Bangladesh J. Bot. **52**(2): 523-528, 2023 (June) Special DOI: https://doi.org/10.3329/bjb.v52i20.68215

HAPLOID DOUBLING EFFECT OF WAXY MAIZE UNDER DIFFERENT ECOLOGICAL ENVIRONMENT

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Keywords: Waxy maize, Haploid, Natural doubling, Colchicine, DMSO

Abstract

The haploid seeds of five waxy maize hybrids, Jingkenuo 2000, Kennian 1, Wannuo 2000, Jikenuo 99 and Jikenuo 20, were treated with natural doubling and colchicine + DMSO chemical doubling. Results showed that the average doubling rates of Jingkenuo 2000, Wannuo 2000, Kennian 1, Jikenuo 20 and Jikenuo 99 were 0.30, 0.18, 0.53, 1.30 and 1.05%, respectively. The haploid doubling rates of Nanbin test site were higher than those of Shuangyang test site under different ecological environment. The haploid doubling rates of different doubling methods were different. Results showed that the doubling effect of needling growth point method was slightly better than that of soaking bud method, but the difference was very small, so it could be used flexibly. For different concentrations of colchicine solution, 0.08 mg/l solution had the best doubling effect on the haploid of waxy maize, with an average doubling rate of 7.96%, and the highest doubling rate of 10.70%.

Introduction

Waxy maize, also known as sticky maize, is controlled by a single recessive gene (wx), which is located on chromosome 9. 1. The waxy maize's endosperm contains a starch which is 100% amylopectin (Li *et al.* 2016, Chen *et al.* 2021). Its unique taste and nutritional value were gradually favored by the public. At present, the domestic market demand is increasing gradually, and due to the higher economic benefits farmers are expanding the planting area continuously (Yu *et al.* 2018). So, it is imperative to develop high yielding maize varieities to meet the demanding needs. However, conventional breeding takes a long time; about 6-8 generations to obtain homozygous waxy maize inbred lines, which greatly delays the speed of waxy maize inbred line breeding, new variety development and promotion (Deng *et al.* 2017).

The development and application of maize haploid breeding technology has significantly accelerated the breeding speed of excellent maize inbred lines. The haploid breeding technology has been industrialized in the breeding of ordinary maize, but the research and application in the breeding of waxy maize are relatively few (*Guo et al.* 2020, Li *et al.* 2020). In order to explore the universality of the application of haploid technology in waxy maize breeding, in the present study waxy maize haploids were used as experimental materials to compare the doubling rate of waxy maize haploids in different time periods, different natural doubling and chemical doubling sites, and different chemical concentrations, so as to provide reference for peers to carry out waxy maize haploid breeding efficiently.

Materials and Methods

Glutinous maize haplotypes were obtained from Jingkenuo 2000, Wannuo 2000, Kennian 1, Jikenuo 99 and Jikenuo 20 by inducing line Jikenou 115. Each haploid seed was divided into three groups, each group had about 1000 seeds, which were used for natural doubling, bud soaking

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doubling and needle acupuncture at the growth point doubling, respectively. The two test sites for natural doubling were located in Shuangyang maize breeding test Site (Shuangyang Test Site) of Jilin Agricultural Science and Technology University and Nanbin breeding base test site (Nanbin Test Site) of Hainan Province. The herbicide doubling test was conducted at Shuangyang test site. The chemical doubling agent was colchicine + 2.5% DMSO, all purchased from Changchun Baijin Biotechnology Co., Ltd.

Haploid grain was purple top white embryo, diploid was purple top purple embryo. The leaf sheaths of haploid seedlings at 2~3 leaf stages were green, while those of diploid seedlings were purple. Haploid leaves were narrow and erect, plants were thin and grow slowly, plant height and ear position were significantly lower than diploid. When haploid seedlings grow to 4-5 leaf stages, further identify haploid seedlings with weak growth vigor and green leaf sheaths for further experimental research (Xu *et al.* 2013, Feng *et al.* 2021).

Normal field sowing, unified manual management under natural growth conditions, pollination and self pollination of the plant were observed when it was pollinated.

The 500 ml colchicine solution with concentration of 0.02, 0.04, 0.08 and 0.10 mg/l, respectively with 2.5% DMSO as penetration aid was prepared. The haplotypes of Jikenuo 20, Jingkenuo 2000 and Jikenuo 99 were treated.

The seeds were soaked for towel roll sprouting method was used to accelerate the roots at 30℃. When the root tip grew to 1cm, the seeds were taken out of the towel roll, the temperature was redused to 24℃, the seeds were put into an enamel plate, water was sprayed to maintain humidity, and use film to seal for germination. When the bud grew to 1.5 cm, the sheath was cut off at the top of the bud by 1-2 mm, exposing a small mouth. They were immersed them in the medicament solution at room temperature, washed with clean water for 60 min after soaking, and then they were placed in the seedling tray. When the seedlings grew to 4-5 leaves, they were transplanted to the field (Wang *et al.* 2013, Jiang *et al.* 2014,Wang *et al.* 2015).

When the haploid seedlings grew to the trilobal stage, the seedlings were selected with good and consistent growth, respectively. The prepared colchicine solution was injected into the growth point at the scutellum node of the seedling stem with a micropipette, about 1 mL per plant (Jiang *et al.* 2014).

The natural doubling rate and chemical doubling rate of haploid were calculated (Cheng *et al.* 2017). The calculation formula of doubling rate, as follows:

Doubling rate = (number of bearing haploid plants/total number of surviving haploid plants) × 100%.

DPS7.1 statistical software was used to process the test data.

Results and Discussion

The natural doubling rate of the five waxy maize varieties tested in Nanbin test site for two consecutive years was higher than that in Shuangyang test site (Table 1). The average doubling rate of the haploid grain of Jingkenuo 2000 in Nanbin test site was 0.60%, but failed in Shuangyang test site. The average doubling rate of Wannuo 2000 haploid grain in Nanbin test site was 0.35%, but failed in Shuangyang test site. The average doubling rate of the haploid grain of Kennian 1 was 1.00% at Nanbin test site and 0.05% at Shuangyang test site. The average doubling rate of haploid grain of Jikenuo 99 was 1.90% at Nanbin test site and 0.20% at Shuangyang test site. The average doubling rate of haploid grain of Jikenuo 20 was 2.30% in Nanbin test site and 0.30% in Shuangyang test site. Based on the results of experiments at different locations, the reason for the difference in the natural doubling rate of waxy maize haploid might be the differing

temperature and sunlight impacting on the male spike powder dispersion, and, reducing the natural doubling rate of the haploid.

Table 1. Haploid natural doubling rate of waxy maize in different locations.

Varieties	2021		2022		Nanbin average	Shuangyang Average	Average
	Nanbin	Shuangyang	Nanbin	Shuangyang			
Jingkenuo 2000	0.50	0	0.7	0	0.60	0	0.30
Wannuo 2000	0.30	0	0.4	0	0.35	0	0.18
Kennian 1	0.90	0	1.10	0.10	1.00	0.05	0.53
Jikenuo 99	1.80	0.30	2.00	0.10	1.90	0.20	1.05
Jikenuo 20	2.20	0.20	2.40	0.40	2.30	0.30	1.30
Average	1.14	0.10	1.32	0.12	1.23	0.11	

The haploid natural doubling rates of Jingkenuo 2000 and Wannuo 2000 were very low, 0.30 and 0.18%, respectively. The natural doubling rate of Kennian 1 was slightly higher, but only 0.53%. The haploid doubling rate of Jikenuo 20 and Jikenuo 99 had obvious advantages. The natural doubling rate of Jikenuo 20 reached 2.40%, and the average doubling rate was 1.30%. The natural average doubling rate of Jikenuo 99 was 1.05%. Therefore, three varieties with higher natural doubling rate, Kennian 1, Jikenuo 20 and Jikenuo 99, were further analyzed.

The chemical doubling effect of colchicine solutions with four different concentrations (0.02, 0.04, 0.08, 0.10 mg/l) was found better than that of natural doubling (Tables 2-3). . With the increasing concentration of colchicine solution, the haploid doubling effect of the three varieties under different treatment methods increased continuously, reaching the highest at 0.0 mg/l, and lowest at 0.02 mg/l, which inhibited the plant, showing a trend of increasing first and then decreasing as a whole. However, there are also special circumstances. For example, when the needle acupuncture at the growth point method was used for the haploid of Jikenuo 99 in Shuangyang test site and the bud soaking method was used for the haploid of Kennian 1 in Nanbin, the doubling effect of colchicine solution of 0.10 mg/l was higher than that of colchicine solution of 0.08 mg/l.

The haploid seeds of three waxy maize varieties of different genetic lineages, Kennian 1, Jikenuo 99 and Jikenuo 20, were treated with colchicine solution of 0.08 mg/l concentration (Table 4). The haploid doubling rate of Jinkenuo 99 was 6.75 times, that of Jikenuo 20 was 7.38 times, and that of Kennian1 was the highest, (13.58 times). To sum up, the sensitivity of waxy maize haploid materials with different genetic backgrounds to colchicine solution at this concentration was different.

Five waxy maize hybrids with different genetic relationships were selected in the experiment. Among them, Jingkenuo 2000 and Wannuo 2000 belong to the mainstream waxy maize varieties in south of China. Under the condition of natural doubling, the natural doubling rates of Jingkenuo 2000 and Wannuo 2000 were very low. Between 0 and 0.7%, the average doubling rates of Jingkenuo2000 and Wannuo 2000 were 0.30 and 0.18%, respectively. The other three varieties, Kennian1, Jikenuo 99 and Jikenuo 20, were the main varieties in northeast of China. Under natural conditions, their doubling rate was high. Kennian1's doubling rate was between 0 and 1%. The highest natural doubling rate of Jikenuo 20 was 2.40%, with an average doubling rate of 1.3%. The natural average doubling rate of Jikenuo 99 was 1.05%. To sum up, different genetic materials have certain effects on the haploid doubling rate of waxy maize.

Table 2. Comparison of haploid doubling rate of waxy maize under different treatments in different locations.

Varieties	Medicament Conc. (mg/l)	Shuangyang		Nanbin		Average doubling rate of budsoaking method	Av. doubling rate of acupuncture	Overall average doubling rate
		Bud soaking	Acupuncture growth point method	Bud soaking	Acupuncture growth point method			
Jikenuo 99	0.02	5.53	7.36	6.38	6.25	5.96^{D}	6.81^{D}	6.38
	0.04	8.97	8.30	8.39	8.96	8.68^{C}	9.13^{B}	8.91
	0.08	9.58	9.15	10.19	9.62	9.89^{A}	9.39^{B}	7.09
	0.10	9.35	9.39	8.37	8.84	8.86^{B}	9.12^{B}	8.99
Jikenuo 20	0.02	5.75	7.52	7.17	6.91	6.46^{D}	7.22^{C}	6.84
	0.04	7.00	8.16	8.72	8.53	7.86^{D}	8.35^{B}	8.10
	0.08	8.66	10.31	8.71	10.70	8.69^{C}	10.51^{A}	9.60
	0.10	9.86	9.73	8.95	9.58	9.41^{B}	9.66^{B}	9.53
Kennian 1	0.02	2.60	3.82	3.54	4.31	3.07^{E}	4.07^{D}	3.57
	0.04	5.91	4.42	6.28	5.33	6.10^{D}	4.88^{D}	5.49
	0.08	7.71	5.26	7.23	8.60	7.47^{D}	6.93^{D}	7.20
	0.10	6.41	5.80	8.24	8.33	7.33^{D}	7.07^{C}	7.20

Capital letters indicated that the difference level is extremely significant.

Table 3. Doubling rate of waxy maize haploid seeds treated with colchicine at the concentration of 0.8 mg/l.

Varieties	Reagent conc. (mg/l)	Shuangyang		Nanbin		Average
		Bud soaking	Acupuncture growth point method	Bud Soaking	Acupuncture growth point method	
Jikenuo 99	0.08	9.58	9.15	10.19	9.62	7.09
Jikenuo 20	0.08	8.66	10.31	8.71	10.70	9.60
Kennian1	0.08	7.71	5.26	7.23	8.60	7.20
Average		8.65	8.24	8.71	9.64	7.96

Table 4. Under the concentration of 0.8 mg/l, the doubling rate of haploid seeds of different basic materials increased by multiple incomplete.

Haploid	Multiplication of doubling rate of bud soaking method	Doubling rate increase multiple of acupuncture growth point method	Average value
Kenian1	14.09	14.93	13.58
Jikenuo 99	9.41	9.43	6.75
Jikenuo 20	6.68	7.47	7.38

Natural doubling is used as the control for doubling rate.

At the same level, there was a certain difference in haploid doubling rate between Shuangyang and Nanbin experimental sites. Nanbin test site belongs to marine climate, with hot days and cool nights, and large temperature difference between day and night. Based on the climate, ecological and climatic characteristics and the condition that artificial irrigation could be carried out at any time, it was more suitable for the growth of maize plants, with a coordinated

flowering period, which was conducive to the male spike scattering, and to a certain extent, it improved the success probability of natural doubling (Wang *et al.* 2015). To sum up, different ecological conditions have a certain impact on the haploid doubling rate of waxy maize, which was consistent with the research results of other researchers (Xu *et al.* 2013, Jiang *et al.* 2014, Cheng *et al.* 2017).

The application of colchicine + DMSO solution could significantly improve the haploid doubling rate, but the treatment of haploid seeds or seedlings will also affect the doubling effect. In this experiment, two treatment methods, namely, acupuncture at the growth point- and bud soaking method, were used. At different concentrations of colchicine, the doubling rate of acupuncture at the growth point method was slightly better than that of bud soaking method, but the difference was very small. With the increase of the concentration of medicament, the doubling rate of bud soaking method would also be higher than that of acupuncture at the growth point method.

From the perspective of treatment method, the treatment method of acupuncture at the growth points is relatively direct, which can make colchicine solution injected at growth points, inhibit the separation of homologous chromosomes and complete haploid doubling for cells in mitotic stage. The principle of bud soaking was to immerse the whole seedling in colchicine solution, so that the buds and roots of seedlings can passively absorb colchicine solution to achieve the same purpose, and the sensitivity to chemicals is poor. However, from the analysis of the experimental data, there was little difference between the two processing methods on the whole, and they could be flexibly used.

The haploid doubling rate under natural conditions was extremely low. Colchicine could effectively interfere with the mitotic process of cells, promote haploid doubling, restore diploid plants, and obtain maize DH lines (Feng *et al.* 2021) . This study showed that with the increasing concentration of colchicine solution, the haploid doubling effect of the three waxy maize varieties under different treatment methods increased continuously, reaching the highest at the concentration of 0.08% mg/l, and decreasing at 0.1% mg/l. Colchicine has strong toxicity. At high concentration, it would be toxic to haploid materials and inhibit plants, thus reducing haploid doubling rate. The effect of colchicine with different concentrations on haploid doubling rate was first increased and then decreased. However, there were also several special cases, which may be caused by the different tolerance of different genetic basic materials to colchicine toxicity (Liu *et al.* 2014).

From the results of the present experiment, it may be said that the doubling of waxy maize haploid will be affected by many factors. In this experiment, there were fewer test materials, more concentration gradient settings were lacking, and there were only two test points, which would affect the validation test results. Therefore, the experimental design should be further improved and the mechanism of haploid doubling of waxy maize should be further studied in the subsequent experiments. In conclusion, different basic materials will affect the haploid doubling rate, and selecting the appropriate doubling location and method can effectively improve the haploid doubling rate of waxy maize.

Acknowledgements

This work was supported by the Scientific Research Project Fund of Jilin Provincial Education Department (JJKH20230441KJ) and the PhD research startup foundation of Jilin Agricultural Science and Technology University (#2022721).

References

Cheng J, Zheng C X, Pan M N and Chen R Y. 2017. Effects of the Ecological Condition and Herbicide on Chromosome Doubling of Haploid Maize. J. Anhui Agricul. Univ. **44**(01): 114-118.

Chen J J, Li X N, Lv G H, Wu Z X and Guo G J. 2021. Combining Ability Effect and Genetic Parameter Analysis of Agronomic Traits of waxy maize. Acta Agricul. Jiangxi. **33**(01): 1-5+11.

Deng K P, Li X Y, Jing G X, Lu B, Xing Z and Zhao R G. 2017. Preliminary Study on Efficient Waxy Maize Haploid Doubling Metho. Seed. **36**(01): 82-85.

Feng H Y, Yao B J, Chen M and Gao L. 2021. Haploid Breeding Techniques: Research Progress. Chinese Agricul. Sci. Bull. **37**(30): 1-6.

Guo S L, Wei X, Wei L M, Zhu W H, Zhang Q J, Guo J S, Zhou B, Wang Y Z, Han X H, Zhang X, Wang Z H, Chen Y H and Lu X M. 2020. Analysis of Technique and Mechanism Relate to Induction and Doubling of Haploid in Maize. J. Maize Sci. **28**(03): 52-59+65.

Jiang L, Ci J B, Cui X Y, Zhang Y, Nan N,Li J Z and Yang W G. 2014. Study on Induction Rate and Doubling Rate of Maize Haploid under Different Ecological Conditions. J. Jilin Agricul. Univ. **36**(02): 139-143.

Jiang L, Ci J B, Nan N and Yang W G. 2014. Primary Report of Acupuncturing Growing Point Method with Colchicines in Three Leaf Stage to Maize Haploid. Seed. **33**(12): 27-29.

Liu J, Yin X H, Liu J, Liu X, Li H and Jing X Q. 2014. Doubling Effect of Maize Haploid Using Different Treatment Methods. J. Maize Sci. **22**(04): 8-12.

Li J M, Jiang L ,Yu H Y, Yu X M, Gao H Y, Xu H, Qin Z X and Chen X. 2020. Study on the Natural Doubling Effect of Haploid Maize Based on Different Ecological Climate. J. Jilin Agricul。Sci. Technol. Univ. **29**(01): 1-3.

Li X Y, Jiang L, Wang X Q ,Jing G X, Xing Z, Deng K P and Zhao R G. 2016. Haploid Inducing and Doubling of Waxy Maize. J. Northwest A&F Univ. (Nat. Sci. Ed.). **44**(08): 90-96.

Wang H, Li J Z, Zhang J W, Ci J B, Jiang L, Zhang Y and Yang W G. 2013. Study on Doubling Efficiency of Maize Haploids Bud Soaked by Herbicide and Colchicine. J. Jilin Agricul. Univ. **35**(04): 384-388.

Wang Z Y, Zheng S Y, Wu J X, Wang J R and Zhang G B. 2015. Study on Natural and Chemical Doubling Rate in Maize Haploid. J. Maize Sci. **23**(06): 18-20+26.

Xu L, Wang S X and Feng J Y. 2013. Study on Natural Doubling Effect of Maize Haploid in Different Ecological Environments. J. Hebei Agricul. Sci. **17**(3): 63-65.

Yu H Y, Jiang L, Mou Q, Li K Z and Chen D Y. 2018. Comparison of Haploid Doubling Method of Waxy Maize in Two Ecological Environments. Seed. **37**(05): 91-93.

(Manuscript received on 12 February, 2023; revised on 09 June, 2023)