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CHROMOSOME DOUBLING OF WILD X CULTIVATED SUNFLOWER INTERSPECIFIC HYBRIDS AND ITS DIRECT EFFECT ON BACKCROSS SUCCESS

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SUMMARY

Embryo cultured interspecific hybrid seedlings of 19 wild diploids and tetraploids crossed with cultivar P21 were subjected to a colchicine treatment to evaluate its effectiveness on chromosome doubling and on backcross seed set. Colchicine treatment resulted in doubled heads on over 30% of treated plants and 10% of all heads examined. Chromosome doubling increased the proportion of large pollen grains and pollen fertility. In diploid hybrids, chromosome doubling increased pollen fertility from 4% to 51% and large pollen grains from 3% to 96%. A similar trend was observed for the triploid hybrids. Seven non-treated tetraploid hybrids had 28% fertile pollen and 98% of all pollen grains were small. Chromosome doubling of diploid hybrids increased sib-pollinated seed set from 0 to 3 seeds/head, and backcross seed set using the F_1 as female and male with P21 was increased from 0.9 and 8.4 to 6.4 and 80.4 seeds/head, respectively. Similarly, doubled triploid hybrids improved sib-pollinated seed set from 0 to 3.5 seeds/head and respective reciprocal backcross seed set from 0.2 and 3.7 to 1.5 and 5.6 seeds/head. Non-treated tetraploid hybrids backcrossed as female and male with P21 set 5.3 and 2.3 seeds/head, respectively. The colchicine chromosome doubling method proved to be a simple and effective method of improving the fertility of interspecific hybrids involving wild diploid or tetraploid species and can be routinely used to improve the efficiency of interspecific gene transfer.

INTRODUCTION

Wild sunflower (Helianthus) species have provided excellent germplasm for improvement of cultivated sunflower (H. annuus L.) (Fick, 1978; Thompson et al. 1981). Major improvements, including Verticillium wilt resistance, downy mildew resistance, rust resistance, recessive branching, and cytoplasmic male-sterility, can be traced to wild germplasm. However, utilization of many species has been limited by interspecific incompatibility and F_1 sterility. Embryo culture (Chandler and Beard, 1983) helped to secure many difficult interspecific hybrids. The low F_1 fertility of some cross combinations still impedes further improvement.

Dewey (1980) emphasized the utility of induced polyploidy as a bridge for interspecific gene transfer. Jan and Chandler (1988) successfully doubled chromosomes of P21 x H. bolanderi Gray F_1 hybrids, and increased seed set on doubled heads. Chromosome doubling, using colchicine applied to apical meristems of young seedlings, has also been demonstrated in cultivated sunflowers, (Dhesi and Saini, 1973; Gupta and Roy, 1979; Jan et al, 1988) and in diploid perennial species and their interspecific hybrids (Heiser and Smith, 1964). This paper examined the success of a modified colchicine chromosome doubling technique on 19 embryo-cultured wild x cultivated interspecific hybrids, and the effect of induced doubling on backcross seed set.

MATERIALS AND METHODS

F_1 seedlings of 19 embryo-cultured interspecific hybrids between wild diploid or tetraploid Helianthus species and P21, were treated with colchicine. Seven

untreated F_1 hybrids of hexaploid species x P21 were also examined. These seven hybrids had relatively high fertility and thus colchicine treatment did not seem necessary. Five-day-old immature hybrid embryos were cultured on artificial medium following the basic procedures outlined by Chandler and Beard (1983). For the colchicine treatment, each peat pot, wrapped in aluminum foil with extruding seedlings, was inverted and the apical meristems of the seedlings submerged into a 0.15% colchicine solution with 0.2% DMSO (dimethyl sulfoxide) for 5 hr in the dark. Seedlings were then washed and transplanted into pots. Chromosome doubling of each head was verified by pollen grain size and stainability (Alexander, 1969). A head with over 90% small (30μ) pollen grains was considered non-doubled (ND). A partially doubled (PD) head had 10 to 60% large (40μ) pollen grains and a doubled (D) head had more than 60% large pollen. Interspecific hybrids were sib-pollinated within each cross and were reciprocally crossed with P21 to produce backcross seeds.

RESULTS

Table 1 shows the degree of chromosome doubling as measured by pollen grain size and stainability of all interspecific hybrids and P21. For the wild diploid x P21 hybrids (2x), chromosome doubling substantially increased the number of large pollen grains. A similar trend was found for the wild tetraploid x P21 hybrids (3x). Wild hexaploid x P21 hybrids (4x) had 28% stainable pollen grains. A few naturally doubled heads in this group increased the number of large pollen grains but did not improve overall pollen stainability. Colchicine treatment induced useful levels of chromosome doubling for most of the 2x and 3x hybrids (Table 2). On the average, complete chromosome doubling was induced on 38.4% of all treated plants, or 11.5% of all heads examined. Chromosome doubling of two non-treated *H. strumosus* x P21 hybrids (4x) was not expected. Those plants may represent a rare natural occurrence or have been mistakenly treated with colchicine. Chromosome doubling increased BC_1F_1 seed set considerably for the wild diploid x P21 hybrids (Table 3). There was a 7-fold increase when F_1 's were used as maternal parents and a 10-fold increase when F_1 's were used as pollinator. Sib-pollinated ND heads produced very few seed; whereas, some of the D heads had reasonably good seed set. Chromosome doubling of tetraploid x P21 hybrids also improved backcross and sib-pollinated seed set. The hexaploid x P21 hybrids had good backcross seed set and chromosome doubling was not necessary.

DISCUSSION

Chromosome doubling did not cause noticeable morphological changes. Doubled sectors had larger and taller disk florets than non-doubled sectors of partially doubled heads. This difference was not apparent when comparing completely doubled heads with non-doubled heads. Chromosome doubling increased pollen stainability, however, the proportion of large pollen grains varied greatly among hybrids and may have been influenced by the surrounding environment. The increased pollen grain size directly reflected chromosome doubling and provided a reliable criterion for classifying treated plants.

Our previous colchicine treatment on P21 x *H. bolanderi* hybrids (Jan and Chandler, 1988) and on P21 (Jan et al. 1988) used germinated seedlings at the 2-true-leaf stage. Since the treatments of most embryo cultured seedlings were much later than the 2-true-leaf stage, a lower chromosome doubling frequency was expected. However, in treated wild diploid x P21 hybrids 44.5% of all plants had 1 or more doubled heads, accounting for 15.2% of all heads examined. The colchicine treated P21 x *H. bolanderi* hybrids resulted in doubled heads on 32% of the plants and 21% of the heads. The high frequency of chromosome doubling

using embryo-cultured older seedlings indicated considerable flexibility in time of colchicine treatment on highly branched interspecific hybrids. There was a steady increase of backcrossed seed set as chromosome doubling progressed on wild diploid x P21 and tetraploid x P21 hybrids. This increased seed set is extremely important for interspecific hybrids that normally produce no backcrossed seed, including crosses involving H. mollis, H. nutallii, H. maximiliani, H. arizonensis, and H. salicifolius. Sib-pollination of doubled heads was believed to produce new amphiploids similar to the amphiploid of P21 x H. bolanderi produced by Jan and Chandler (1988). Those amphiploids could serve as germplasm sources to breeders interested in those wild species. Whether these amphiploids could serve as genetic bridges for improved gene flow between wild species and cultivated lines requires further evaluation.

Non-doubled hexaploid x P21 hybrids had reasonable backcross seed set. Further chromosome doubling of this group would not serve any practical purpose for interspecific genetic transfer. Sib-pollination of the two doubled H. strumosus x P21 heads did not produce seeds. Since there is not a naturally existing octoploid Helianthus species identified, it is possible that hexaploidy is the highest optimum ploidy level for this genus. Further increase to octaploidy would likely result in less adapted plants. Sib-pollination of doubled tetraploid x P21 heads produced hexaploid amphiploids. Our data (unpublished) on H. hirsutus x P21 amphiploids indicated good pollen stainability and sib-pollinated seed set. Careful selection of tentative parental species for interspecific hybridization and amphiploid production would enhance the sunflower scientists' understanding of sunflower evolution and contribute to the future improvement of this crop.

CONCLUSION

A simple and efficient treatment for inducing chromosome doubling on embryo-cultured interspecific hybrids was described. Chromosome doubling of interspecific hybrids between distantly related wild diploid or tetraploid species and cultivated sunflower increased pollen grain size, pollen stainability and seed set. This method is extremely helpful for those interspecific hybrids which do not normally produce backcross seeds. To prevent backcross failure on valuable interspecific hybrids, this chromosome doubling procedure should be routinely considered.

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Table 1. Percent pollen grain size and fertility of non-doubled (ND), partially-doubled (PD) and doubled (D) heads of interspecific hybrids and P21.

F ₁ Pedigree	ND Heads				PD Heads				D Heads				
	Pollen Grain				Pollen Grain				Pollen Grain				
	Large		Small		Large		Small		Large		Small		
	S	† F	S	F	S	F	S	F	S	F	S	F	
----- % -----													
<u>(2x) (2x) = 2x, D = 4x</u>													
H. debilis x P21	-	-	-	-	-	-	-	-	-	-	-	-	
H. mollis x P21	-	-	-	-	-	-	-	-	-	-	-	-	
H. mollis x P21	2	0	97	1	14	1	85	0	26	73	1	0	
H. grosseserratus x P21	5	0	95	0	-	-	-	-	54	44	2	0	
H. nuttallii nut x P21	0	0	99	1	5	21	73	1	18	69	13	0	
H. maximiliani x P21	0	0	73	27	-	-	-	-	36	64	0	0	
H. maximiliani x P21	2	0	96	2	-	-	-	-	-	-	-	-	
H. gracilentus x P21	0	0	99	1	30	30	40	0	45	44	11	0	
H. pumilus x P21	1	1	97	1	14	20	65	1	45	52	3	0	
H. cusickii x P21	2	1	96	1	20	25	52	3	49	44	6	1	
H. arizonensis x P21	1	1	96	2	14	22	64	0	24	74	2	0	
H. occidentalis plan. x P21	2	0	95	3	11	1	87	1	76	24	0	0	
H. occidentalis occ. x P21	4	0	96	0	14	4	82	0	67	27	6	0	
H. divaricatus x P21	2	2	90	6	10	15	73	2	60	37	0	3	
H. salicifolius x P21	6	2	92	0	18	2	80	0	-	-	-	-	
H. floridanus x P21	2	1	97	0	11	15	73	1	-	-	-	-	
	\bar{X}	2.1	0.6	94.1	3.2	14.6	14.2	70.4	0.8	45.4	50.2	4.0	0.4
<u>(4x) (2x) = 3x, D = 6x</u>													
H. hirsutus x P21	4	4	86	6	11	15	72	2	26	60	13	1	
H. hirsutus x P21	3	2	91	4	14	14	71	1	50	37	13	0	
H. strumosus x P21	1	1	93	5	-	-	-	-	-	-	-	-	
	\bar{X}	2.7	2.3	90.0	5.0	12.5	14.5	71.5	1.5	38.0	48.5	13.0	0.5
<u>(6x) (2x) = 4x, D = 8x</u>													
H. strumosus x P21	1	1	56	42	-	-	-	-	-	-	-	-	
H. californicus x P21	0	1	62	37	-	-	-	-	-	-	-	-	
H. tuberosus x P21	2	1	66	31	6	6	78	10	-	-	-	-	
H. tuberosus x P21	0	0	67	33	-	-	-	-	-	-	-	-	
H. rigidus rig x P21	2	3	90	5	6	14	74	6	-	-	-	-	
H. rigidus rig x P21	0	0	80	20	-	-	-	-	-	-	-	-	
H. resinosus x P21	3	1	77	19	20	3	71	6	92	1	0	7	
	\bar{X}	1.1	1.0	71.1	26.7	10.7	7.7	74.3	7.3	92.0	1.0	0	7.0
P21, 2x	0	0	0	100	-	-	-	-	50	39	11	0	

† S = sterile or non-stainable.

F = fertile or stainable.

Table 2. The effectiveness of colchicine treatments on sunflower interspecific hybrids for chromosome doubling.

Pedigree	Total Plants (heads)	ND Plants (heads)	PD Plants (heads)	D Plants (heads)	Chromosome Doubling Frequency		
					ND	PD	D
					Plants (heads)		
					no.	%	
<u>(2x) (2x) = 2x</u>							
H. debilis x P21	2(15) †	2(6)	1(2)	2(7)	100.0(40.0) ‡	50.0(13.3)	100.0(46.7)
H. mollis x P21	2(13)	2(5)	2(7)	1(1)	100.0(38.5)	100.0(53.8)	50.0(7.7)
H. mollis x P21	3(13)	2(9)	1(4)	0(0)	66.7(69.2)	33.3(30.8)	0(0)
H. grosseserratus x P21	10(24)	8(19)	2(3)	2(2)	80.0(79.2)	20.0(12.5)	20.0(8.3)
H. nuttallii nut. x P21	3(31)	3(21)	3(7)	2(3)	100.0(67.7)	100.0(22.6)	66.7(9.7)
H. maximiliani x P21	6(27)	6(22)	2(4)	1(1)	100.0(81.5)	33.3(14.8)	16.7(3.7)
H. maximiliani x P21	7(44)	6(27)	4(6)	3(11)	85.7(61.4)	57.1(13.6)	42.9(25.0)
H. gracilentus x P21	8(58)	5(15)	6(19)	7(24)	62.5(25.9)	75.0(32.8)	87.5(41.4)
H. pumilus x P21	7(63)	7(42)	3(4)	5(17)	100.0(66.7)	42.9(6.3)	71.4(30.0)
H. cusickii x P21	12(42)	8(16)	3(3)	10(23)	66.7(38.1)	25.0(7.1)	83.3(54.8)
H. arizonensis x P21	9(99)	9(58)	3(6)	4(35)	100.0(58.6)	33.3(6.1)	44.4(35.4)
H. occidentalis plan. x P21	8(28)	8(23)	1(5)	0(0)	100.0(82.1)	12.5(17.9)	0(0)
H. occidentalis occ. x P21	1(7)	1(4)	1(1)	1(2)	100.0(57.1)	100.0(14.3)	100.0(28.6)
H. divaricatus x P21	17(92)	12(54)	10(32)	5(6)	70.6(58.7)	58.8(34.8)	29.4(6.5)
H. salicifolius x P21	1(14)	1(13)	1(1)	0(0)	100.0(92.9)	100.0(7.1)	0(0)
H. floridanus x P21	2(8)	2(8)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
	\bar{x}				89.5(63.6)	52.6(18.0)	44.5(15.2)
<u>(4x) (2x) = 3x</u>							
H. hirsutus x P21	18(54)	11(24)	10(26)	4(4)	61.1(44.4)	55.6(48.1)	22.2(7.4)
H. hirsutus x P21	25(242)	19(189)	10(38)	8(15)	76.0(78.1)	40.0(15.7)	32.0(6.2)
H. strumosus x P21	35(222)	33(162)	16(38)	15(22)	94.3(73.0)	45.7(17.1)	42.8(9.9)
	\bar{x}				71.6(65.2)	47.1(27.0)	32.3(7.6)
<u>(6x) (2x) = 4x</u>							
H. strumosus x P21	7(26)	8(24)	0(0)	2(2)	100.0(92.3)	0(0)	28.6(7.7)
H. californicus x P21	1(5)	1(5)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
H. tuberosus x P21	9(65)	9(65)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
H. tuberosus x P21	3(29)	3(29)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
H. rigidusrig. x P21	7(43)	6(36)	4(7)	0(0)	85.7(83.7)	57.1(16.3)	0(0)
H. rigidus rig x P21	9(44)	9(44)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
H. resinosus x P21	8(48)	8(48)	0(0)	0(0)	100.0(100.0)	0(0)	0(0)
	\bar{x}				97.9(96.6)	8.2(2.3)	4.1(1.1)

†Total plants investigated (number of heads analyzed).

‡Percentage of plants showing at least 1 head of class (percent of total heads in class)

Table 3. Effect of chromosome doubling on seed set of sunflower interspecific hybrids when crossed as seed (♀) and pollen (♂) parent with P21 and sib-pollinated.

F ₁ Pedigree	Backcross Seed Set, Seeds/Head						Sib-pollinated Seed Set	
	NO		PD		D		NO Sib	D Sib
	♀	♂	♀	♂	♀	♂		
<u>(2x) (2x) = 2x</u>								
<i>H. debilis</i> x P21	2.3	-	-	-	3.8	14.0	0	0
<i>H. mollis</i> x P21	0	-	1.0	-	9.0	60.0	0	0
<i>H. mollis</i> x P21	2.3	5.3	0	-	-	84.0	-	-
<i>H. grosseserratus</i> x P21	1.0	-	1.5	2.0	1.0	33.2	-	5.0
<i>H. nuttallianus</i> x P21	0.2	-	1.3	-	0	-	0	0.5
<i>H. maximiliani</i> x P21	1.0	4.9	0.7	-	8.0	30.0	0	-
<i>H. maximiliani</i> x P21	0.3	1.0	12.6	-	1.0	-	0.1	0.4
<i>H. gracilentus</i> x P21	0.9	0	7.1	-	7.1	8.0	0	5.9
<i>H. pumilus</i> x P21	0.8	46.0	6.0	-	24.1	185.0	0	12.4
<i>H. cusickii</i> x P21	0.3	3.3	0.3	-	1.8	300.0	0	0.2
<i>H. arizonensis</i> x P21	0.1	3.0	2.8	-	2.9	72.0	0	0
<i>H. occidentalis plan</i> x P21	0.8	2.5	0.5	-	-	-	0	-
<i>H. occidentalis occ</i> x P21	0	-	0	-	0	-	-	-
<i>H. divaricatus</i> x P21	0.9	3.0	0.3	-	17.5	18.0	0	-
<i>H. salicifolius</i> x P21	0.2	-	1.0	-	-	-	-	-
<i>H. floridanus</i> x P21	3.6	15.0	0	42.0	-	-	0	-
\bar{X}	0.9	8.4	2.3	22.0	6.4	80.4	0.008	3.0
<u>(4x) (2x) = 3x</u>								
<i>H. hirsutus</i> x P21	0.6	3.0	1.0	-	3.5	4.5	0.1	1.0
<i>H. hirsutus</i> x P21	0	-	0.2	-	0	-	0	7.0
<i>H. strumosus</i> x P21	0.1	8.0	0.3	-	1.1	12.4	0	2.5
\bar{X}	0.2	3.7	0.5	-	1.5	5.6	0.03	3.5
<u>(6x) (2x) = 4x</u>								
<i>H. strumosus</i> x P21	8.7	2.5	-	-	-	1.0	0.1	0
<i>H. californicus</i> x P21	3.4	4.5	-	-	-	-	0	-
<i>H. tuberosus</i> x P21	1.7	0	-	-	-	-	0.3	-
<i>H. tuberosus</i> x P21	4.8	3.0	-	-	-	-	0.8	-
<i>H. rigidus rig</i> x P21	0.8	0.5	5.3	14.0	-	-	0.2	-
<i>H. rigidus rig</i> x P21	6.8	2.3	-	-	-	-	0.7	-
<i>H. resinasus</i> x P21	10.9	3.3	-	1.0	-	-	2.3	-
\bar{X}	5.3	2.3	5.3	7.5	-	1.0	0.6	0