+86)53183179088.

Previous studies showed that the callus induction and regeneration system is the major one used in wheat transformation, and tissue culture capacities of different explants had great effects on transformation efficiency (Pellegrineshi et al., 2002). To date, various explants such as immature embryos (Barro et al., 1999; Fennell et al., 1996; Wu et al., 2009), inflorescences (Caswell et al., 2000; Kavas et al., 2008; Maddock et al., 1983), isolated anthers (Brisbe et al., 2000; Soriano et al., 2008), isolated microspores (Mejza et al., 1993; Liu et al., 2004), mature embryos (Wang et al., 2009; Patnaik et al., 2006; Özgen et al., 1998, 2001), and shoot tips (Haliloglu et al., 2006; Viertel et al., 1996), leaf base (Wang et al., 2004) were successfully used for tissue culture, and immature embryos, 14 days after pollination with the size about 1.0 mm, were the most common explants used for transformation.

The transformation efficiency was much more depended on the callus induction and regeneration frequency, which were quite different among cultivars with different genetic backgrounds (Zale et al., 2004; Maddock et al., 1983; Raziuddin et al., 2010; Sharma et al., 1995). Therefore, it is very important to select an ideal cultivar as recipient. Bobwhite is a very nice cultivar used worldwide for genetic transformation (Hu et al., 2003; Cheng et al., 1997), while Yangmai 158 and Xinchun 9 are also excellent cultivars in immature embryo culture and they have been successfully used for producing transformants in China (Ye et al., 2005; Zhang et al., 2007, 2009), but all the aforementioned cultivars are spring wheat, and it takes long time to transfer the integrated alien genes into winter wheat by several generations backcrosses. Therefore, to accelerate the breeding progress, donor plants used for genetic transformation should not only have excellent tissue culture capacity but also desirable agronomic traits.

So, it is a promising pathway to directly introduce the alien genes into current released wheat cultivars or advanced lines with winter growth habit. Among the four wheat growing regions in China, Huang-Huai wheat region is the largest one with the largest production and largest sowing area, accounting for 70% of China wheat production (He et al., 2001; Zhuang et al., 2003). Only the cultivars with winter growth habit can survive the winter freezing temperature in this region. Thus, lots of transformation works make use of winter wheat as the donor plants in China (Ding et al., 2009; Zhang et al., 2009). Shandong which is the second largest wheat production area in China, release cultivars with excellent agronomic traits every year, these cultivars have broad adaptations in Huang-Huai wheat region. To understand the tissue culture capacity of these genotypes, immature embryos, inflorescences and anthers isolated from 50 cultivars released in 2009 were cultured on their corresponding medium, the callus induction and regeneration ability of those explants were evaluated, and the relationships of tissue culture capacity among

different explants were first reported in the present study, which provided very useful information in explants and cultivars selection during genetic transformation.

MATERIALS AND METHODS

A total of 50 cultivars with excellent agronomic traits, released in 2009, were selected from the Wheat Germplasm Bank of Shandong Academy of Agricultural Science, and planted in an irrigated experimental field located in Jinan, in 2009 to 2010 cropping season according to local management practices. Spikes from main tiller were harvested at 14th days after pollination (DAP). The isolated seeds were washed with 70% ethanol for 30 s and sterilized in 1% hypochlorous acid for 10 min. Immature embryos with the exact size of 1 ± 0.1 mm were incubated on the SD2 induction medium: Murashige and Skoog (MS) (1962) salts supplement with thiamine 1 mg/L, glutamine 150 mg/L, 2,4-dichlorophenoxyacetic acid (2,4-D) 2 mg/L, sucrose 3%, agar 0.7%, pH 5.8, keep in dark at 25°C, and four days later the axis was removed completely with the sharp scalpel under microscope and placed the scutellum on the same induction medium. The callus were transferred to fresh-made medium every two weeks, and four weeks after incubation in dark at 25°C, the induced callus were transferred to regeneration medium 1/2MS supplement with 2 mg/L trans-zeatin, incubated at 25°C under the light intensity 40 $\mu\text{mol/s.m}^2$, with 16 h day/ 8 h night. The green shoot formation and the regenerated plantlets were scored on the 35th day after the regeneration. Inflorescences were isolated when its size reached 6 ± 1 mm. Each one was cut into several pieces with the size about 2 mm, then incubated on the W4 induction medium (Risacher et al., 2009): MS basal medium supplement with 2,4-D 2 mg/L, glutamine 500 mg/L, casein 100 mg/L, sucrose 20 g/L, agarose 6 g/L, keep in dark at 25°C for five weeks and changed to the fresh-made medium every two weeks. Record the numbers of induced callus, and transfer induced callus to regeneration medium: MS without any hormone, incubated at 25°C under the light intensity 40 $\mu\text{mol/s.m}^2$, 16 h day/8 h night, and the numbers of green plantlets regenerated were recorded and calculated on 35th day after being transferred to regeneration medium.

Due to more than one plantlets were generated from one callus in some cultivars, and in order to decrease the bias in the fertile ability assessment of the regenerated green plantlets, only one plantlets regenerated from each induced callus of immature embryos and inflorescences was further transferred to pots with KLASMAN compost, vernalization at 4°C in the low temperature growth room for 40 days, then transferred to another growth room until harvest and grew under 16°C day/20°C night with the photoperiod 16 h day/8 h night, 500 $\mu\text{mol/s.m}^2$ light intensity. The agronomic traits were observed during the whole growing period and the produced seeds from each plant were recorded individually. Anthers with the majority of microspores at the mid- to late-uninucleate stage were isolated and inoculated to C17 induction medium: MS salts supplement with glycine 2 mg/L, nicotinic acid 0.5 mg/L, thiamine hydrochloride 1 mg/L, pyridoxine hydrochloride 0.5 mg/L, D-biotin 1.5 mg/L, kinetin 0.5 mg/L, 2,4-D 2 mg/L, sucrose 90 g/L, agar 7 g/L, pH 5.8. They were kept in dark for three days at 30°C, and then incubated at 25°C for four weeks. The induced embryoids or callus were calculated under the microscope and transferred to regeneration medium: MS salts supplement with glycine 2 mg/L, nicotinic acid 0.5 mg/L, thiamine hydrochloride 1 mg/L, pyridoxine hydrochloride 0.5 mg/L, D-biotin 1.5 mg/L, kinetin 2 mg/L, indole -3-butyric acid (IBA) 0.5 mg/L, sucrose 30 g, agar 7 g/L, pH 5.8, under the light intensity 40 $\mu\text{mol/s.m}^2$, with 16 h day/8 h night. The regeneration plantlets were scored once a week and the regeneration plantlets were calculated at the 35th day after being transferred to regeneration medium. Numbers of regenerated green

Figure 1. The callus induced from immature embryos and the agronomic performance of the regenerated plants. **A.** The callus induced from immature embryos after three weeks culture. **B.** The agronomic performance of the regenerated plantlets in growth room.

plantlets and albinos were calculated in four weeks.

One of the plantlets regenerated from each callus was further transferred to planting pots with KLASMAN compost, and vernalization at 4°C in the low temperature growth room for 40 days and then transferred to another growth room. With the occurrence of new tiller, the ploidy level of plantlets were analyzed by observing 10 stomatal guard cell length of each plant under the microscope at 400× magnification, following the method described by Sood et al. (2003): Take out the haploids from compost and wash the root completely. Trim the roots and leaves to about 3 cm, and put the trimmed plantlets into doubling solution (5% dimethyl sulfoxide (DMSO), 0.2% cochicine) for 4 h with continuous aeration to the doubling solution. Together with the automatically doubled haploids, the treated haploids were washed thoroughly with fluid water for 5 h and re-planted to compost with 5 g/L osmotic fertilizer, grew in the growth room under 16°C day/20°C night with the photoperiod 16 h day/8 h night, and 500 $\mu\text{mol/s.m}^2$ light intensity. The agronomic traits were observed during the whole growing period and the produced seeds from each plant were recorded individually.

In the aforementioned experiments, three replicates were made for each cultivar, and in each replicate, 30 immature embryos, 25 inflorescences and 100 anthers were isolated for tissue culture, respectively. Statistical analysis was performed using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, Version 9.1).

RESULTS

The tissue culture capacity of immature embryos

Five days after inoculation, globular stick up can be observed on the surface of scutellum under the microscope, the friable yellow-white embryonic structures appeared in some callus from different cultivars two weeks later, and the ratio of this structure in different cultivars are quite different. Some embryonic structure developed into green shoots on the induction medium in three weeks (Figure 1A). All of the embryos with the size

of about 1 mm were developed into callus, so the induction ratio was 100% in all cultivars. The induced callus together with the developed shoots, were transferred to the regeneration medium four weeks after the axis removed. The regeneration frequency of the induced callus were significantly different at the level of $P < 0.05$ (data not shown), with the regeneration frequency more than 90% in six cultivars, accounting for 10% of the surveyed cultivars, including three cultivars Yangmai158, Bobwhite, Liao0802 with a regeneration frequency of 100%, respectively. While 36 cultivars had the regeneration frequency less than 60%, accounting for 72%, and the lowest two cultivars, SN09-30 and 034700, had the regeneration frequencies of 26.7 and 27.3%, respectively (Table 1). Eight cultivars had the regeneration frequencies from 60 to 80%, accounting for 16%. After vernalization, the regenerated plantlets were transferred to the growth room, and 39 out the 50 surveyed cultivars obtained the matured seeds, while 11 cultivars had some plants with no pollen which cannot get seeds, and the ratios of the fertile plants in those cultivars were more than 96% (Table 1). The plantlets which cannot get the mature seeds were mainly dwarf mutants with no pollen. No obvious agronomic trait change was observed in fertile plantlets (Figure 1B).

The tissue culture capacity of inflorescences

The inflorescences with the length about 6 mm were isolated and cut into three pieces with the size about 2 mm, and then inoculated to the induction medium. One week later, the initiated callus can be observed along the spike's edge, and three weeks later, the embryonic structures were visible with naked eyes (Figure 2A). Numbers of the induced callus were calculated on the

Table 1. Tissue culture capacities of immature embryos, inflorescences and isolated anthers from different cultivars.

Field entry	Cultivar	FRIM (%)	FFIM (%)	FRYS (%)	FFYS (%)	NCINAN	NRGP	FRGP (%)	CANC (%)	FFAN (%)
1	Luyuan185	51.3	98.7	45.3	100.0	16.3	12.3	75.5	12.3	86.5
4	Liao 0801	30.7	100.0	25.3	100.0	22.7	16.3	72.1	16.3	89.8
8	Lin 061	50.0	100.0	44.0	97.0	15.0	11.3	75.6	11.3	97.1
11	Luzi 0318-1	52.0	100.0	44.0	100.0	10.7	6.3	59.4	6.3	94.7
12	Liao 0802	100.0	98.7	97.3	100.0	12.3	7.7	62.2	7.7	91.3
13	05428	31.3	100.0	29.3	100.0	15.3	9.0	58.7	9.0	96.3
18	Qifeng No.3	82.7	99.2	81.3	100.0	14.7	10.3	70.5	10.3	96.8
19	Tainong 7018	32.7	100.0	32.0	100.0	8.7	6.0	69.2	6.0	94.4
25	Wennong 18	96.0	100.0	97.3	97.3	22.0	14.3	65.2	14.3	95.4
27	Tainong 8681	83.3	99.2	80.0	100.0	18.0	15.0	83.3	15.0	95.6
28	SN055849	51.3	100.0	49.3	100.0	23.7	14.0	59.2	14.0	92.9
31	Wennong 19	30.0	100.0	36.0	100.0	20.0	11.3	56.7	11.3	91.2
32	Deyou 06-1	54.7	100.0	56.0	100.0	15.3	11.7	76.1	11.7	94.3
34	Tainong 8968	66.7	100.0	62.7	100.0	23.3	18.7	80.0	18.7	94.6
35	055319	55.3	100.0	41.3	100.0	18.0	13.7	75.9	13.7	95.1
37	9921	58.7	100.0	54.7	100.0	16.7	11.3	68.0	11.3	97.1
38	Shannong 06-205	34.0	100.0	36.0	100.0	13.7	12.0	87.8	12.0	97.2
39	Shannong 71	42.0	100.0	34.7	96.2	14.7	12.7	86.4	12.7	94.7
43	Shannong 044	65.3	100.0	70.7	94.3	14.7	12.0	81.8	12.0	91.7
44	Zhongzuo 155	46.0	100.0	46.7	100.0	32.7	24.3	74.5	24.3	86.3
46	035373	83.3	99.2	81.3	100.0	26.3	16.7	63.3	16.7	92.0
47	Bpt06037	36.7	100.0	26.7	100.0	43.3	38.3	88.5	38.3	94.8
51	Heng 4399	85.3	99.2	81.3	100.0	28.7	21.3	74.4	21.3	96.9
54	034700	27.3	100.0	28.0	100.0	28.0	17.0	60.7	17.0	90.2
55	07PS22	91.3	100.0	86.7	96.9	33.0	17.3	52.5	17.3	92.3
57	990512	74.7	100.0	66.7	100.0	33.3	24.7	74.0	24.7	98.7
59	By18	32.7	100.0	30.7	100.0	10.3	8.3	80.7	8.3	96.0
61	BPT0536	90.7	100.0	96.0	100.0	24.7	16.3	66.2	16.3	85.7
62	Shannong 05-0149	33.3	100.0	33.3	100.0	17.3	12.7	73.1	12.7	97.4
67	Zinong 0907	46.0	98.6	57.3	100.0	13.3	5.7	42.5	5.7	88.2
69	Kenxing No.1	48.0	100.0	25.3	100.0	10.3	7.0	67.7	7.0	90.5
70	SN09-30	26.7	100.0	33.3	100.0	9.0	7.3	81.5	7.3	90.9
71	055318	46.0	100.0	50.7	97.4	12.7	10.0	79.0	10.0	96.7

FRIM, Average frequency of immature embryos regeneration in three replicates; FFIM, average frequency of fertile plantlets regenerated from immature embryos; FRYS, average frequency of inflorescences regeneration in three replicates; FFYS, average frequency of fertile plantlets regenerated from young spikes; FCINAN, average callus induction frequency from isolated anthers in three replicates; NRGP, average numbers of regenerated green plantlets in three replicates; FRGP, average frequency of regenerated green plantlets in three replicates; ANCP, anther culture capacity; FFAN, average frequency of fertile plantlets regenerated from the induced callus of isolated anthers.

Table 1. Continued.

73	Shannong D919	85.3	100.0	93.3	100.0	25.3	18.3	72.4	18.3	87.3
77	SN738	40.0	100.0	25.3	100.0	55.7	46.0	82.6	46.0	95.7
79	Shannong 0801	36.7	100.0	34.7	100.0	42.3	33.3	78.7	33.3	96.0
81	Xingnong No.9	34.0	100.0	34.7	100.0	25.3	19.0	75.0	19.0	91.2
82	Dehan 06-3	32.0	97.9	65.3	100.0	33.0	27.3	82.8	27.3	97.6
83	Yangmai 158	100.0	98.7	96.0	98.6	24.7	14.7	59.5	14.7	93.2
84	K35	54.0	100.0	54.7	100.0	91.0	68.3	75.1	68.3	95.1
85	Ji8802	45.3	100.0	46.7	100.0	88.3	74.7	84.5	74.7	96.0
86	Weimai No.7	52.0	96.2	53.3	95.0	86.3	72.7	84.2	72.7	96.3
87	Jimai 20	40.0	100.0	36.0	100.0	15.7	12.3	78.7	12.3	91.9
88	Jimai 22	47.3	100.0	41.3	100.0	18.0	13.3	74.1	13.3	97.5
89	Yannong 19	46.7	100.0	48.0	100.0	32.7	23.3	71.4	23.3	92.9
90	Linmai No.4	58.0	100.0	65.3	95.9	36.7	25.7	70.0	25.7	93.5
91	Liangxing 99	34.7	100.0	38.7	100.0	19.7	14.7	74.6	14.7	93.2
92	Liangxing 66	36.7	100.0	46.7	100.0	21.3	12.7	59.4	12.7	94.7
93	Bobwhite	100.0	99.3	88.0	100.0	24.7	15.3	62.2	15.3	93.5
94	Linmai No.6	46.0	100.0	40.0	96.7	14.0	8.0	57.1	8.0	83.3

35th day, just before the callus was transferred to regeneration medium. The regenerated plantlets were induced in two weeks and stop to produce new plantlets after four weeks (Figure 2B). The statistical analysis indicated that, similar to immature embryos, the callus induction frequency of inflorescences with the size about 6 mm were 100%, but the regeneration frequency of the induced callus differed significantly at the level of $P < 0.05$ (data not shown). In 50 surveyed cultivars, 34 cultivars had the frequency less than 60%, accounting for 68%, and the lowest one was Liao0801 (25.3%), 11 cultivars had the frequencies from 40 to 90%, accounting for 22%, and five cultivars had the frequencies more than 90%, accounting for 10%. Among them, the top two cultivars were Liao0802 and Wennong18 with the equal regeneration frequency of 97.3%. After vernalization, the regenerated plantlets were transferred to the growth room, and 40 out the 50

surveyed cultivars obtained the matured seeds, while 10 cultivars had some plants with no pollen which cannot get seeds, and Shannong044 was the cultivar with the lowest ratio (94.3%) of the fertile plants (Table 1). Same with the plantlets regenerated from callus of immature embryos, the plantlets regenerated from the callus of young spikes which cannot get the mature seeds were mainly the dwarf mutants with no pollen. No obvious agronomic trait change was observed in fertile plantlets.

The tissue culture capacity of isolated anthers

100 anthers of each replicate were inoculated on the induction medium, three weeks later the embryonic callus were observed with naked eyes, and they were transferred to the regeneration medium when the callus grew to 1 mm. The

regenerated plantlets appeared in three weeks (Figure 3A). The results show that the embryonic callus can be obtained in all the cultivars, but differed from immature embryos and inflorescences, the induction frequency of those from different cultivars were significantly different, while the regeneration of green plantlets were also significantly different. The induction frequencies were less than 25% in 33 cultivars, accounting for 66%, 14 cultivars had the frequencies from 25 to 60%, accounting for 25%. While Ji8802, Weimai7 and K35 were the top three with induction frequencies more than 75%. However, the albinos regenerated from induced callus were unable to survive (Figure 3B). As shown in Table 1, the regeneration frequencies of green plantlets varied in a large spectrum, ranging from 42.5 to 88.5%. Among them, K35 had the highest callus induction efficiency, but its regeneration frequency of green plantlets was

Figure 2. Callus formation and regeneration of the inflorescences. **A.** The callus formation after three weeks induction. **B.** The regeneration of the induced callus from inflorescences.

Figure 3. Regeneration of the callus induced from isolated anthers and the agronomic performance of the regenerated plants. **A.** The regeneration of the induced callus from isolated anthers. **B.** The agronomic performance of the regenerated plants in growth room.

lower than Ji8802, leading to an inferior final anther culture capacity to Ji8802. Consequently, the tissue culture capacity of anther was co-determined by both the efficiency of callus induction and the regeneration frequency of green plantlets. The regenerated green plantlets were transferred to the compost and grew to three tillers. After the chromosome number checking and the doubling treatment of the haploids, the plantlets were transferred to vernalization to grow in growth room. As shown in Table 1, the ratios of the fertile plantlets (FFAN) surveyed 50 cultivars ranging from 83.3% (Linmai No.6) to 98.7% (990512). The failure of chromosome doubling

was the main reason that resulted to no seed. Some dwarf mutants were also observed in some cultivars (data not shown).

The correlations of the tissue culture capacities of different explants

The correlations of tissue culture capacities among inflorescences, immature embryos and isolated anthers were analyzed, and the results indicate that the capacities of tissue culture from immature embryos and

Table 2. Correlations of different factors involved in tissue culture capacity.

Parameter	RFYS	RFIM	FCINAN	FRGP	ANCP
RFYS	1				
RFIM	0.93	1			
FCINAN	0.017	-0.004	1		
FRGP	-0.26	-0.26	0.28	1	
ANCP	-0.04	-0.057	0.96	0.40	1

FRYS, Frequency of inflorescences regeneration; RFIM, frequency of immature embryos regeneration; FCINAN, callus induction frequency from isolated anthers; NRGP, numbers of regenerated green plantlets; FRGP, frequency of regenerated green plantlets; ANCP, anther culture capacity.

inflorescences were highly correlated ($r=0.92$, $P<0.01$). However, both of them have low correlations with anther culture capacity (Table 2), indicating that we can predict tissue culture capacity of immature embryos with that of inflorescences; but it makes no sense for anther culture capacity. The anther culture capacity was highly correlated with the callus induction frequency ($r=0.96$, $P<0.01$), and comparatively lower correlated with the frequency of regenerated green plantlets ($r=0.40$, $P<0.01$), while the coefficient between the frequency of regenerated green plantlets and that of induced callus from isolated anthers was 0.28 ($P<0.01$). Accordingly, the anther culture capacity was co-determined by callus induction frequency and green plantlets regeneration frequency, of which callus induction frequency was more important.

DISCUSSION

The present study indicates that the immature embryos with the proper size from different genetic background respond to induction medium very nicely, but the regeneration capacity of the induced callus differed greatly, so tissue culture capacities of the immature embryos were mainly determined by their regeneration frequency. The result disagrees with some previous reports which stated that the induction frequency of immature embryos was genotype dependent (Barro et al., 1999; Fennell et al., 1996; Maddock et al., 1983). This divergence maybe aroused from the size of immature embryos or the components of the induction medium used in different experiments. Previous studies also found that not all of the immature embryos with the size less than 0.5 mm or more than 1.5 mm can be induced into callus and the best response was within the size around 1 mm (Sharma et al., 1982). In the present study, the size of immature embryos were selected strictly in 1 ± 0.1 mm, and the SD₂ induction medium used herein was proved to be a quite good medium for callus induction. All were induced into callus which indicated that the callus induction of immature embryos was genotypes-independent and the regeneration efficiency of the

induced callus can represent the tissue culture capacity of immature embryos.

Genotype independent of callus induction frequency from inflorescences was also in disagreement with some previous studies (Maddock et al., 1983; Sharma et al., 1995), but consistent with the report of Redway et al. (1990). The difference may also be related to the medium used and the development stage of the inflorescences. Medium composition, callus age and vernalization period of donor plants were the main factors reported, which had great effects on inflorescences regeneration capacity (Kavas et al., 2008; Maddock et al., 1983; Pellegrineschi et al., 2003). Our preliminary studies indicate that the inflorescences which have yellowish transparent color with the length less than 2 mm cannot be induced into callus at all, and those with the sizes from 3 to 5 mm responded to induction medium, but not all of them can develop into callus, whereas those with the sizes from 5 to 8 mm can be initiated into callus easily. Based on these preliminary experiments, only the inflorescences with the sizes from 5 to 7 mm were used for callus induction in the present study, and the results indicate that the callus induction of inflorescences in this specific development stage were genotype independent. W4 medium was first used herein for inflorescences callus induction and the high efficiency callus induction ability indicated that W4 is a vigorous medium for inflorescences callus induction. Therefore, apart from the induction medium and other effectors, the tissue culture capacity of inflorescences was mainly determined by regeneration frequency of the induced callus.

In the present study, Ji8802, Weimai7 and K35 were the top three cultivars with outstanding anther culture capacity. Among them, Ji8802 has strong winter wheat growth habit which need at least 40 days vernalization at 4°C and Weimai7 is also a winter wheat cultivar that need about 30 days vernalization at 4°C. This characteristic made them very sensitive to the vernalization conditions, such as the lasting time and temperature. On the other hand, for Weimai7, the spikes often break through the cover leaf and expose to the open air when its microspores develop to the mid- to late-uninucleate stage, that lead to the isolated anthers which are always

spoiled by contaminations. Compared with Ji8802 and Weimai7, K35 is a cultivar not sensitive to vernalization and photoperiod, and can survive the freezing temperature of Huang-Huai wheat region in winter. Therefore, in terms of the anther culture capacity and growth habit, K35 is an optimal cultivar for anther culture.

The present study first reports the correlations of the tissue culture capacities between immature embryos, inflorescences and isolated anthers. The immature embryos were the most popular explants used in wheat genetic transformation, due to their good response to tissue culture. Inflorescences were harvested from younger plants, so it can reduce growth space and save time. According to the previous reports, physiological status of donor plants appears to have less influence on explants response in culture (Barro et al., 1999). The new finding of the close relationship between the tissue culture capacity of inflorescences and immature embryos, provided us very useful information in predicting the tissue culture capacity of different explants for a specific cultivar, reciprocally. However, anther culture capacity is mainly determined by the callus induction frequency and the ratio of the green plantlets. Some cultivars have highest callus induction frequency, but the ratio of regenerated albinos was also higher than the others. The final capacity of this sort of cultivars was not the highest, while some cultivars with average callus induction frequency and lower ratio of regenerated albinos sometimes had excellent anther culture capacity. So far the mechanism involved in the formation of albinos was veiled, and there is need for more works to be focused on how to decrease the ratio of albinos during the wheat anther culture.

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