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**Transformation of the Wild Tetraploid Oats into
Domesticated Forms**

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Transformation of the wild tetraploid oats into domesticated forms

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Abstract

The exploitation of crops wild relatives in breeding is based on extracting specific characteristics of economic value from them and incorporating them into cultivated varieties. In this research an alternative approach was attempted. The tetraploid ($2n=28$) wild oats, Avena magna and A. murphyi, were domesticated by transferring to them, via hybridization, the domesticated syndrome of the common oat, A. sativa ($2n=42$). Hybrid seeds were obtained only when the latter served as the female parent. The pentaploid hybrids were totally self-sterile but produced a few seeds when pollinated with pollen from the tetraploid parents. The BC plants exhibited variation in chromosome number, spikelet morphology and fertility. A few partially fertile plants with the domesticated syndrome have been selected. Morphologically they were distinguished by spikelet non-shedding, yellow and glabrous lemmas and reduced awn formation. In the F_2 generation, tetraploid, fertile derivatives containing the domesticated syndrome were selected. They were crossed with their parental wild species and the F_1 hybrids were fully fertile. In the F_2 families involving A. magna, each of the four spikelet characteristics segregated as a single gene. In one F_2 family all four characteristics formed one linkage group, and two linkage groups in the other two families. The domesticated tetraploids were again crossed with the common oat and a few more domesticated tetraploids were selected. Domesticated A. magna was crossed with the diploid A. strigosa, var. Saia, and following chromosome doubling, a stable fertile synthetic hexaploid was selected.

Objectives of the original research proposal

The main objective of the research was to develop new source of germ plasm in oats by transforming the wild tetraploid species A. magna and A. murphyi into cultivated forms through the introgression of agronomically important traits from selected A. sativa cultivars.

Introduction

Plant domestication is the process of selecting characteristics which have been favored under cultivation but are usually of low selective value, or even lethal in the wild. In grain crops, selection of types which have lost their seed dispersal mechanism marks the beginning of domestication and expansion of the crop. Selection of similar types is also a prerequisite in modern plant domestication, as in the case of lupin (Schwanitz 1966, Gladstones 1967). In both prehistoric and modern domestication the raw material has been rare mutations occurring in wild populations. These rare mutants were selected for the most part unintentionally by the Neolithic farmers, but are prime targets for modern plant domesticators. In this research we attempted a novel approach for domesticating wild plants. Instead of searching for desirable domestic characters in the wild populations we introduced them from the cultivated species.

The common oat, Avena sativa, is an allohexaploid ($2n=6X=42$) originating from the A. sterilis-A. fatua complex of wild and weedy forms ($2n=42$), which are interfertile with the common oat. The

diploid and tetraploid ancestors of the hexaploid oats have not yet been identified (Ladizinsky 1994).

Although the recently discovered tetraploid ($2n=28$) oats A. magna and A. murphyi are akin to A. sterilis, neither of them can be regarded as the tetraploid progenitor of the hexaploid oats. In the last few years A. magna was regarded as a synonym for an older taxon A. maroccana (Baum 1977), but recent evidence has shown that the two are separate biological entities (Ladizinsky 1993). Both A. magna and A. murphyi show resistance to rust and powdery mildew and are exceptionally rich in protein. This genetic diversity could be exploited by introgressing it into the common oat, or by reverse introgression of the domesticated syndrome of the common oat into these wild tetraploids (Ladizinsky and Fainstein 1977). Characteristics of the common oat such as spikelet non-shedding, yellow and glabrous lemmas and lack of awns, are each controlled by a single gene (Marshall and Shaner 1992) and the transmission of which into the tetraploid species can apparently be achieved.

Among other factors, massive introgression of that kind is conditioned by the degree of homology, or homoeology, between the chromosomes of the species involved. The proportions of the tetraploid genomes which can be affected by introgression from A. sativa is indicated by the number of chiasmata in the pentaploid hybrids as compared with their numbers in the tetraploid species. Accordingly, about 75% and 62% of A. magna and A. murphyi genomes, respectively, can be modified by introgression from the common oat (Ladizinsky 1992). However, the potential of a specific A. sativa

characteristic to be introgressed can be determined only following hybridization experiments.

Materials and methods

In selecting common oat cultivars for the hybridization experiments an attempt was made to include lines and cultivars with unique diversity. Ogle, 86-4189, 86-4407, 86-5698 and 86-6404 have been selected for their tolerance to barley yellow dwarf virus (BYDV); Dolphin, besides being BYDV tolerant is a dwarf variety, and Tibor and Pennuda are naked oat varieties. Two A. magna accessions M1 and M3-1 were employed in the initial crosses and M21, M26 and M29 were used for backcrosses. Accessions 4471 and 4096 of A. murphyi were employed in the initial crosses and for backcross they have been supplemented by accessions 40-1 and 43-2 for the backcrosses.

Crosses and backcrosses

In all crosses the common oat served as the female parent. Reciprocal crosses have never yielded hybrid seeds. Hybrid seeds were significantly smaller than those produced by selfing and any contamination could be eliminated by size during seed harvest. Backcrosses were accomplished by planting the F_1 hybrids among plants of the tetraploid pollen donor parent allowing natural cross-pollination to occur.

Chromosome study

Chromosome number of backcross plants and F_2 derivatives was examined in root tips following a 24 h treatment of cold water,

fixation in 3:1 absolute ethanol-glacial acetic acid, and staining with Feulgen reagent. Meiotic chromosomes were examined in pollen mother cells following fixation in 3:1 absolute ethanol-glacial acetic acid and acetocarmine staining.

Pollen and seed fertility

Pollen fertility was examined in mature anthers following acetocarmine staining. Regularly shaped and darkly stained grains were considered normal. Seed fertility was determined as no. of seeds per spikelet. It was very low on the F_1 plants and was estimated in the segregating generations.

Statistical analysis: Linkage -1 program (Suiter et al. 1983) was used to test the fit to 3:1 segregation and two-point linkage.

Results

Production of domesticated *A. magna*

First hybridization cycle: The *A. sativa* X *A. magna* pentaploid hybrids were raised in 1989 and were totally self-sterile when kept in the greenhouse. Pollen fertility was less than 0.1% and the anthers did not dehisce. When grown in the field among the *A. magna* accessions they were heavily infected by BYDV, though less so than the tetraploid plants. Consequently, some of the *A. magna* plants died as early as the vegetative stages and only a few flowering tillers emerged and dispersed their pollen. Spikelet morphology of the pentaploid hybrids was intermediate to their parents. Spikelets remained attached to the pedicels at maturity, like in the common oat. Lemma color was brownish gray and partially pubescent, and awns were formed only on the lower floret of the spikelet. Although

the pentaploid hybrids were highly sterile they did produce a few seeds. At maturity, a seed-bearing spikelet could be distinguished from a sterile one by its greener glumes and darker lemma color. Altogether 33 backcross seeds were collected from the pentaploid hybrids.

The BC1 plants: These plants were grown in autumn 1989 in the greenhouse and exhibited variation in chromosome number, morphology and fertility. The tetraploid chromosome number ($2n=28$) was observed in three plants, but $2n=29$ to $2n=33$ were most common numbers. A few plants had the pentaploid or hexaploid chromosome numbers. Spikelet morphology varied considerably among the BC plants. About half of the plants dropped their spikelets at maturity as the wild parent does. This was expected because spikelet shedding is governed by a single gene in the hexaploid oat and spikelet shedding among the BC plants was similar to that of a test cross. The BC plants also varied with regards to lemma color, lemma pubescence and the degree of awn development. Nearly all the BC plants were sterile. Although pollen stainability varied considerably (10%-89%), only three plants set some seeds. A plant designated Aa2 ($2n=32$) had the highest pollen stainability and produced 47 seeds. The other two plants, also aneuploids produced three to four seeds.

F₂ BC: Progeny of the Aa2 plant were grown in a nethouse during winter-spring 1990. Thirty five seeds germinated and 33 plants reached maturity. The chromosome number was checked in 16 plants, of which 11 were tetraploids. At maturity, 25 plants retained their

spikelets as A. sativa, and eight dropped them like the wild parent. Segregation of spikelet shedding fit a single gene 3:1 ratio, $\chi^2=0.01$, $p=0.9$, indicating the Aa2 BC plant to be heterozygous.

Four F₂ plants were morphologically almost indistinguishable from the common oat and possessed the following four main characteristics of the domesticated syndrome: Spikelet non-shedding, glabrous lemma, yellow lemma and a weakly developed awn on the lower floret or no awns at all. By progeny testing two plants appeared to be homozygous and two heterozygous for spikelet shedding.

Crosses between wild and domesticated A. magna

To study the genetic nature of the domesticated A. magna and to obtain a deeper insight into the introgression process, the domesticated and wild forms were crossed with one another. Three domesticated and three wild lines were involved in these crosses. Hybrid seeds were easily obtained in both cross directions. The F₁ hybrids developed normally and their spikelet morphology was intermediate to that of the parents. Chromosome pairing at meiosis was normal and 14 bivalents were regularly formed. Pollen stainability and seed set were normal.

Spikelet shedding, lemma color, lemma pubescence and awn formation segregated in all three F₂ families. The various characteristics were grouped as follows: lemma color was classified as yellow or as various degrees of coloration, lemma pubescence as

Table 1: Segregation of four spikelet characteristics in F² families of hybrids between wild and domesticated *A. magna* (χ^2 , 3:1)

Family	Spikelet shedding	Lemma color	Lemma pubescen.	Awn/spikelet
	shed. nonshed	yellow dark	glabrous pubes.	2 1
M21 x Aa2-3	37 93	36 94	29 101	37 93
	$\chi^2=0.83$, $p=0.36$	$\chi^2=0.50$, $p=0.46$	$\chi^2=0.50$, $p=0.46$	$\chi^2=0.83$, $p=0.36$
M29 x Aa2-13	41 97	17 121	17 121	41 97
	$\chi^2=1.63$, $p=0.20$	$\chi^2=11.83$, $p=0.00$	$\chi^2=11.83$, $p=0.00$	$\chi^2=1.63$, $p=0.20$
Aa2-16 x M26	46 119	33 132	32 133	44 121
	$\chi^2=0.72$, $p=0.39$	$\chi^2=2.20$, $p=0.13$	$\chi^2=2.76$, $p=0.09$	$\chi^2=0.24$, $p=0.62$

Table 2: Linkage of spikelet characteristics in *A. magna* wild x domesticated F₂ families

Characteristic & families	χ^2	df	P	r	SE
Spikelet shed.-lemma color					
M21 X Aa2-3	12.38	1	0.00	0.216	0.083
M29 x Aa2-13	2.79	1	0.09	0.428	0.068
Aa2-16 x M26	12.66	1	0.003	0.166	0.075
Spikelet shed.-lemma pubesc.					
M21 x Aa2-3	0.02	1	0.98	0.494	0.060
M29 x Aa2-13	2.79	1	0.09	0.428	0.067
Aa2-16 x M26	12.09	1	0.000	0.169	0.075
Spikelet shed.-awn formation					
M21 x Aa2-3	12.38	1	0.000	0.007	0.087
M29 x Aa2-13	13.80	1	0.000	0.000	
Aa2-16 x M26	13.68	1	0.000	0.035	0.007
Lemma color-awn formation					
M21 x Aa2-3	11.01	1	0.010	0.220	0.083
M29 x Aa2-13	3.48	1	0.062	0.421	0.069
Aa2-16 x M26	11.78	1	0.000	0.172	0.075
Lemma color-lemma pubesc.					
M21 x Aa2-3	0.03	1	0.846	0.490	0.060
M29 x Aa2-13	13.80	1	0.000	0.000	
Aa2-16 x M26	5.12	1	0.023	0.172	0.074
Lemma pubescens-awn format.					
M21 x Aa2-3	0.008	1	0.929	0.498	0.060
M29 x Aa2-13	3.48	1	0.062	0.421	0.069
Aa16 x M26	11.25	1	0.000	0.176	0.074

glabrous or as various degrees of hairiness, and awn formation as two awns per spikelet, absence of awns or a single awn per spikelet. Each of the four characteristics segregated as a single gene in M21 x Aa2-3 and Aa2-16 X M26 families. Spikelet shedding and number of awns per spikelet segregated as single genes also in M29 X Aa2-13 family, but lemma color and lemma pubescence deviated significantly from the expected 3:1 ratio (Table 1). Gene symbols in the hexaploid oats also can be adopted for the domesticated A. magna, giving the designation of Ba, Lc, Lp and A for spikelet shedding, lemma color lemma pubescence and awn formation, respectively. The four characteristics were linked to one another in the Aa2-16 X M26 family (Table 2). In the M21 X Aa2-3 family lemma pubescence was removed from this linkage group and in the M29 X Aa2-13 family, spikelet shedding and awn formation formed one group and lemma pubescence and lemma color another group. The different linkage groups in the three families was unexpected because the three domesticated lines derived from a single BC plant with normal meiosis, and chromosome pairing was also normal in the three F₁ hybrids. This discrepancy could be explained by assuming that the parental lines of the F₂ families differed by intercalary translocations which could not be detected at metaphase I of meiosis. The average recombination values of the four genes in the three F₂ families suggest a tight linkage of 1.4 map units between Ba and A genes and a linkage of 8 units between Lc and Lp. The gene order seems to be Ba-A-Lc-Lp. Ba is equally distant from Lc and Lp, (19.1 units), whereas A is closer to Lc than to Lp (13.6 and 17.6

units respectively).

Second hybridization cycle

Fertile tetraploid hybrid derivatives exhibiting domesticated syndrome were utilized in the second hybridization cycle with selected A. sativa lines. Here too, hybrid seeds were obtained only when the common oat served as the female parent. In the winter of 1992, the F₁ hybrids were grown in a greenhouse to protect them from BYDV and were surrounded by Aa2-3 plants as pollen donors. The cooling system of the greenhouse provided moderate wind which assisted in dispersing the pollen grains.

The F₁ plants were tall and vigorous and produced 6-25 tillers per plant. Total yield was much higher this time and 593 BC seeds were collected. However, the proportion of fertile florets remained very low. Here fertile florets were also plumper and their lemmas darker as compared to the sterile florets. On average, 8.9 seeds were obtained per plant, but hybrids of some combinations did not produce even a single seed while others produced more than 20 seeds on a single plant (Table 3). The number of florets per panicle varied on the F₁ hybrids in the range of 80-120, and an average of 100 florets is a reasonable estimate. Assuming an average of 15 panicles per F₁ plant, the estimated seed set was 8.9/1500, or 0.5%.

BC2: Four hundred BC seeds were germinated in the autumn of 1992, and chromosome numbers were counted in 237 of them. Although chromosome number in the majority of the seedlings was in the range

Table 3: Backcross seeds collected on pentaploid hybrids naturally pollinated by Aa2-3 (2n=28)

Hybrids	No. plants	Total No. seeds	Seeds/plant
86-6404 x Aa2-3	5	7	1.4
86-6404 x Aa2-13	7	91	13.0
86-4407 x Aa2-13	7	133	19.0
86-4407 x Aa2-16	8	92	11.5
86-5698 x Aa2-3	3	17	5.6
86-5698 x Aa2-13	2	43	21.5
86-5698 x Aa2-16	8	76	9.5
Dolphin x Aa2-3	3	13	4.3
Dolphin x Aa2-16	10	0	0
Tibor x Aa2-3	5	113	22.6
Pennuda x Aa2-3	8	8	1.0
Total	66	593	8.9

Table 4: Frequency distribution of chromosome numbers among BC plants of the second hybridization cycle involving A. magna

2n

Family	28	29	30	31	32	33	34	35	36-52	Total
10*	5	5	4	3	6	5	7	5	7	47
11	8	8	6	9	4	5	2	3	4	49
12	4	4	6	9	7	2	4	3	3	42
13	6	4	4	3	2	1	4	4	2	30
14	11	9	12	3	5	4	3	0	0	47
15	1	4	4	1	2	0	4	3	3	22
Total	35	34	36	28	26	17	24	18	19	237

* 10 = (86-6404 x Aa2-13) x Aa2-3
 11 = (86-4407 x Aa2-16) x Aa2-3
 12 = (86-5698 x Aa2-16) x Aa2-3
 13 = (Tibor x Aa2-3) x Aa2-3
 14 = (86-4407 x Aa2-13) x Aa2-3
 15 = (86-5698 x Aa2-13) x Aa2-3

of $2n=28$ to 35 (Table 4), the tetraploid number was found in only 15% of them. The plants developed normally and they all exhibited the domesticated syndrome. This was expected because both parents shared this syndrome. Of the 380 growing BC plants, only four set some seeds. These plants were all tetraploid with over 70% stainable pollen. Ten progeny were grown from each of the partially fertile BC plants. Most of them were almost as fertile as their tetraploid parents and exhibited moderate variation in panicle shape and glume size.

Domesticating A. murphyi

Avena murphyi domestication was performed according to the experimental procedure used for A. magna. Hybrid seeds were also obtained only when the hexaploid parent was used as the female. Accession no. 4471 of A. murphyi was crossed with the cultivated lines Ogle and 86-6404, and the F_1 hybrids were grown in the field surrounded by plants of the wild parent, accession no.s 4471 and 4096. The A. murphyi plants were severely infected by BYDV and many of them died at vegetative stages. The F_1 hybrids were more tolerant to BYDV but also showed severe symptoms. As a result, only 12 BC seeds were collected on the pentaploid hybrids. Morphologically these hybrids were characterized by non-shedding spikelets, a single awn per spikelet and glabrous yellow lemmas. These latter two lemma characteristics were shared by the parental lines.

The 12 BC seeds were germinated and chromosome numbers were

counted in root tips. Only one BC plant was tetraploid. In the rest they varied in the range of $2n=29$ to 33. None of the BC plants produced seeds.

Another attempt to develop domesticated A. murphyi was made in 1991. Cultivar Ogle and line 86-4189 were crossed with accession no. 4471 of A. murphyi. Forty-five pentaploid hybrids were grown in the greenhouse to protect them from BYDV, and were surrounded by plants of the tetraploid parent, accession no.s 4471 and 4096 as pollen source. The F_1 hybrids were vigorous but highly sterile and only 102 seeds were collected from them, about two seeds per plant.

The BC plants were germinated in the autumn of 1991 and chromosome numbers were counted in 41 of them. None of these had the tetraploid number, a single plant had $2n=29$ and in the rest of the plants the $2n$ numbers varied in the ranges of 30 to 35. Despite their uneven chromosome numbers, six BC plants produced some seeds. Among them, plants Ai1-5 and Ai1-7 with $2n=32$ and $2n=33$, respectively, Ai2-29 with $2n=35$ and Ai2-35 having $2n=31$.

As expected, spikelet shedding segregated among the BC plants. At maturity, 39 plants shed their spikelets while in 36 they remained attached to the pedicels. This segregation pattern fit the 1:1 ratio of a monohybrid test cross ($\chi^2=0.12$, $p=0.9$). Among the partially fertile BC plants which retained their seeds were Ai2-29 and Ai2-35 and they were used for further selection of domesticated A. murphyi. Additional spikelet characteristics of these plants were glabrous, yellow lemmas and a single, weakly developed awn per spikelet on the lower floret. Plants with shedding spikelets always

Table 5: Frequency distribution of chromosome numbers among F₂ families of (A. sativa x A. murphyi) x A. murphyi

2n							
Family	27	28	29	30	31	32	Total
Ai2-29 (2n=35)	-	6	4	2	7	1	20
Ai2-34	-	4	1	1	-	1	7
Ai2-35 (2n=31)	1	23	20	9	1	-	54
Ai2-39	-	1	4	2	1	-	8

15a

Table 6: Ranges of number of seeds per spikelet (n=20) in plants having the same chromosome number in F₂ families of (A. sativa X A. murphyi) x A. murphyi cross

Family	2n					
	28	29	30	31	32	
Ai2-29	0-2.57	0-1.90	0	0-1.44	-	
Ai2-34	0-0.32	0	0	-	0	
Ai2-35	0-2.50	0-2.70	0-1.96	1.47	-	
Ai2-39	1.90	0-1.10	0.67	0.18	-	

had two awns per spikelets and they developed as in the wild parent.

The F₂ families: Chromosome numbers varied within each of the four F₂ families and among them. In the Ai2-35 family, which derived from a 2n=31 plant, individuals with 2n=28 were most common (Table 5). Most interesting, however, is the Ai2-29 family which originated from a 2n=35 plant. Here again, 2n=28 was counted in about 1/3 of the family members.

Spikelet shedding segregated in each of the four F₂ families, indicating that the four BC plants were heterozygous for this characteristic. The fertility of the F₂ plants also varied considerably. Plants with the tetraploid chromosome number exhibited the whole range, from complete sterility to nearly normal seed set (Table 6). By progeny testing of the most fertile F₂ plants which also retained their spikelets at maturity, a number of fertile domesticated lines were selected.

Fertile domesticated A. murphyi plants were crossed with the wild form. The F₁ hybrids developed normally, 14 bivalents were regularly formed at meiosis and they were fertile. They retained their spikelets at maturity and had a single awn per spikelet.

BYDV tolerance in the domesticated forms

The strict sensitivity to BYDV of both A. magna and A. murphyi was a major factor in the selection of common oat lines for the hybridization experiments. It also forced us to grow the BC plots and segregating generations under an insect-proof net. The

domesticated tetraploids were tested for BYDV tolerance but all were sensitive.

Another approach for introducing BYDV tolerance into the tetraploid domesticated forms is to search for tolerant types among natural populations of both A. magna and A. murphyi. During the summer of 1993, 10 A. magna and nine A. murphyi collections from IGER Institute, Aberystwyth, UK, were screened for BYDV tolerance. Each of these collections was composed of 20-50 individuals which had maintained their identity. None of the A. magna collections showed BYDV tolerance, but it was found in two A. murphyi collections, Cs43 and Cs45. In the spring of 1993 additional material consisting of the two wild tetraploids was collected by the principal investigator in Morocco. The material was tested for BYDV tolerance in 1994. This time one A. magna accession, collected near Ouazane, exhibited excellent tolerance in about one third of the individuals examined. The BYDV tolerant types of both species will be used in crosses to transfer this tolerance to the domesticated types.

Protein content of the domesticated tetraploids

Protein content of some of the domesticated tetraploids and their parental lines, all of which grew under uniform conditions, was examined in the summer of 1994 (Table 7). Protein contents of two A. sativa lines were 18.05% and 18.75%, while those of A. magna and A. murphyi were up to 24.75% and 23.69% respectively. The three examined magna-derivatives had protein contents in the range of the

wild tetraploid. In a single murphyi-derivative however, groat protein content was similar to that of the common oat.

Table 7. Protein content in parental speceis and tetraploid derivatives

Species & line	Seed weight (gr)	Protein %
<u>A. sativa</u> 86-6404	0.608	18.75
Ogle	0.523	18.05
<u>A. magna</u> M1 (wild)	0.525	24.75
M3 (wild)	0.517	24.40
Aa2-3 (domest)	0.555	23.19
Aa2-13 (domest)	0.532	23.30
Aa2-16 (domest)	0.552	19.89
Ba 13-13 (domest)	0.523	24.07
Ba 14-10 (domest)	0.526	24.02
<u>A. murphyi</u> 4771 (wild)	0.519	23.69
Ai2-35 (domest)	0.490	18.39

Production of synthetic hexaploid oats

The hexaploid oats, including A. sativa, are allopolyploids but their diploid and tetraploid ancestors have not been identified. Genetically and morphologically A. magna, and to lesser

extent A. murphyi, are closer to the hexaploid oats than any other tetraploid oat. Nevertheless, they too cannot be regarded as the tetraploid ancestors of the hexaploid oats (Ladizinsky 1994). The potential of the common oat as a crop plant depends upon the genetic diversity which is stored in its three genomes. However, at the hexaploid level other genomic combinations may have better potential as crop plants. This assumption has remained purely theoretical until the production of the domesticated tetraploids A. magna and A. murphyi. These domesticated tetraploids enable, at the first time, the creation and testing of synthetic hexaploids which involve different tetraploid and diploid species. Such synthetic hexaploids are expected to exhibit non-shedding spikelets and reduced awn formation, but lemma color and lemma pubescence depend on the phenotype of the diploid oat involved. They can probably be used as raw material to create new cultivated hexaploid oats, and also as a bridge for gene transfer from diploid species to the common oat. While the creation and testing of synthetic hexaploids was not included in the proposed project, it was added as soon as the domesticated A. magna was in our hand.

Domesticated A. magna, designated Aa2-3, and the diploids A. longiglumis and A. strigosa var. Saia were employed in the production of the synthetic hexaploids. The latter is a cultivated type known as slim oat and is grown mainly as a fodder plant. In all crosses the diploid species served as female parents because their anthesis took place about 2 h later than that in A. magna. Chromosome doubling in the triploid hybrids was attempted when the

plants reached five to six tiller stage. All but one tiller cut and 0.05% aqueous colchicine solution, in tubes, was applied to the cut tillers for 5 h. Successful treatment was indicated at maturity by seed-bearing panicles. Othrewise, no seeds were produced.

(Domesticated *A. magna* Aa2-16 X *A. longiglumis*)₂: The wild diploid oat *A. longiglumis* used to produce the synthetic hexaploid, had a sparse panicle and a relatively small number of spikelets, hairy, yellow lemmas, a developed awn on each floret and at maturity each floret formed a dispersal unit. Panicle and spikelet characteristics of the synthetic hexaploids exhibited a combination of the parental characteristics. Lemmas were yellow and nearly glabrous, a weak awn emerged from the lower floret of the spikelet and at maturity all the spikelets remained attached to the pedicels. Chromosome association at meiosis was irregular and two to three associations of four chromosomes (quadrivalents) were observed in almost every cell. Pollen stainability was about 80% and considerable variation in seed set was observed (Table 8). The seeds were extremely large with hull percentages similar to those in the diploid parent.

(*A. strigosa* var. Saia X Domesticated *A. magna* Aa2-3)₂: Cultivar Saia is characterized by a dense panicle, single floret spikelets, slightly geniculated weak awns and glabrous brown-gray lemmas. Aa2-3 had open panicle, two florets per spikelet and glabrous yellow lemmas. The awns lacked in spikelets of the upper part of the panicle and were only weakly developed on the lower floret of the spikelet at the lower portion of the panicle. Variety Saia exhibits

outstanding BYDV tolerance and is resistant to many crown rust races. Panicle shape and spikelet characteristics of the synthetic hexaploid were similar to those of the tetraploid parent. Lemmas

Table 8. Seed characteristics of synthetic hexaploid oats and their parents

Lines	Spikelets/ panicl	Seeds/ spikelet	20 seed weight (g)	Hull %
Saia	96±8.32	1.08±0.06	0.36±0.08	25
<u>A. longiglumis</u>	27±2.41	2.15±0.12	0.24±0.09	75
Aa2-3	89±7.12	2.01±0.03	0.48±0.05	56
(Saia X Aa2-3) ²	78±11.5	1.86±0.06	0.63±0.06	54
(longig X Aa2-3) ²	56±7.05	1.22±0.38	0.79±0.11	79

were mostly yellow but with much darker tips. When grown in the field the synthetic hexaploid was as BYDV tolerant as var. Saia, and nearly rust free.

Chromosomes of this synthetic hexaploid paired almost regularly at meiosis. Of the 30 cells examined 21 bivalents were formed in 27, and in the other three cells 19 bivalents + one quadrivalent were formed. Pollen stainability was above 90% and in most of the spikelets both florets produced seeds (Table 8).

Crosses between the common oat and the synthetic hexaploids

Crosses in this combination were aimed at studying: a) crossability between the two hexaploids, b) the nature of the F_1 hybrids, and c) potential gene flow between the two hexaploids.

A. sativa X (Saia X A. magna Aa2-3)²:

Crossability in this combination was good and hybrid seeds were easily obtained in both cross directions. The F_1 hybrids developed normally but were highly sterile. Chromosome association at metaphase I was extremely irregular and 7-11 univalents, 5-13 bivalents, 1-4 trivalents, 0-3 quadrivalents and an occasional pentavalent were observed in various cells. Pollen stainability was 14% and the hybrids produced no seeds in the greenhouse. However, when they were grown in the field surrounded by plants of the synthetic hexaploid, they set a few seeds with 0-3 seeds per panicle being collected.

The development and fertility of 70 BC plants was tested. They all developed normally and exhibited variation in panicle shape, glume and spikelet size. Pollen stainability was in the range of 30%-70% but seed set was very poor with the number of seeds produced being 0-10 per plant.

Discussion

The present study demonstrates how the domesticated syndrome of a crop plant can be transferred to its wild relatives which are

members of the crop's secondary gene pool and have a different chromosome number. The main obstacles to gene flow between the hexaploid common oat and the two wild tetraploids are as follows:

1. Hybrid seeds can be produced only when the common oat is the female parent;
2. Extremely low seed set on the pentaploid hybrids, even when they are pollinated by the parental species;
3. A low proportion of BC plants which produce seeds.

Since hybrid seeds can only be produced in one cross direction, all the tetraploid derivatives contain the A. sativa cytoplasm. Repossession of the tetraploid cytoplasm can be achieved at later stages by crossing the wild tetraploids with their domesticated derivatives. The production of hybrid seeds only in the 6(X) X 4(X) cross direction, in the second hybridization cycle as well, suggests that no cytoplasmic or genetic factors affect hybrid seed development. In this regard the ploidy of the endosperm is apparently a major factor, and it is supported by the smaller size of the hybrid seeds as compared with seeds produced by selfing. It would be interesting to compare endosperm development in the parental species embryos to that in hybrids from the two cross directions.

Complete self-sterility of the pentaploid hybrids is due to low pollen fertility, which does not enable anther dehiscence and release of the few viable pollen grains. Chromosome numbers in the viable female gametes of these pentaploids can be deduced by subtracting the pollen chromosome number, 14, from the $2n$ of the BC plants. It appears that chromosome number per se does not affect

gamete viability, at least that of female gametes. Instead, the main factor is apparently the genetic content of the individual chromosomes. Furthermore, a chromosome constitution that confers gamete viability in the pentaploid hybrids does not necessarily guarantee fertility of the resultant BC plants. In fact, none of the $2n=28$ BC plants produced seeds whereas the partially fertile aneuploids evidently contained the complete set of 14 pairs which could be selected as stable tetraploid forms in the F_2 generation.

Besides fertility of the interspecific hybrids and their derivatives, introgression also depends upon the degree of homology between the chromosomes of the species involved and the rate of crossing over. The recombination potentials between A. sativa chromosomes and those of A. magna and A. murphyi were relatively high, 75% and 62%, respectively (Ladizinsky 1992). These values, however, cannot predict the potential of introgressing a specific gene, or characteristic, from the common oat to the wild tetraploid species. Recovery of stable fertile tetraploid derivatives exhibiting the domesticated syndrome of the common oat indicates that the genes controlling the relevant characteristics reside in chromosome segments which are considerably homologous to one another in the three oat species. Each of the following spikelet characteristics: non-shedding, lemma color, lemma pubescence and awn formation is controlled by a single gene in the common oat (Marshall and Shaner 1992) and the control is the same also in domesticated A. magna. Furthermore, these genes are linked to one another in the common oat, and appeared to be linked also in Aa2-16

line of domesticated A. magna. It is likely that the domesticated syndrome was transferred as a block to the Aa2-16 line, and as two separate segments to the Aa2-3 and Aa2-13 lines.

Potential use of the domesticated tetraploids.

Establishing domesticated forms of A. magna and A. murphyi naturally raises the question of their usefulness for oat research and production. At the moment, their impact may be visualized in three different areas: they may become crops in their own right, they may be used to produce synthetic hexaploids, and they may provide a useful bridge for gene transfer from diploid oat species to the common oat.

Tetraploid oat varieties: It is premature to assess the potential of the domesticated tetraploids as a new crop. Much work is needed before the first commercial tetraploid variety can be released. This endeavor must include transfer of additional diversity from the common oat and the initiation of breeding within each of the domesticated tetraploids. It is also difficult to predict any advantage of these tetraploids over the common oat. In this regard, however, wheat is an encouraging model. Both hexaploid and tetraploid wheats are extensively grown, usually for different purposes and in different areas. The hexaploid wheat is grown mainly for bread-making and it is common in cooler areas, whereas the tetraploid wheat is used for pasta and grows in warmer areas. Both wild tetraploid oats grow naturally in warm habitats in

Morocco and southern Spain. Their domesticated derivatives may be more successful than the common oat in such habitats. Both A. magna and A. murphyi are known for the high protein content in their groats, 25%-40% more than in leading common oat cultivars. It is encouraging to note that this high protein content was maintained in some of their domesticated derivatives and this parameter may be one of the main features of the tetraploid varieties. An obvious disadvantage of the domesticated tetraploid lines is their high sensitivity to BYDV. So far we have been unsuccessful in transferring the tolerance occurring in some common oat varieties. The discovery of BYDV-tolerant types in both wild tetraploids during this study is encouraging because the transfer of BYDV tolerance within the tetraploid level is expected to be much simpler and quicker than that from hexaploids.

Synthetic hexaploids: The common oat is an allohexaploid but its diploid and tetraploid ancestors, as of all other hexaploid oats, have not yet been identified. Similarly, there is no way of knowing if the genomic composition of the common oat is optimal for achieving the highest possible performance in this crop. The domesticated tetraploid forms enable, for the first time, the synthesis and testing of new hexaploids with different genomic combinations. Of the two synthetic hexaploids produced in this study, the one involving the diploid A. strigosa var. Saia seems most promising. Not only is var. Saia a domesticated form, but also it is rust resistant and BYDV-tolerant. Furthermore, when A. strigosa is involved in the creation of an allopolyploid it has a

marked effect on the regularity of chromosome pairing at meiosis. In such raw allopolyploids A. strigosa chromosomes pair with their homologues and not with homoeologous chromosomes, resulting with bivalent pairing (Ladizinsky 1974). This is why the synthetic hexaploid involving var. Saia exhibited much more regular meiosis than that involving A. longiglumis, and was consequently more fertile.

Reproductively, the synthetic hexaploid (Saia X Aa2-3)² is strongly isolated from the common oat. The F₁ hybrids are self-sterile and even when backcrossed with viable pollen of the synthetic hexaploid they produce only a few seeds per plant. The BC plants are also highly sterile, but further study is needed to determine to what extent fertility is restored in the F₂ generation. The apparent isolation between the common oat and the synthetic hexaploid may be of agronomic importance. Throughout its growing area the common oat is accompanied by its weedy hexaploid relatives, with which it is fully interfertile. When a herbicide-resistant common oat cultivar, for example, grows adjacent to A. sterilis or A. fatua the herbicide-resistance gene may quickly be established in the wild forms making them even more serious weeds. This is not likely to happen when the synthetic hexaploid grows adjacent to either of these weedy hexaploids.

The domesticated tetraploids as a bridge for gene transfer:

Reproductively, the diploid oat species are strongly isolated from the common oat. F₁ hybrids can be obtained only with great difficulty, usually via embryo culture, and are totally sterile.

Gene transfer from the diploids to the common oat must involve chromosome doubling either before or after production of the hybrids. Both A. magna and A. murphyi cross freely with the diploid oats and following chromosome doubling fertile or partially fertile hexaploids are formed. When the domesticated tetraploids are used in these experiments, the synthetic hexaploids have many of the common oat characteristics and following hybridization between the two hexaploids a greater proportion of the progeny is expected to contain desirable characteristics.

The taxonomic status of the domesticated tetraploids: Since the domesticated tetraploids seem to have some economic value, they have to be named to ease communication among oat workers. Indeed, the taxonomy of these new types is an interesting question and some guidelines regarding their status may be obtained from the International Code of Botanical Nomenclature (1983). According to article H.3.1 of the ICBN, a hybrid between representatives of two or more taxa are nothotaxa and can be named; the epithet of a nothotaxon is a collective name (article H.3.3) and the nothotaxon has the same rank as its postulated or known parent taxa (article H.5.1). Accordingly, each of the domesticated tetraploids has to be regarded as a new species and to receive its own name. This situation has been the rule for many years in regard to crop plants and their wild progenitors. In oat, for example, the common oat is known as A. sativa and its wild progenitors as A. sterilis or A. fatua. Current views on the taxonomic status of crop plants and their wild progenitors consider the two as subspecies of the same

species because they are interfertile and the morphological characteristics of the cultivated form result from selection under domestication. Along this line, the domesticated tetraploids must also be treated as subspecies of their respective wild forms. This, however, requires a new intraspecific division of both A. magna and A. murphyi into two subspecies a wild and domesticated one. we suggest that the domesticated forms of A. magna and A. murphyi will be treated as ssp. domestica and ssp. rigida, respectively. Their complete description will be published elsewhere.

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Description of cooperation

The Israeli investigator developed the domesticated tetraploids and the synthetic hexaploids. The US collaborator conducted the BYDV tests on this material and on collections of wild *A. magna* and *A. murphyi*. In the summer of 1994 the Israeli investigator visited the US to examine the performance of the

domesticated tetraploids and the synthetic hexaploids under local conditions. During that visit we sought ways to further develop the tetraploid oats.

Evaluation of the research achievements

The aim of this project was the creation of stable tetraploid domesticated forms of the two wild oat species A. magna and A. murphyi through hybridization with the hexaploid common oat A. sativa. From each of these species we now possess a number of domesticated lines resulting from the first and second hybridization cycles. In addition, we synthesized two hexaploids which involve the domesticated A. magna and the diploids A. longiglumis and A. strigosa, and examined the relationships between the latter hexaploid and the common oat. This part was in addition to the original research proposal.

The development of domesticated tetraploid stocks of A. magna and A. murphyi is an important event in oat research with obvious economic impact. After appropriate breeding and research, the domesticated tetraploid oats may become crops in their own right. They have the potential of adaption to warmer areas and having higher protein content in their groats as compared to the common oat.

The synthetic hexaploids based on these domesticated tetraploids also have economic potential and their utilization could come sooner than that of the tetraploid types. They can be developed into cultivars or used as a bridge for gene transfer from

diploid oats to A. sativa.

Publications resulting from the research project

1. Ladizinsky, G. 1992. Genetic resources of tetraploid wild oats and their utilization. Proc. 4th Int.Oat Conf. Adelaide. Vol. 2 pp.65-70.
2. Ladizinsky, G. Domestication via hybridization of the tetraploid wild oats Avena magna and A. murphyi (in preparation).
3. Ladizinsky, G. Synthethic hexaploid oats and their economic potential (in preparation).