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Influence of Maize Genotypes on Wheat Haploid Embryos Production in Maize Mediated Cross System

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SIX selected bread wheat double haploid genotypes with same heading time and different genetic background and eight maize genotypes belonging to four maize types (waxy, sweet waxy, sweet and super sweet maize types) were used in this study. The six wheat genotypes pollinated with the eight maize genotypes to produce wheat haploid embryos. The objective of this investigation was to study the effects of different maize genotypes of distinct types on the wheat haploid embryo ratio. The results showed that the same wheat material when pollinated with different maize genotype, the embryo rate differed between 1.5 to 3 times and ranging from 15.43% to 47.80%. Screening high induction rate maize varieties was essential to improve the efficiency of wheat haploid production. The average haploid embryo induction rate among the four maize types was highest in sweet type (33.24%) and lowest in super sweet type (29.59%). The wheat genotype A6 recorded the highest embryo rate percentage (34.11%), while A5 recorded the lowest rate (29.36%). Baitiannuo SQW-1 (B4) found to be the highest for embryo rate, followed by the Yuntianyu 6 (B5) and Zhenhenuo 1(B1), while the lowest was recorded by Yunchaotian 2 (B8) genotype. The maize genotypes in the same type differed significantly regarding the haploid embryo induction rate of wheat genotypes and thus maize types were not consistent in their behavior. So, the key was to select maize genotypes with high induction rate for wheat haploids and not the maize type. The interaction between wheat genotype and maize genotype has a significant effect on the embryogenesis rate. Therefore, if the rate of embryo induction of some wheat materials with a common maize variety is low, it may be possible using the pollination of other maize genotypes to increase the embryogenesis in wheat x maize hybridization.

Key words: Wheat x maize hybridization, Maize genotypes, Embryo rate

Introduction

The establishment of maize pollen induced haploid technology has the characteristics of simple operation, low cost, good induction effect, etc. (Zenkteler and Nitzsche, 1984, Laurie and Bennett, 1986 and 1988). Wheat × maize hybridization is one of the most efficient methods for producing haploid in wheat, which can be used to accelerate germplasm innovation, multi-gene polymerization breeding, molecular mapping group and gene expression and function research, ...etc. (Laurie & Reymondie,

1991, Chen & Chen 1998, Li & Dai 2000; Wang & Zhang 2001, Gu & Yang, 2002, Maluszynski et al., 2003, Gu et al., 2005 and 2006, Hussain et al., 2012, Ferrie, 2017 and Wiśniewska et al., 2019).

It is the precondition and basis for improving the haploid production efficiency of wheat to improve and stabilize the haploid embryo rate in

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the wheat haploid production of hybrid wheat x maize hybridization. Domestic and international studies show that the embryo rate of haploid embryo in wheat is influenced not only by the genotype of wheat material itself (Sun et al. , 1992, Inagaki & Tahir, 1992, Kisan, 1993, Chen et al., 1996, Lefebvre & Devaux, 1996, Inagaki, 1997, Chen et al., 1998, Christina et al., 1998, Wang, 1998, Vinesh et al., 1999, Cherkaout et al., 2000, Maluszynski et al., 2003, Cai et al., 2004, Cai et al., 2005a, Cai et al, 2006; Zhang et al. 2007; Zhang 2010 and Hussain et al., 2012), but also related to many factors, including maize genotype (Sun et al., 1992, Lefebvre and Devaux, 1996, Inagaki, 1997, Christina et al., 1998, Wang, 1998, Vinesh et al., 1999, Maluszynski et al., 2003 and Cai et al., 2005a), pollination (Chen et al., 1998 and Gu et al. 2006), 2,4-d treatment methods (Wang, 1998 and Cherkaout et al., 2000, Ballesteros et al., 2003, Maluszynski et al., 2003, Cai et al., 2004 and Cai et al., 2005), the treatment methods of hybrid spike after pollination, culture conditions (Cherkaout et al., 2000, Ballesteros et al., 2003, Cai et al., 2005 and Gu et al., 2008), ...etc., the most influential is the maize genotype (Chen and Chen 1998, Cai et al., 2004 and Gu et al. 2006).

Many researchers around the world have done some research on different types of maize to screen the maize varieties with high embryo rate (Christina et al., 1998, Cai et al., 2005a,b and Gu et al., 2006). The result was different from one to other; some results showed that the haploid embryo induction rate of popcorn is the highest, while in some studies sweet corn or sweet waxy corn is best. Results were different may be because of the varying wheat materials used in these studies, that is F_1 and F_2 of the hybrid offspring and the gametes is different. On this basis, the selection of maize genotypes with high embryo yield potential for haploid embryo has become a research focus to improve the embryo yield of wheat. So, this study intends to use six wheat double haploid lines and eight different genotypes of maize belonging to four maize types. The objective of this investigation was to study the effects of different maize types and varieties on the yield of wheat haploid embryos.

Materials and Methods

During 2016 season in Yunnan Academy of Agricultural Sciences, Songming Scientific Research Experimental Station, six bread wheat genotypes were selected from 4100 double haploid lines. These six genotypes had the same heading time and different genetic background. For this study, these six genotypes were denoted as A1 to A6.

Four types of maize (waxy, sweet waxy, sweet and super sweet maize types) were used in this study. Eight maize genotypes (from B1 to B8) selected by the Institute of Agricultural Sciences of Yunnan Province from all types are listed in Table 1.

Wheat x Maize hybridization experiment was conducted in the summer of 2017 in Kunming, Yunnan, China. Maize genotypes were sown at three sowing dates, determined according to Gu et al. (2006), with six days interval to ensure availability of sufficient pollen sources throughout the reproductive stage of the wheat genotypes. Wheat genotypes were planted in one sowing date because these genotypes had same heading time. At heading stage at least 48 spikes were selected from each wheat genotypes. The central floret of each spikelet was removed by forceps to produce more space for lateral florets. Wheat anthers were removed manually with the help of fine forceps and spikes were covered with paper bag to prevent cross pollination. After 24 hours of emasculation, fresh maize pollen from eight maize genotypes were collected in paper and pollinated emasculated spikes (Figure 1). Each treatment had six spikes dividing in three replications (2 spikes per repetition). The tillers with pollinated spikes were cut near the soil after 24 hour of pollination and kept in water. The cut spikes sprayed with 100 ppm 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and transferred to nutrition solution. All spikes put in growth chamber with the nutrition solution. After 13-14 days from cutting, spikes were separated for each treatment and green parthenocarpic caryopsis (GPC's) were removed from the florets.

Data were recorded for number of florets pollinated and number of GPCs Developed. Total numbers of haploid embryos were counted using a microscope. The embryo ratio percentage (also called "haploid embryo induction rate") was calculated as follow:

Embryo ratio (%) = Total number of haploid embryos/total number of florets pollinated x100**.** Data for embryo ratio was statistically analyzed using DPS (data processing software) via analysis of variance using complete randomized design.

TABLE 1. Selected maize genotypes used in this study

Maize types	Maize genotypes
Waxy	B1（Zhenhenuo 1）
	B2（Yunnuo 3）
Sweet waxy	B3 (Tiannuo 888)
	B4（Baitiannuo SQW-1）
Sweet	B5（Yuntianyu 6）
	B6 (Zhenhetianyu 1)
Super sweet	B7（Yunchaotian 1）
	B8（Yunchaotian 2）

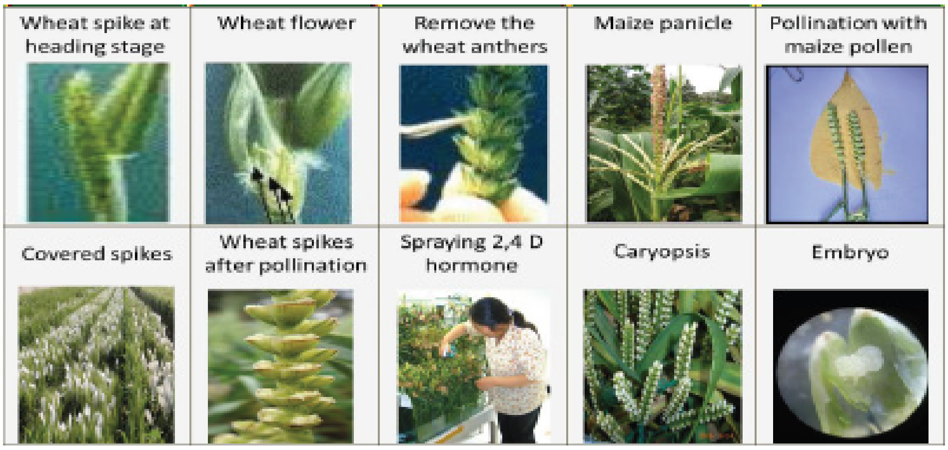

Fig. 1. Simple procedure for haploid embryos plant production by wheat x maize hybridization system

Results and Discussion

Analyses of variance

The analyses of variance (ANOVA) for embryo rate are presented in Table 2. The ANOVA revealed highly significant differences for wheat and maize genotypes. These results indicated that these genotypes were diverse and comparisons between genotypic means became valid. The highly significant differences for wheat x maize genotypic interactions indicated that wheat genotypes responded variably to the different maize genotypes suggesting the importance of assessing particular wheat genotype(s) for specific maize genotype in order to get high haploid embryo rate.

Mean performance

The average of wheat genotypes for number of florets pollinated, number of GPCs developed caryopsis, number of embryos and embryo rate（%） were illustrated in Table 3. Number of pollinated florets ranged from 1474 to 1787 for A3 and A6, respectively. A2 genotype recorded lowest values of number of GPCs and the number of embryos developed. A1 and A6 genotypes recorded the highest values of number of GPCs (1548) and number of developed embryos (525), respectively. According to the results of the average haploid embryogenesis rate of 6 wheat DH lines, embryo development rate ranged from 29.36% to 34.11%. A6 and A3 recorded the highest embryogenesis rate (34.11% and 33.36% respectively), while A5 recorded the lowest rate (29.36%).

Eight maize genotypes were used to pollinate six wheat double haploid lines, and the average rate of embryo for 6 wheat DH Lines, induced by each maize genotype, is given in Table 4. The data

TABLE 2. Analyses of variance for the haploid embryo rate

SOV	df	SS	MS	F	p
Wheat genotypes (A)	5	213.2183	42.6437	53.306916	1E-07
Maize genotypes (B)	7	852.6342	121.8049	152.26267	1E-07
A×B	35	3741.9320	106.9123	133.64612	1E-07
Error	96	76.7967	0.79997		

TABLE 3. Mean values of florets pollinated, GPCs developed caryopsis, embryos developed and embryo rate (%) for six wheat genotypes

Wheat genotypes	Florets pollinated (numbers)	GPCs Developed Caryopsis (numbers)	Embryos Developed (numbers)	Embryogenesis rate（%）
A1	1760	1548	494	31.91 c
A2	1559	1241	405	32.63 bc
A3	1474	1367	456	33.36 ab
A4	1675	1268	409	32.26c
A5	1648	1410	414	29.36 d
A6	1787	1539	525	34.11 a

TABLE 4. Mean values of florets pollinated, GPCs developed caryopsis, embryos developed and embryogenesis rate (%) for eight maize genotypes

Maize genotypes	Florets pollinated (numbers)	GPCs Developed Caryopsis (number)	Embryos Developed (numbers)	Embryogenesis rate（%）
B1（Zhenhenuo 1）	1413	1201	408	33.97 b
B2（Yunnuo 3）	1098	892	290	32.51 c
B3 (Tiannuo 888)	1296	1073	317	29.54 de
B4（Baitiannuo SQW-1）	1371	1205	460	38.17 a
B5（Yuntianyu 6）	1205	1031	357	34.63 b
B6 (Zhenhetianyu 1)	1192	982	297	30.24 d
B7（Yunchaotian 1）	1270	1054	322	30.55 d
B8（Yunchaotian 2）	1158	936	268	28.63 e

showed that there were significant differences in the induction rate of wheat haploid embryos between different genotypes of maize. It ranged from 28.63 to 38.17%, the difference was about 10%. Baitiannuo SQW-1 (B4) was the highest, followed by Yuntianyu 6 (B5) and Zhenhenuo 1 (B1). The lowest value recorded by Yunchaotian 2 (B8) genotype. Among them, Baitiannuo SQW-1(B4) was the promising maize genotype with higher haploid embryo induction rate.

At the same time, different maize genotypes in the same type had significant effect on the haploid embryo induction rate of wheat genotypes (Table 5). For example in sweet waxy type, B3 (Tiannuo 888) and B4 (Baitiannuo SQW-1) were significantly different (29.54% and 38.17%). Also, same results for waxy, sweet and super sweet types were enunciated. Among the four maize types (waxy, sweet waxy, sweet and super sweet), the average haploid embryo induction rate was varied as Sweet Waxy (33.86%) > Waxy (33.24%) > Sweet (32.43%) > Super Sweet (29.59%). However, the difference between two genotypes of sweet waxy type was 8.63% and sweet type was 4.38% (Table 5). However, this difference within the waxy and super sweet types was less.

According to genotypes, the induction rate of haploid embryo was B4 (Sweet waxy) >B5 (sweet) >B1 (waxy) >B2 (waxy) >B7 (super sweet), B6 (sweet) >B3 (Sweet waxy), B8 (super sweet). Since, maize types are not regular in inducing haploid embryos, so the key was to select maize genotypes with high induction rate of wheat haploid not maize type.

For this selection, the results showed that the difference of haploid induction rate between different genotypes in the same maize type (such as sweet waxy and sweet) is greater than that of different maize types. The eight different maize genotypes were significantly different in the rate of haploid embryo induction. There is no regularity between the types, so the key to screening high embryo rate maize varieties is to select maize genotypes rather than maize type. The results were inconsistent with the results of several previous studies. Gu et al. (2006), showed that the induction rate of haploid embryo altered from sweet waxy corn, sweet maize, corn, and common maize. Cai et al. 2005b, studied embryo rate on different wheat F_1 material and placed as sweet corn (35.7%) > flour corn (11.6%) and popcorn (8.7%) > waxy corn (7.3%), as well as popcorn (9.9%) > waxy corn (8.9%) > flour corn (8.2%) > Sweet corn (4.6%). Wang 1998, from F_2 wheat materials obtained the order as sweet corn (8.71) and waxy maize (6.54 %) > flour corn (5.65); Zhang, et al. 2007 concluded that sweet corn (2.99%) > waxy corn (2.27%) > flour corn (2.08%); Wang. et al. 2016 considered as popcorn (9.59%) > Sweet corn (4.82%) and waxy corn (4.35%); The reasons for these differences was because most of the wheat materials used by researchers are cross-descendant materials and in the same material spikes and florets differed. In addition to that, during each of these studies often only one genotype of maize was selected.

TABLE 5. Comparison of haploid embryo induction rate between maize types and genotypes

Maize types	Maize genotypes	Embryogenesis rate (%)	Type means (%)	Variance within type (%)
Waxy	B1（Zhenhenuo 1）	33.97 b	33.24	1.45
	B2（Yunnuo 3）	32.51 c		
Sweet waxy	B3 (Tiannuo 888)	29.54 de	33.86	8.63
	B4（Baitiannuo SQW-1）	38.17 a		
Sweet	B5（Yuntianyu 6）	34.63 b	32.43	4.38
	B6 (Zhenhetianyu 1)	30.24 d		
Super sweet	B7（Yunchaotian 1）	30.55 d	29.59	1.92
	B8（Yunchaotian 2）	28.63 e		

According to ANOVA results (Table 2), the interaction between wheat genotypes and maize genotypes had a significant effect on embryogenesis rate. In this experiment, the same wheat genotype was pollinated by different maize genotypes and the embryogenesis rate was statistically different (Table 6). The embryo ratio in A1 ranged from 41.80 to 21.12 by B3 and B8, respectively. Yuntianyu 6 (B5) and Tiannuo 888 (B3) recorded the highest (41.43) and lowest (19.44) values of embryo ration in A2 genotypes, respectively. B1 recorded the highest value (42.31), while B6 recorded the lowest one (28.56) in A3 wheat genotypes. Baitiannuo SQW-1 (B4) maize genotype was the best one in over all mean and it recorded the highest values with A4 (42.40), A5 (42.88) and A6 (47.80). The lowest embryo rate values in A4, A5 and A6 recorded with B3 (23.19), B2 (17.14) and B8 (15.43), respectively. Therefore, for some wheat materials, if the rate of embryo induction with a common maize variety is low, it may be possible to increase the embryogenesis rate significantly by using the pollination of other maize genotypes.

In this study, by pollinating the same wheat material with different maize genotypes, the embryo rate differed between 1.5 to 3 times. This is in consistent with the previous research results [Gu et al. 2006; Christina et al. 1998; Cai et al. 2005; Wang 1998; Cai et al. 2005b], indicating that screening for the high embryo rate of maize genotypes to improve the production efficiency of wheat x maize hybrid wheat haploid is very important.

In addition, the relationship between wheat genotypes and maize genotypes in this study had a significant effect on the embryo rate, and also there were differences between "general combining ability" and "specific combining ability" among maize genotypes and wheat genotypes for the embryo development. So, not only by screening wheat genotypes we could get higher haploid embryo induction rate with maize genotype (such as B4 in this study), but also the low embryo induction rate of a wheat genotype could be significantly improved by pollinating with different maize genotypes. It provides a new way to improve the embryo yield in wheat x maize hybridization and resultantly haploid production efficiency.

Conclusion

It could be concluded that by pollinating the same wheat material with different maize genotypes, the embryo rate differed between 1.5 to 3 times indicating the important of maize genotypes screening to improve the production efficiency of wheat x maize hybrid wheat haploid**.** The results showed that the difference of haploid induction rate between different genotypes in the same maize type (such as sweet waxy and sweet) is greater than that of different maize types, so the key to screening high embryo rate maize varieties is to select maize genotypes rather than maize type. Although, wheat genotypes could get higher haploid embryo induction rate with B4 maize cultivar, the low embryo induction rate of a wheat genotype could be significantly improved by pollinating with different maize genotypes. Nevertheless, more investigations and research studies are needed for more understanding of the other factors which influence on frequency of fertilization and embryo formation to increase the efficiency of double haploid technique.

TABLE 6. Embryo rate listed as maize genotype x wheat genotype two-way data format

Genotypes	A1	A2	A3	A4	A5	A6
B1	28.88 d	40.37 a	42.31 a	33.54 c	23.76 e	34.88 d
B2	33.63 c	33.63 b	35.52 b	30.87 cd	17.14 f	44.27 b
B3	41.80 a	19.44 d	30.27 de	23.19 f	28.97 d	33.55 d
B4	30.22 d	34.85 b	30.89 de	42.40 a	42.88 a	47.80 a
B5	34.03 c	41.43 a	34.63 bc	33.57 c	24.65 e	39.40 c
B6	37.26 b	35.95 b	28.56 e	30.52 d	26.24 de	22.92 e
B7	28.33 d	26.28 c	32.82 cd	27.28 e	33.91 c	34.70 d
B8	21.12 e	29.09 c	31.95 cd	36.83 b	37.37 b	15.43 f

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تأثير بعض التراكيب الوراثية من الذرة علي معدل انتاج أجنة القمح الاحادية في تقنية التهجين بين القمح و الذرة

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أجريت هذه الدراسة في محطة بحوث سونمنج، كونمنج ، ولاية يونان ، الصين بهدف دراسة تأثير ثمانية تراكيب وراثية من الذرة تتبع طرز مختلفة علي معدل انتاج الأجنة الأحادية للقمح في تقنية تهجين القمح والذرة. تم استخدام ستة تراكيب وراثية من القمح ناتجة من برنامج مضاعفة النباتات الأحادية تتميز بموعيد طرد سنابل واحدة و خلفية وراثية مختلفة. تم التهجين بين الستة تراكيب الوراثية من القمح مع الثمانية تراكيب الوراثية من الذرة للحصول عليى الأجنة الأحادية للقمح. أوضحت النتائج وجود فروق معنوية بين التراكيب الوراثية للقمح و الذرة و كذلك التفاعل بينهم. تراوح معدل انتاج الأجنة من ١٥,٤٣ الي ٤٧,٨٠ في نفس التركيب الوراثي للقمح بالتهجين مع أصناف الذرة المختلفة. مما يدل على اهمية انتخاب أصناف الذرة التي تعطي معدل انتاج أجنة اعلى في تحسين انتاج الاجنة الأحادية. وبمقارنة طرز الذرة المختلفة كذلك أعطت الذرة السكرية اعلى معدل للاجنة (٣٣,٢٤). بينما كان الصنف Baitiannuo SQW-1 (B4) هو الاعلى بين الأصناف في معدل انتاج الأجنة ثم الصنف Yuntianyu 6 (B5) بينما كان الصنف Yunchaotian 2 (B8) هو الاقل في معدل انتاج الأجنة. كما أوضحت التتائج ان التراكيب الوراثية للذرة تحت نفس الطراز اختلفت معنويا في معدل انتاج الأجنة و كانت الفروق داخل الطراز اعلى احيانا من الفروق بين الطرز المختلفة. لذلك انتخاب صنف الذرة الذي يعطي معدل أجنة اعلى هو الافضل من انتخاب طراز معين من الذرة. وجود تفاعل معنوي بين التراكيب الوراثية للقمح وأصناف الذرة يدل على انه يمكن استخدام الصنف B4 بصفة عامة في انتاج الاجنة الاحادية في القمح إلا اذا وجدت نسبة منخفضة من الأجنة لبعض التراكيب الوراثية من القمح فيمكن استخدام أصناف ذرة أخرى.