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Short Communication

High Frequency of Haploid Production of Wheat through Intergeneric Cross with Teosinte

Tomohiko USHIYAMA[1], Takao SHIMIZU[1] and Tatsuo KUWABARA[2]

[1] *Nagano Agricultural Research Center,* Suzaka, Nagano, 382
[2] *Nagano Agricultural Experiment Station,* Suzaka, Nagano, 382

Thirty-nine genotypes of maize (*Zea mays* L.) and one genotype of teosinte (*Zea mays* ssp. *mexicana*) were investigated for the production of haploid plants in common wheat (*Triticum aestivum* L. var. Nishikazekomugi). The method to product them was followed: the detached wheat spikes pollinated by teosinte or maize genotypes were cultured in a solution of 100 mg/*l* 2,4-dichlorophenoxyacetic acid, 10 m*l*/*l* ethanol, 0.8 m*l*/*l* sulfurous acid and 40 g/*l* sucrose for 16 days, and subsequently excised embryos were cultured on B_5 medium. Wheat × teosinte cross was the highest in frequency of embryo obtained (38.5%) and in haploid frequency per pollinated florets (31.5%). It is suggested that teosinte can be used effectively in haploid breeding programs of wheat employing intergeneric crosses.
KEY WORDS: *Triticum aestivum, Zea mays* ssp. *mexicana, Zea mays,* haploid, intergeneric cross.

Introduction

An application of the "bulbosum method" for wheat haploid production is restricted due to the presence of non-crossability genes, *Kr1* and/or *Kr2*, in wheat genotypes (SNAPE *et al.* 1979). However, LAURIE and BENNETT (1988) observed that the wheat genotypes carrying *Kr* gene(s) could produce haploid plants through intergeneric crosses with maize (*Zea mays* L. ssp. *mays*). The frequency of haploid production was increased by treatment with 2,4-dichlorophenoxyacetic acid (2,4-D) (SUENAGA and NAKAJIMA 1989; INAGAKI and TAHIR 1990). On the other hand, USHIYAMA *et al.* (1990) reported that wheat florets crossed with maize pollen contained many seeds lacking embryos as compared with those of the "bulbosum method". Then, they indicated that to alleviate such shortcomings, it was necessary to select maize genotypes with a high crossability with wheat and investigate the concentration and combination of phytohormones for embryo development. In this report, we describe the results of selection of maize and teosinte (*Zea mays* L. ssp. *mexicana*) genotypes as pollen donors for wheat haploid production.

Materials and Methods

Thirty-nine maize genotypes (24 inbred lines and 15 F_1 hybrids) and one genotype of teosinte (2n = 20) were used for this study. All of them originated from Nagano Chushin Agricultural Experiment Station. Twenty plants of each genotype were grown in a greenhouse kept at a min. temperature of 20°C. At anthesis, pollen grains were collected from them and mixed well within each genotype. The wheat cultivar Nishikazekomugi was selected

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as the female plant, because it showed rather high crossability with maize genotypes without the application of phytohormones as campared with other wheat genotypes (USHIYAMA *et al.* 1990). Wheat plants eared in a greenhouse kept at a min. temperature of 15°C under natural day length conditions. After emasculation, wheat florets were crossed with freshly collected pollen grains from each of maize or teosinte genotypes. The method of intergeneric crossing followed the "bulbosum method" of USHIYAMA (1989), and then the detached wheat spikes were cultured in the solution of KATO *et al.* (1990), except that 100 mg/*l* 2.4-D, 10 m*l*/*l* ethanol, 0.8 m*l*/*l* sulfurous acid (about 6%) and 40 g/*l* sucrose were added. The embryo was excised from caryopsis which grew over two thirds of the glume length. Those obtained embryos were transferred onto agarose-solidified B_5 medium (GAMBORG *et al.* 1968), and incubated at 18~25°C under fluorescent light for 10 hours per day.

Results and Discussion

Table 1 shows the number of seeds obtained and plants regenerated. Caryopses pollinated with maize or teosinte were classified into two types: true seed with embryo, and "seed" in which no embryo was identified under a dissecting microscope (embryo-less seed).

Among the wheat × maize crosses, the frequencies of true seeds ranged from 2.2% to 29.9%, and those of embryo-less seeds ranged from 21.7% to 59.9%. In the case of wheat × teosinte cross, the corresponding values were 38.5% and 37.0%, respectively. It should be noted that no genotype of maize exceeded the frequency of true seed set in wheat × teosinte cross and 26 out of 39 maize genotypes showed a higher frequency of embryo-less seed set of wheat than that of teosinte.

Frequencies of plant regeneration per embryo cultured ranged from 0 to 85.7% in maize pollination and 81.8% in teosinte. Although the maize line "CM44" showed a higher frequency of plant regeneration of wheat than teosinte, this line produced the most frequently embryo-less seeds among the pollen parents, and the percentage of regenerated plants per pollinated floret was very low (2.5%). The frequency of regenerated plants per pollinated floret was the highest in wheat × teosinte cross (31.5%), which was about three times the highest value (11.6%) obtained in wheat × maize (F131). The above stated differences in seed setting and plant regeneration between teosinte and maize were statistically significant (Table 2).

It is concluded that teosinte displayed a much higher potentiality of haploid production with wheat than the maize genotypes although the environment for the pollen parents, teosinte and maize, was not favorable due to insufficient light and dense planting in the current study. Therefore, it is suggested that the use of teosinte may be suitable for wheat haploid breeding programs through intergeneric crosses.

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Table 1. Frequency of haploid production of wheat in crosses of a wheat genotype "Nishikazakomugi" with 39 maize genotypes and a teosinte genotype

Pollen source	No. of florets pollinated	No. of seeds set (%)		No. of plants regenerated	
		with embryo	without embryo	(%)[1)]	(%)[2)]
Teosinte genotype					
Teosinte(1651)[3)]	200	77(38.5)	74(37.0)	63(81.8)	(31.5)
Maize genotype					
F131	198	33(16.7)	87(43.9)	23(69.7)	(11.6)
Kiwase	170	24(14.1)	47(27.6)	15(62.5)	(8.8)
P3552	258	44(17.1)	101(39.1)	21(47.7)	(8.1)
CM109	266	43(16.2)	149(56.0)	20(46.5)	(7.5)
Pioneer P80 Lisa	378	78(20.6)	149(39.4)	28(35.9)	(7.4)
CM139	190	35(18.4)	82(43.2)	14(40.0)	(7.4)
SD25	166	25(15.1)	54(32.5)	12(48.0)	(7.2)
A509	410	70(17.1)	178(43.4)	29(41.4)	(7.1)
Falconer Semi-Dent	302	45(14.9)	108(35.8)	20(44.4)	(6.6)
ND240	140	17(12.1)	45(32.1)	9(52.9)	(6.4)
ND100	204	20 (9.8)	104(51.0)	13(65.0)	(6.4)
W117Ht	124	18(14.5)	66(53.2)	7(38.9)	(5.6)
CM7	196	32(16.3)	77(39.3)	11(34.4)	(5.6)
Black Mexican	250	48(19.2)	121(48.4)	14(29.2)	(5.6)
Mejiro Tokibi	288	57(19.8)	136(47.2)	16(28.1)	(5.6)
G4589	200	28(14.0)	84(42.0)	11(39.3)	(5.5)
Seneca Wampan	294	58(19.7)	141(48.0)	16(27.6)	(5.4)
V3	224	67(29.9)	90(40.2)	12(17.9)	(5.4)
Bai Baomi (202)[3)]	158	23(14.6)	56(35.4)	8(34.8)	(5.1)
Begentchoukskaya	194	45(23.2)	87(44.8)	9(20.0)	(4.6)
Extra Early Adams	304	31(10.2)	102(33.6)	14(45.2)	(4.6)
W182B-2 (946)[3)]	220	27(12.3)	101(45.9)	10(37.0)	(4.5)
Hongse Baomi (81)[3)]	178	28(15.7)	67(37.6)	8(28.6)	(4.5)
A556	282	36(12.8)	162(57.4)	12(33.3)	(4.3)
CM47	192	14 (7.3)	115(59.9)	8(57.1)	(4.2)
W182BN	472	35 (7.4)	158(33.5)	19(54.3)	(4.0)
CM174	274	35(12.8)	121(44.2)	11(31.4)	(4.0)
CM39	230	26(11.3)	81(35.2)	9(34.6)	(3.9)
SX239	210	31(14.8)	108(51.4)	8(25.8)	(3.8)
Oh43	316	26 (8.2)	150(47.5)	12(46.2)	(3.8)
Seneca Brone	252	30(11.9)	131(52.0)	9(30.0)	(3.6)
A34	235	14 (6.0)	85(36.2)	8(57.1)	(3.4)
W64AHt	254	22 (8.7)	58(22.8)	8(36.4)	(3.1)
CM44	236	7 (3.0)	138(58.5)	6(85.7)	(2.5)
Span Cross	186	18 (9.7)	51(27.4)	4(22.2)	(2.2)
PF157	184	15 (8.2)	91(49.5)	3(20.0)	(1.6)
RB259	358	8 (2.2)	141(39.4)	4(50.0)	(1.1)
A654	180	5 (2.8)	39(21.7)	2(40.0)	(1.1)
W182B-1 (728)[3)]	194	6 (3.1)	66(34.0)	0 (0.0)	(0.0)
Subtotal in maize	9367	1224(13.1)	3927(41.9)	463(37.8)	(4.9)

1) Frequency of plants regenerated from the embryos obtained.
2) Frequency of plants regenerated from the florets pollinated.
3) Identification code is referred to the number given to the maize germplasm smaples preserved at Nagano Chushin Agricultural Experiment Station.

Table 2. Contingency X^2 analysis for variation in the rate of seed setting and plant regeneration in wheat × teosinte and maize

Item	d. f.	X^2	P
a) Seed setting (no seed: true seed: embryoless seed)			
Teosinte vs maize	2	112.877	>0.001
Within maize genotypes	76	708.693	>0.001
b) Plant regeneration per embryos cultured (regenerated: not regenerated)			
Teosinte vs maize	1	58.204	>0.001
Within maize genotypes	38	93.000	>0.001
c) Plant regeneration per florets pollinated (regenerated: not regenerated)			
Teosinte vs maize	1	266.322	>0.001
Within maize genotypes	38	4063.006	>0.001

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属間交雑（コムギ×テオシント）によるコムギ半数体の高頻度作出

牛山智彦[1)]・清水孝夫[1)]・桑原達雄[2)]

([1)] 長野県農業総合試験場，須坂市，〒382

[2)] 長野県農事試験場，須坂市，〒382)

属間交雑を利用したコムギ半数体の作出頻度を高めるため，コムギとの交雑能力の高いトウモロコシを選抜することを目的として，コムギ（品種　ニシカゼコムギ）とトウモロコシ 39 品種・系統，テオシント 1 品種を交雑し，受粉直後に切除穂を 100 m*l*/*l* の 2，4-ジクロロフェノキシ酢酸，10 m*l*/*l* のエタノール，8 m*l*/*l* の亜硫酸水，40 g/*l* の蔗糖を含む水溶液で生育させた．結実した種子から胚を摘出し，無菌的に培養した結果，テオシント（*Zea mays* ssp. *mexicana*）は，胚形成率 38.5%，受粉小花当たり植物体再生率 31.5% と高頻度に半数体を誘導し，得られた胚の再生率が 81.8% と非常に高い値を示した．以上からコムギ半数体作出のための属間交雑の花粉親としてテオシントを用いることは極めて有用である．