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INTERACTION EFFECTS IN HEXAPLOID WHEAT INVOLVING *AEGILOPS COMOSA* CHROMOSOMES AND CYTOPLASM

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ABSTRACT

Interaction studies of nucleus and cytoplasm in alloplasmic hybrids involving one or two chromosomes of cytoplasm donor, *Aegilops comosa* (2n = 14), and genomes of seven varieties of common wheat, *Triticum aestivum* (2n = 42), were made. The plants normally exhibited delayed heading, reduced vigour, complete male sterility and partial or complete male fertility. On the contrary, however, some of the 43- and 44-chromosome plants showed considerable restoration of male fertility with normal or increased vigour and set normal seeds after selfing. Meiotic analysis showed two critical *comosa* chromosomes involved in interaction for fertility restoration which were identified to be median or submedian and satellite (nucleolus organizing) chromosomes. In addition, some undesirable side effects on the morphology of some plants were also observed which need rectification.

Key words: *Triticum aestivum, Aegilops comosa*, alloplasmic addition lines, nucleo-cytoplasmic interaction, fertility restoration.

Nucleo-cytoplasmic hybrids or alloplasmic plants involving interaction of different cytoplasms of *Aegilops* species and common wheat genomes are known to manifest tremendous morphological variations, the most common being delayed maturity, reduced vigour and fertility or complete male sterility [1–5]. Some of the alloplasmic plants have shown resistance to insect pests [6]. The male sterility resulted from such interaction provides feasibility of producing commercial hybrid seed [7, 8]. But a major limitation has been to find satisfactory fertility restorer R-lines. Several authors in an interaction study reported restoration of male fertility in the presence of some extra chromosomes of the cytoplasm donor species added to wheat genomes [1–3, 9, 10]. In our nucleo-cytoplasmic interaction study programme, *Aegilops comosa* has been used which is known to possess outstanding resistance to stripe rust of wheat [11] and high grain protein content [12]. The

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normal genomes of common wheat (*Triticum aestivum*) with *Aegilops comosa* cytoplasm show delayed maturity, reduced plant vigour, and complete male sterility [13]. Maan [14] observed bushy growth habit and normal vigour in alloplasmic wheat with *Ae. comosa* cytoplasm. However, interaction effects of *Ae. comosa* cytoplasm and genomes of addition lines of hexaploid *T. aestivum* wheats with *comosa* chromosomes are not well known. The present experiment was conducted to study the nature of interaction with regard to the morphological features and male sterility, and fertility restoration system in alloplasmic hybrids involving one or two chromosomes of cytoplasm donor *Ae. comosa* and genomes of breadwheat.

MATERIALS AND METHODS

Nucleo-cytoplasmic hybrids or alloplasmic plants having genomes of three exotic *Triticum aestivum* (2n=42, AABBDD) cultivars, viz. Chinese Spring, Selkirk and S 615, and cytoplasm of *Aegilops comosa* Sibth. et Sm Strain-2 (2n=14, MM) with its one or two chromosomes, were initially developed at the Laboratory of Genetics, Kyoto University, Japan, following the nucleus substitution technique of Kihara [1]. The first F_1 hybrids (2n=42, AABBDM) from the cross (*Ae. comosa* x *Triticum turgidum* var. *nigrobarbatum*, 2n=28 (AABB) x *T. aestivum* genomes, constituted by fertilization of unreduced female gametes (n=21, ABM) with normal male gametes (n=21, ABD) were backcrossed with *T. aestivum* genomes, the recurrent pollen parents. The resulting hybrids (2n=49, AABBDDM) were again backcrossed with the recurrent pollen parents. From the nucleus substitution backcross 2 (SB_2) progenies thus emerged, the alloplasmic plants with extra M-chromosomes (2n=42+1, 42+2) were screened at the seedling stage by checking the chromosome number in root-tip cells. These plants were maintained by a series of backcrosses with normal genome donors. The alloplasmic lines were designated as per system of Tsunewaki [14]. The recurrent genome donors were considered as standard controls. Observations were recorded on various morphological characteristics including self- and cross-seed fertility in SB_3 and SB_4 generations.

In another set of crosses, a large number of male sterile plants with 43 and 44 chromosomes in each line of Chinese Spring, Selkirk and S 615 were crossed twice with four improved Indian wheat (*T. aestivum*) varieties, viz. Kalyan Sona, WL 711, C 306 and HD 2160 as recurrent genome donors, presumably from diverse sources of origin, so as to substitute *Ae. comosa* cytoplasmic background. Data on relative fertility of the alloplasmic plants thus produced were recorded in SB_1 generation. These plants could not be examined for their chromosome number but were presumed to possess one or two alien M-chromosomes from the cytoplasm donor, since the alien chromosomes are preferentially transmitted through the female gametes [15]. Some of the alloplasmic plants exhibiting fertility restoration in advanced SB generations were also analysed for the types of extra chromosomes of *Ae. comosa* involved in interaction.

RESULTS AND DISCUSSION

Morphological features. The alloplasmic progenies in the present experiment could not be classified as per the types of *Ae. comosa* chromosomes involved in interaction because they still segregated for some of the characters. This may be due to incomplete restoration of genomes of the recurrent pollen parents as the number of backcrosses are still small. Consequently, the data were recorded on average performance of morphological characters of the normal and alloplasmic heterozygous plants of common wheat Chinese Spring, Selkirk and S 615, having *Ae. comosa* cytoplasm and one or two of its chromosomes in SB_3 (*comosa*-CS^4 and SB_4 [(*comosa*-SK^5; (*comosa*)-$S615^5$] generations (Table 1). The alloplasmic plants in general exhibited delayed heading by 8 to 20 days, reduction in height, reduction in top-internode length, and number of spikelets per spike. These results are in agreement with those reported in different nucleo-cytoplasmic hybrids with *T. aestivum* normal genomes and *Aegilops* species cytoplasms [10, 13, 16]. But conversely, a few plants in certain lines showed early heading, increased height and general vigour, particularly in

Table 1. Average performance of alloplasmic wheat plants in SB_3 and SB_4 generations

Line	2n	No. of plants	Days to heading	Height (cm)	No. of tillers	Top internode (cm)	Spike length (cm)	Spikelets per spike
(*Comosa*)-CS^4	43	21	114.9 (101–126)	78.6 (52–111)	11.6 (5–27)	23.3 (13–34)	9.1 (6.5–12.5)	37.2 (30–46)
	44	18	120.3 (111–124)	83.5 (57–105)	11.7 (5–26)	24.3 (10–34)	9.8 (8–12)	37.1 (30–44)
Normal CS	42	8	103.5 (101–104)	100.6 (95–109)	11.4 (6–18)	33.0 (27–38)	8.8 (7–10)	43.3 (38–50)
(*Comosa*)-SK^5	43	22	132.4 (123–148)	73.0 (51–94)	11.2 (5–24)	21.0 (11–27)	11.8 (8–14)	41.3 (26–50)
	44	12	132.4 (127–140)	72.3 (62–97)	9.6 (6–20)	19.3 (19–29)	10.3 (9–14.5)	36.0 (28–42)
Normal SK	42	8	116.4 (114–120)	104.7 (96–114)	15.5 (10–25)	30.6 (24–38)	14.5 (13–16)	52.3 (46–56)
(*Comosa*)-S 615^5	43	40	132.0 (120–145)	82.4 (56–104)	12.3 (5–52)	20.1 (13–30)	15.1 (10–22)	37.1 (30–48)
	44	17	130.1 (120–138)	81.1 (64–114)	14.8 (4–30)	22.0 (17–35)	13.7 (10–18.5)	14.1 (24–50)
Normal S 625	42	8	116.4 (113–122)	113.3 (102–127)	15.6 (7–30)	33.8 (25–38)	17.3 (15–19)	48.8 (46–50)

CS—Chinese Spring; SK—Selkirk; (*Comosa*)—*Aegilops comosa* cytoplasm.

Range given in parentheses in all tables.

44-chromosome plants, increased spike length and number of tillers per plant or in some cases apparently no change was observed in comparison to their respective normal controls. In addition, some plants, mostly in S 615 lines, manifested characters remarkably different from the normal ones. Their frequencies are: 1) necrosis and chlorosis (36.4%), the necrotic plants died while those with chlorosis were either very weak or recovered to normal state prior to heading; 2) darker leaves than the normal (10.6%); 3) waxiness of culm, leaves and glumes (7.6%); 4) grassy type (2.5%); 5) twisting of flag leaf (4.5%); 6) bifurcation of spikes (1.5%, Fig. 1: 1); 7) gappy spikes (6.0%, Fig. 1: 2, middle); 8) branched spikes (1.5%, Fig. 1: 2, right); and 9) red pigmentation on culms, rachis, glumes, etc., the only character which occurred in all the three substitution lines of Chinese Spring, Selkirk and S 615 with frequencies 5.0, 69.7 and 8.3%, respectively.

Occurrence of these characters and those represented in Table 1 is obviously attributable to the 43- and 44-chromosome genomes of *T. aestivum* interacting with the cytoplasm of *Ae.*

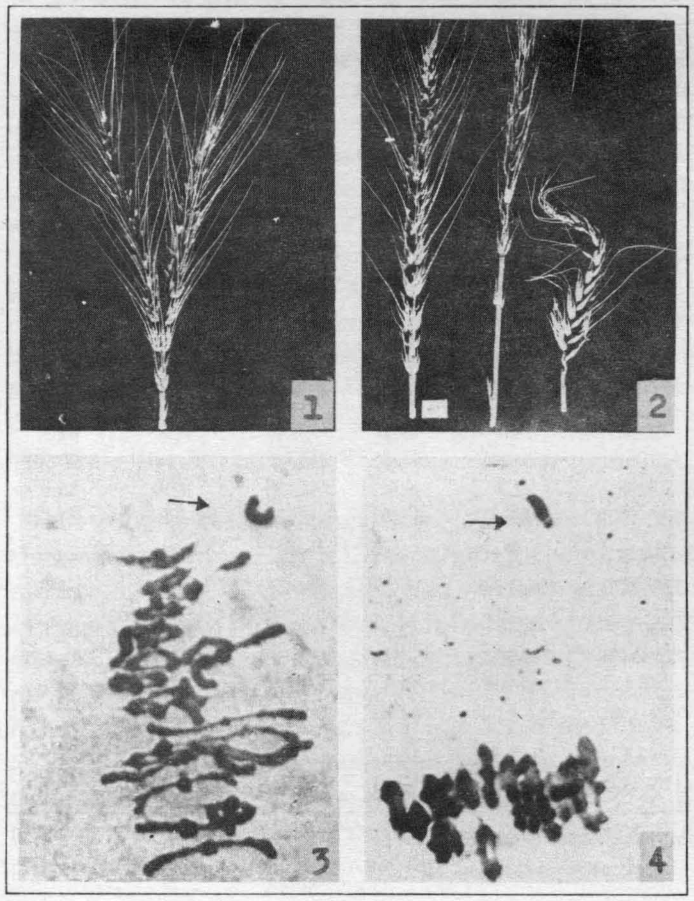

Fig. 1.
Spikes (1, 2) and PMCs at M I with 21 bivalents + 1 univalent (3, 4) of alloplasmic plants in S 615 line. 1) Bifurcated spike; 2) normal, gappy and branched spikes, respectively; 3) median/submedian, and 4) satellite chromosomes of *Ae. comosa* marked with arrows.

comosa depending on the types of *comosa* chromosomes involved. However, complementary genes in interaction [17] and genes contributing branching character from *T. turgidum* and grassy nature from *Ae. comosa* still remaining in alloplasmic hybrids due to small number of backcrosses to wheat [18] may also be the cause of some abnormalities observed.

Fertility. Table 2 contains data on selfed and crossed seed fertility. Most alloplasmic heterozygotes in all the addition lines showed complete or high degree of male sterility and partial to complete female fertility. Besides, five plants with 43 and two plants with 44 chromosomes in the progenies of Chinese Spring line, which in previous generations exhibited complete male sterility, were found to be highly fertile. They set normal seeds when selfed. This characteristic is, interestingly, in contrast to the alloplasmic hybrids with normal *T. aestivum* genomes (2n=42) and *Ae. comosa* cytoplasm which had shown complete male sterility [13]. Thus, it is apparent that the critical alien *comosa* chromosomes involved in interaction had the gene(s) for fertility restoration. Further, the alien chromosomes in completely male sterile plants may either be lacking the fertility genes or may express the character in later generations. Similar fertility restoration has also been observed [1–3, 9, 10] in alien addition alloplasmic wheats. In the present experiment, the 43-chromosome plants were more fertile and thus produced more selfed seeds than the 44-chromosome plants (Table 2). According to Fukasawa [9] the degree of fertility is dependent on interaction between the cytoplasm and combination of certain chromosomes and not always on the number of univalents. This may be the cause of higher degree of fertility restoration in 43-chromosome plants than those with 44 chromosomes.

Table 2. Average selfed and crossed seed fertility of alloplasmic wheat plants in SB_3 and SB_4 generations

Line	2n	Seed setting (%)	
		self	cross
(*Comosa*)-CS^4	43	25.2 (1.8–84.2)	35.3 (4.5–81.3)
	44	20.5 (10.8–30.2)	44.6 (9.3–60.7)
Normal CS	42	91.3 (82.5–96.0)	—
(*Comosa*)-SK^5	43	0.0	25.2 (0.0–54.2)
	44	0.0	36.2 (0.0–66.7)
Normal SK	42	73.0 (62.5–77.6)	—
(*Comosa*)-S 615^5	43	0.0	21.7 (0.0–63.6)
	44	0.0	29.1 (0.0–60.0)
Normal S 615	42	83.0 (72.7–88.0)	—

Some of the 43-chromosome plants in the Chinese Spring line with high male fertility and normal or increased vigour were meiotically analysed for the critical *comosa* chromosomes involved in interaction. They were identified to be median or submedian and satellite (nucleolus organizing) chromosomes, lying apart at M I stage (Fig. 1: 3, 4). Similarly, in a 44-chromosome plant one of the extra chromosomes was satellited. This indicated that at least two chromosomes of *Ae. comosa* possess fertility genes which in their own cytoplasmic background can restore male fertility to wheat satisfactorily. The existence of fertility gene(s) on the satellite chromosome 1B of *T. aestivum* has been reported [19].

Female fertility, as assessed on the basis of cross seed set on individual spike, varied enormously (Table 2). The spike bearing 1 to 3 seeds were considered partially fertile and those without a single seed completely sterile. It has been suggested that the alien chromosomes in their own cytoplasmic background were preferentially transmitted through the male and female gametes and are essential for normal fertilization [10, 15]. Thus, it can be explained that the partial fertility observed in the present experiment may be a consequence of inviability of the gametes or their failure to effect fertilization without alien chromosomes. However, multivalent formation also observed in the present (unpublished) materials which may affect fertility cannot be completely ruled out.

Fertility of alloplasmic Indian wheat varieties. Observations were made on alloplasmic hybrids of four indigenous improved varieties, viz. Kalyan Sona, WL 711, C 306 and HD 2160 involving *Ae. comosa* cytoplasmic background in SB_1 generation. Most of the plants showed complete male sterility and partial female fertility as judged from the percentage of crossed seed set on them. On the contrary, 6 plants in WL 711, 6 in C 306, and 5 in HD 2160 lines showed fertility restoration even in SB_1. The data pertaining to selfed and crossed seed fertility are represented in Table 3. It seems that these improved genotypes either possess fertility restoring genes against sterility caused by *comosa* cytoplasm or the alien chromosomes with fertility genes might have been transmitted preferentially through the female gametes from the cytoplasm donor plants utilized primarily in the cross with each variety. Thus, they have demonstrated an appreciable improvement in interaction for fertility restoration in spite of the small number of crosses. Morphologically, the alloplasmic plants in all the lines showed delayed maturity and reduction in vigour as compared to the Chinese Spring, Selkirk and S 615 lines.

Table 3. Seed fertility of alloplasmic plants involving Indian wheat varieties in SB_1 generation

Line	No. of plants	Seed setting (%)	
		self	cross
(*Comosa*)-Kalyan Sona	26	0.0	23.5 (3.4–50.0)
Normal Kalyan Sona	10	92.3 (89.1–95.5)	—
(*Comosa*)-WL 711	15	13.4 (8.0–18.8)	25.3 (4.2–52.9)
Normal WL 711	10	86.8 (83.3–88.9)	—
(*Comosa*)-C 306	32	11.7 (1.5–20.0)	33.8(6.7–69.2)
Normal C 306	10	89.6 (86.7–92.7)	—
(*Comosa*)-HD 2160	10	9.8 (4.8–13.9)	37.9 (21.4–73.1)
Normal HD 2160	10	88.6 (87.3–92.5)	—

These are preliminary observations, but it can be suggested that the nucleo-cytoplasmic interaction involved *T. aestivum* genomes and *Ae. comosa* cytoplasm with one or two *comosa* chromosomes may improve in later generations; and thus the possibility exists for isolation of satisfactory male sterile and related counterpart fertility restorer R-lines.

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