

Introgression of Desirable Alien Chromatin from Wild Avena Species into Cultivated Oat (*Avena sativa* L.) and Its Identification through Agro-Morphological and Molecular Techniques

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Abstract

The genetic enhancement of polyploid oats poses a significant challenge for crop production improvement. This study aimed to introgress desirable agronomic and quality traits from wild '*Avena*' species into cultivated *A. sativa* L. We utilized three diploid species (*A. strigosa* L., *A. longiglumis* L., and *A. orientalis* L.), one tetraploid species (*A. barbata* L.), and three hexaploid species (*A. sterilis* L., *A. byzantina* L., and *A. sativa* L.) for interspecific and interploidy crosses. Interspecific crosses were advanced to BC₁F₁ and BC₂F₁ generations over three *Rabi* seasons at CSK Himachal Pradesh Agricultural University, India. Phytohormone application, embryo culture, and colchicine treatment facilitated interploidy crosses, while intraploidy crosses were performed without manipulation. We developed 41 embryos from *A. strigosa* × *A. sativa*, 18 from *A. longiglumis* × *A. sativa*, and 14 from *A. orientalis* × *A. sativa*, with 15 plants surviving colchicine treatment (34.88% survival rate). SSR primers confirmed hybridity, and molecular screening of BC₁F₁ and BC₂F₁ generations showed donor alleles in 16 of 26 BC₂F₁ plants. The BC₂F₁ derivatives exhibited significant improvements over the recurrent parent, including up to 75% higher beta-glucan content, 41% higher protein content, 11.28% reduced maturity days, and 70.96% higher seed yield. These advancements underscore the potential of BC₂F₁ derivatives in oat breeding programs for trait introgression and crop improvement.

1. Introduction

Cereal production is strategically crucial to the global economy. The primary aim of breeding programs has traditionally been to develop cultivars with enhanced agronomic performance, such as high grain yield, superior grain quality, and resistance to diseases and lodging (Tadesse et al., 2019). However, in today's rapidly changing environment, adaptability to varying environmental conditions has become an increasingly important trait (Nelson et al., 2007). While genetic improvement of diploid cereal species like rice, barley, and maize is relatively straightforward (Hisano et al., 2021), the enhancement of polyploid crops such as hexaploidy wheat and oats presents significant challenges.

Oat (*A. sativa* L.), an important annual forage crop of the *Rabi* season, demonstrates wide adaptability to diverse growing conditions. It offers excellent nutritional value for both animal and human consumption, making it a valuable dual-purpose crop (Ahmad et al., 2014). Oats are well-suited to a variety of soil types and outperform many other small-seeded cereals, particularly on acidic and alkaline soils (Wibbererley, 2006). The genus '*Avena*' is extensive, encompassing both wild and cultivated polyploid species with a basic chromosome number of $n = 7$ (Liu et al., 2017). The regions of the Himalayas, in particular, provide an ideal environment for oat cultivation, thanks to the crop's adaptability, rapid regrowth, and superior nutritional value. In many parts of the Himalayan region, oats serve as a vital forage crop in summer and are also grown as a multipurpose crop for fodder and grain (Sood et al., 2016).

Over time, elite germplasm tends to lose valuable genetic variation, prompting breeders to explore the rich allele diversity found in crop wild relatives (CWR) and landraces stored in seed banks (Mohler et al., 2023). Despite the challenges involved, these ancestral species hold the potential to introduce new

alleles that improve disease resistance, abiotic stress tolerance, and overall yield and quality in cultivated crops (Zhang et al., 2017). Wild species related to cultivated oats often contain desirable genetic variation that can be transferred into cultivated oats through interspecific crossing techniques (Imam and Allard, 1965). Progenitor species and wild relatives, especially those at lower ploidy levels, have preserved a high degree of useful genetic variation. The success of plant breeders in utilizing this alien variation for crop improvement depends largely on the degree of relatedness between the cultivated species and the wild donor species (Kashyap et al., 2022). However, transferring desirable genes from diploid and tetraploid '*Avena*' species into hexaploid oat cultivars is a complex and challenging task (Mohler et al., 2023).

The integration of beneficial genes from wild relatives into cultivated oat species is a key objective for plant breeders aiming to broaden the genetic base and enhance desirable traits (Mohler et al., 2023). Despite strong sterility barriers and the difficulty of chromosome pairing between species with different ploidy levels, several studies have reported significant improvements in agronomic and quality traits through crosses with these wild species. Techniques such as embryo rescue (Rogo et al., 2023) and colchicine treatment (Eng and Ho, 2019) are often required to overcome the challenges of interploidy transfer, with greater success typically observed when the lower ploidy species is used as the female parent (Huang et al., 2022). In recent years, considerable progress has been made in this area, with several successful gene transfers reported. Pollination followed by auxin treatment and embryo rescue has become a well-established approach for producing interspecific hybrids in oats.

Given these advancements and the potential benefits, the present investigation aims to introgress desirable alien chromatin from wild '*Avena*' species into cultivated oat, utilizing both agro-morphological and molecular techniques to evaluate and identify the successful introgressions. This research holds promise for enhancing the genetic diversity and agronomic traits of cultivated oats, contributing to the development of more resilient and productive oat cultivars.

2. Materials and Methods

The present investigation was carried out during the *Rabi* seasons from 2017-18 to 2020-21 at the Experimental Farm and Molecular Cytogenetics and Tissue Culture Lab, CSK HP Agricultural University, Palampur (India). The experimental material consisting of three accessions of wild diploid species *viz.*, *A. strigosa* L., *A. longiglumis* L. and *A. orientalis* L., one tetraploid species *i.e.* *A. Barbata* L. and two hexaploid wild species *viz.*, *A. sterilis* L., *A. byzantina* L. and one cultivated *A. sativacv.* HJ-8 were used to develop the interspecific crosses (Table 1).

Table 1
Detail of the material used in the present study

Sr. No.	Species	Accession No.	Chromosome number (2n)	Source
1.	<i>Avena barbata</i>	HFO 58	28	CCS HAU, Hisar
2.	<i>Avena byzantina</i>	HFO 60	42	CCS HAU, Hisar
3.	<i>Avena orientalis</i>	HFO 103	14	CCS HAU, Hisar
4.	<i>Avena longiglumis</i>	HFO 498	14	CCS HAU, Hisar
5.	<i>Avena strigosa</i>	HFO 505	14	CCS HAU, Hisar
6.	<i>Avena sterilis</i>	HFO 508	42	CCS HAU, Hisar
7.	<i>Avena sterilis</i>	HFO 878	42	CCS HAU, Hisar
8.	<i>Avena sativa</i>	HJ-8	42	CSKHPKV, Palampur

2.1 Crossing Plan

During *Rabi* 2017-18, interspecific crosses between wild '*Avena*' species and cultivated *A. sativa* cv. HJ-8 were attempted (Fig. 1). Hybrid embryos developed through crossing diploid and tetraploid species with *A. sativa* were rescued and cultured on suitable MS medium followed by colchicine treatment under controlled conditions. The procedure to develop interploidy crosses, hormone treatment, embryo culture and colchicines treatment was used as per Rines et. al. (2007). Plants developed through embryo culture were maintained in the lab under artificial conditions till maturity and F₁ seeds were harvested after ripening. The crosses between wild hexaploid and cultivated *A. sativa* were developed and harvested at maturity as they are fully compatible.

F₁ plants were grown and back crossed twice with cultivated *A. sativa* cv. HJ-8 to generate BC₁F₁ and BC₂F₁ generation during *Rabi*, 2018-19 and 2019-20, respectively in the field conditions (Fig. 1). BC₂F₁ generation along with parents were evaluated in the pots during *Rabi*, 2020-21 for various agro-morphological and quality traits under field conditions.

2.2 Embryo culture of interploidy hybrids

The caryopses from crossed panicles were harvested and washed thoroughly using *Tween-20* under tap water to avoid any sort of contamination/infection. At the time of embryo rescue in Laminar Air Flow Chamber, surface sterilization of caryopses was done using 0.1 per cent HgCl₂ for 1–2 minutes followed by three washing with autoclaved distilled water. The embryos were then excised from sterilized seeds and transferred to the test tubes containing working MS medium. Half strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with essential amino acids (Table 3.2 and 3.3) was used for the rescue of interploidy hybrid embryos as per suggested by Rines et al., 2003. Cultured embryos were placed in the dark section of Plant Growth Chamber (PGC) at 20 ± 2°C with 75 per cent humidity for regeneration for about a week till the roots and shoots initiated. The regenerated plantlets

were then shifted to the other section of the PGC at $20 \pm 2^\circ\text{C}$ with 10/14 hrs light/dark profile with 75 per cent relative humidity for proper development of plantlets. The plantlets regenerated from embryo culture were treated with IBA (Indole butyric acid) and NAA (Naphthalene acetic acid) solution of 100 ppm concentration each for 2 minutes for profuse rooting. Potting mixture was prepared by mixing of coco peat, vermiculite and perlite in the proportion of 3:1:1 v/v. The plantlets were then transferred to the soil less media in small pots (5 cm diameter) for proper rooting and shooting. Appropriate conditions were maintained for proper growth. At 3–4 leaf stage, some plantlets were treated with 0.05% and remaining were treated with 0.10% colchicine with 2% DMSO for 4 hours. DMSO acts as osmoregulator. After attaining 10–12 leaf stage, the treated plants were transferred to potting mixture.

2.3 Data recorded

Observations were recorded on five randomly selected competitive plants of parents for all the traits, and each plant data recorded in case of BC_2F_1 generation. The agro-morphological characters were included days to 50% flowering, plant height (cm), number of leaves per plant, tillers per plant, leaf area (cm^2), spikelets per panicle, green fodder yield/ plant (g), leaf: stem ratio, dry matter yield/ plant (g), days to 75% maturity, biological yield/ plant (g), seed yield/ plant (g), harvest index, 1000 grain weight (g), seed length (mm), protein content (%) and beta- glucan content (%). The protein content for each genotype was analysed by the quantitative method given by Lowry et. al. 1951. Beta-glucan content (%) was determined by the extraction method given by Wood et al. (1977) and estimation method given by Semedo et al. (2015).

2.4 Molecular marker analysis

To detect the hybridity of F_1 plants from different interspecific crosses, 25 SSR primer pairs were screened for polymorphism among the parental 'Avena' species. Details of these primers, including their sequences, are provided in Supplementary Table 1. Polymorphic SSR markers were used for hybridity test in F_1 and tracing the introgressions in BC_1F_1 and BC_2F_1 . Extraction of Plant genomic DNA, PCR amplification and Agarose gel electrophoresis were done in Molecular Cytogenetics and Tissue Culture Lab, Department of Genetics and Plant Breeding, CSK HPKV, Palampur. The PCR amplification was performed in a mixture containing 1.5 ul of DNA template, 0.5 ul of each forward and reverse primers, 0.1 ul of Taq polymerase (Takara Pvt. Ltd.), 2.0 ul of dNTP mix, 2 ul of PCR Buffer + mgcl_2 and 6.4 ul of sterile water with final volume of 12 ul per reaction.

2.5 Statistical Analysis

Data recorded for various traits was pertained to test whether the mean difference between mean values of plants is significant or not over individual cross mean as well as overall mean of BC_2F_1 generation using simple t-test performed as:

$$\text{Student's t-test} = \frac{\bar{X}_d}{\text{SE}(X_d)} \text{ (at } n - 1 \text{ d.f.)}$$

Where,

$\bar{X}_d$ = mean-difference between two sets of related samples

SE (X_d) = Standard error of mean difference

n = Number of related samples

The significance of backcross derivatives and parents was tested at tabulated value of (n-1) degree of freedom at 5% level.

3. Results

3.1 Interspecific hybridization in *Avena* species

A total of 73 F_1 hybrid plants were successfully developed from six interspecific crosses (Table 2). All crosses were successful except for the *A. barbata* $\times$ *A. sativa* cross, which did not result in embryo formation. The successful hybrids were backcrossed with the recipient parent twice, resulting in 24 plants in the BC_1F_1 generation and 26 plants in the BC_2F_1 generation. For the cross between *A. orientalis* and *A. sativa*, 5 seeds were produced in the F_1 generation, followed by 2 seeds in BC_1F_1 and 3 seeds in BC_2F_1 . A greater number of seeds were obtained from the *A. longiglumis* $\times$ *A. sativa* cross, with 28 F_1 seeds, 9 BC_1F_1 seeds, and 5 BC_2F_1 seeds. Similarly successful was the *A. strigosa* $\times$ *A. sativa* cross, which yielded 27 F_1 seeds, 6 BC_1F_1 seeds, and 6 BC_2F_1 seeds. When crossing *A. sativa* with *A. byzantina*, the resulting generations produced 5 F_1 seeds, 2 BC_1F_1 seeds, and 4 BC_2F_1 seeds. Two different cultivars of *A. sterilis* were crossed with *A. sativa*: cv. HFO 508 yielded 4, 3, and 4 seeds in F_1 , BC_1F_1 , and BC_2F_1 generations respectively, while cv. HFO 878 produced 4, 2, and 4 seeds in the corresponding generations.

Table 2
Plants developed in F₁, BC₁F₁ and BC₂F₁ generations of
different interspecific crosses

Crosses	Number of Plants		
	F ₁	BC ₁ F ₁	BC ₂ F ₁
<i>A. barbata</i> × <i>A. sativa</i>	0	0	0
<i>A. orientalis</i> × <i>A. sativa</i>	5	2	3
<i>A. longiglumis</i> × <i>A. sativa</i>	28	9	5
<i>A. strigosa</i> × <i>A. sativa</i>	27	6	6
<i>A. sativa</i> × <i>A. byzantine</i>	5	2	4
<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO 508	4	3	4
<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO 878	4	2	4
Total	73	24	26

3.2 Embryo culture in Interploidy hybridization

In the interploidy crossing program, species with lower ploidy levels were used as female parents, while species with higher ploidy levels were used as male parents, as this approach has a higher success rate according to previous studies. From 839 caryopses, a total of 73 hybrid embryos were developed. Specifically, 41 embryos were derived from the *A. strigosa* × *A. sativa* cross, 18 from *A. longiglumis* × *A. sativa*, and 14 from *A. orientalis* × *A. sativa*. These embryos were cultured on suitable MS medium and incubated in the dark for germination. The hybrid plant regeneration frequency was 61.64%, with 45 of the 73 embryos germinating within 7–10 days. Of these, 43 embryos developed into seedlings with 3–4 leaves.

3.3 Evaluation of BC₂F₁ derivatives

The BC₂F₁ derivatives were assessed for a range of agro-morphological and quality traits during the *Rabi* season of 2020-21, alongside parent lines. The evaluation revealed significant variations in key traits among the derivatives (Tables 3 and 4). Days to 50% flowering ranged from 117 to 142 days, with several plants, including P₁ of *A. orientalis* × *A. sativa* (124 days) and P₂ and P₃ of *A. longiglumis* × *A. Sativa* (122 and 124 days, respectively), flowering earlier than the overall BC₂F₁ mean (130.30 days). Whereas, *A. strigosa* (123 days) was significantly superior for this trait among the parents.

Table 5
Promising BC₂F₁ plants selected for important agro-morphological and quality traits.

Sr. No.	Cross	Plant number			
		Seed yield per plant (g)	Biological yield per plant (g)	Days to 75% maturity	Beta-glucan content (%)
1.	<i>A. orientalis</i> × <i>A. sativa</i>	P ₁	P ₁ , P ₃	P ₁	P ₃
2.	<i>A. longiglumis</i> × <i>A. sativa</i>	P ₃ , P ₄ , P ₅	P ₃	P ₁ , P ₂ , P ₃	P ₁ , P ₂ , P ₃
3.	<i>A. strigosa</i> × <i>A. sativa</i>	P ₁ , P ₂ , P ₅	P ₁ , P ₄	P ₁ , P ₂ , P ₃ , P ₅ , P ₆	P ₁ , P ₃ , P ₄
4.	<i>A. sativa</i> × <i>A. byzantina</i>	P ₁	P ₁ , P ₃	P ₁	P ₃
5.	<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO-508	P ₁ , P ₄	P ₁ , P ₄	-	P ₁
6.	<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO-878	P ₃	P ₂ , P ₃	-	-

Plant height among the BC₂F₁ derivatives varied from 58 cm to 95 cm. Plants P₁ of *A. orientalis* × *A. sativa* (80.00 cm) and P₃ of *A. longiglumis* × *A. Sativa* (95.00 cm) were notably taller than the overall mean (74.0 cm). *A. strigosa* (82.00 cm) and *A. sterilis* cv. HFO-508 (83.00 cm) showed significant superiority for plant height. Similarly, leaf number ranged from 22 to 60, with *A. strigosa* (48.00), *A. byzantina* (52.00), and *A. sterilis* cv. HFO-878 (52.00) exhibiting superior performance.

The number of tillers per plant varied from 5 to 14, with plants P₃ of *A. longiglumis* × *A. sativa* (14.00) and P₁ and P₅ of *A. strigosa* × *A. sativa* (12.00 and 10.00, respectively) showing significant superiority. Leaf area ranged from 13.87 to 56.00 cm², with plants P₃ of *A. orientalis* × *A. sativa* (26.50 cm²), and P₄ and P₅ of *A. longiglumis* × *A. sativa* (56.00 and 37.33 cm², respectively) demonstrating superior values. Number of spikelets per plant varied from 28 to 76, with several plants across different crosses showing significant superiority.

Green fodder yield per plant ranged from 72 to 118 g. *A. strigosa* (110.00 g) and *A. longiglumis* (106.00 g) were exhibited superiority among the parents. The leaf:stem ratio ranged from 0.22 to 0.65, with significant superiority in several plants including P₃ of *A. orientalis* × *A. sativa* (0.39), P₂ of *A. strigsa* × *A. sativa* (0.40). Dry matter yield per plant varied from 15.54 to 32.96 g, with *A. byzantina* (24.09 g), *A. strigosa* (29.77 g) and *A. longiglumis* (31.10 g) showing significant superiority.

Days to 75% maturity ranged from 155 to 178 days, with several BC₂F₁ derivatives showing earlier maturity than the overall mean. Biological yield per plant varied from 27.06 to 42.90 g, with *A. sterilis* cv. HFO-508 (36.87 g) and *A. sterilis* cv. HFO-878 (36.34 g) showing significant superiority. Seed yield per

plant ranged from 3.11 to 7.30 g, with several plants, including those from *A. orientalis* × *A. sativa*, showing superior values.

Harvest index ranged from 8.64 to 17.97%, with significant superiority in several plants from cross *A. orientalis* × *A. sativa* and *A. longiglumis* × *A. sativa*. Thousand-grain weight varied from 28.30 to 35.40 g, with *A. orientalis* (32.70 g), *A. byzantina* (34.40 g), and *A. longiglumis* (35.70 g) showing significant superiority. Seed length ranged from 11.95 to 14.15 mm, with *A. orientalis* (15.12 mm), *A. byzantina* (14.60 mm), *A. longiglumis* (10.40 mm), and *A. strigosa* (10.15 mm) showing superior values.

Protein content ranged from 7.3–11.00%, and all wild accessions except *A. sterilis* cv. HFO-878 demonstrating superior values than overall mean and cultivated species *A. sativa*. Beta-glucan content varied from 2.89–5.42%, with *A. longiglumis* (4.43%) and *A. strigosa* (4.62%) showing significant superiority. Plant P₃ (3.97%) of cross *A. orientalis* × *A. sativa*; P₁ (5.42%), P₂ (4.31%) and P₅ (4.70%) of cross *A. longiglumis* × *A. sativa*; P₁ (4.74%), P₃ (4.00%) and P₄ (4.95%) of *A. strigosa* × *A. sativa*; P₃ (3.94%) of *A. byzantina* × *A. sativa*; P₁ (4.10%) of *A. sativa* × *A. sterilis* cv. HFO-508 showed significant superiority for this trait over overall mean.

Overall, the BC₂F₁ derivatives exhibited promising traits for introgression into cultivated oat genotypes, potentially enhancing their performance. These findings align with previous research highlighting the benefits of interspecific crosses in improving plant traits (Murphy, 1981).

3.4 Molecular Screening

Molecular characterization of all '*Avena*' species accessions utilized 25 SSR primers. Among these, five SSR primers displayed polymorphism between wild species and the cultivated oat variety, *A. sativa* cv. HJ-8. Each wild species exhibited unique polymorphic primers, except for MAMA_4, which was polymorphic in both *A. orientalis* and *A. sterilis* cv. HFO-878 (Table 6). The polymorphic primers confirmed the hybridity of F₁ plants from interploidy crosses, demonstrating the presence of alleles from both parent species (Plate 2). For intraploidy crosses, hybridity was verified in five plants of *A. sativa* × *A. byzantina*, four plants of *A. sativa* × *A. sterilis* HFO-508, and four plants of *A. sativa* × *A. sterilis* HFO-878, all showing alleles from both parent species.

Table 6
List of SSR primers polymorphic for different *Avena* species

S. No.	Primer	Cross	Annealing Temp. (°C)	No. of Alleles
1.	AM87	<i>A. sativa</i> × <i>A. byzantina</i>	52.59	2
2.	MAMA_4	<i>A. orientalis</i> × <i>A. sativa</i>	52.30	2
3.	MAMA_5	<i>A. longiglumis</i> × <i>A. sativa</i>	53.09	3
4.	AM115	<i>A. strigosa</i> × <i>A. sativa</i>	51.17	2
5.	Astavea	<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO 508	54.35	2
6.	MAMA_4	<i>A. sativa</i> × <i>A. sterilis</i> cv. HFO 878	52.30	2

In the BC₁F₁ generation, all plants from intraploidy crosses presented amplicon segments from both parent species, whereas in interploidy crosses, all plants of *A. orientalis* × *A. sativa* possessed alleles from both parents. However, in the cross *A. longiglumis* × *A. sativa*, plants 4, 7, and 8 out of 9 lacked the amplicon region from the donor parent P₁.

In the BC₂F₁ generation, the cross *A. sativa* × *A. byzantina* yielded four plants, three of which exhibited the amplified region of primer AM87 from both parents (Plate 3). Similarly, two plants each in the crosses *A. sativa* × *A. sterilis* cv. HFO-508 and *A. sativa* × *A. sterilis* cv. HFO-878 demonstrated amplicon regions from the donor parent. These plants were notably superior in seed yield per plant, biological yield per plant, and plant height. In the interploidy cross *A. orientalis* × *A. sativa*, only one plant showed the allelic region for SSR marker MAMA_4 from both parents, and this plant excelled in days to 50% flowering, seed yield per plant, and tillers per plant compared to the recurrent parent *A. sativa* cv. HJ-8. Similarly, in the cross *A. longiglumis* × *A. sativa*, only plant P₁ exhibited the allelic region from the donor parent, and it was significantly superior in beta-glucan content, protein content, and days to 75% maturity over the recurrent parent. In the cross *A. strigosa* × *A. sativa*, three plants showed the allelic region from the donor parent and were also significantly superior in beta-glucan, protein content, and days to 75% maturity.

4. Discussion

The successful introgression of desirable chromatin from wild '*Avena*' species into cultivated oat underscores the potential of interspecific and interploidy hybridization in enhancing the genetic diversity and agronomic traits of cultivated oats (Tinker et al., 2022). CWRs can significantly expand the genetic diversity of crops and introduce beneficial traits (Brozynska et al., 2016; Zhang et al., 2017). However, their direct use in breeding programs is often challenging due to the presence of undesirable traits like, linkage drag (peng et al., 2014) and common breeding barriers (Kashyap et al., 2022) with cultivated

crops. Cross compatibility is notably low in hexaploid × diploid 'Avena' hybrids (Latta et al., 2019). A significant challenge in transferring genes from wild diploid and tetraploid oat species into hexaploid cultivars is successfully producing healthy, vigorous inter-ploidy F₁ hybrids (Aung et al., 2010). These hybrids must maintain all chromosomes from both the cultivated and wild parents, ensuring that the gene of interest is effectively expressed (Aung et al., 2010). Few studies have focused on transferring traits from wild relatives to cultivated oats, particularly for agronomic characteristics (Hermann et al., 2014), nutritional traits (Ahokas and Manninen, 2000), crown rust resistance (Rines et al., 2007; Aung et al., 2010), and powdery mildew resistance (Yu and Hermann, 2006) and they got positive result by successful introgression.

4.1 Molecular Screening

This study generated a significant number of F₁ hybrids from various interspecific crosses, although the cross between *A. barbata* and *A. sativa* was unsuccessful due to the absence of embryo formation. The subsequent backcrossing efforts yielded promising BC₁F₁ and BC₂F₁ generations, indicating the feasibility of incorporating wild 'Avena' chromatin into cultivated backgrounds.

Embryo culture proved crucial in rescuing hybrid embryos from interploidy crosses, with a notable regeneration frequency of 61.64%. The development of 43 viable seedlings from 73 germinated embryos highlights the effectiveness of this technique in overcoming barriers associated with interploidy hybridization. These results align with previous studies, which suggest that using species with lower ploidy levels as females in crosses increases the likelihood of success. The hybrids demonstrated a broad range of agro-morphological variations, with several BC₂F₁ derivatives exhibiting significant improvements in traits such as plant height, number of tillers, leaf area, green fodder yield, seed yield, and other agronomic characteristics.

4.2 Evaluation of BC₂F₁ derivatives

The evaluation of BC₂F₁ derivatives in our study revealed significant variations in key agro-morphological and quality traits, highlighting the potential of introgressing desirable traits from wild 'Avena' species into cultivated oat (*A. sativa* L.). The observed variability in days to 50% flowering, ranging from 117 to 142 days, demonstrates the potential for modifying flowering times through interspecific crosses. Notably, plants derived from *A. orientalis* × *A. sativa* and *A. longiglumis* × *A. sativa* exhibited earlier flowering compared to the overall BC₂F₁ mean, suggesting that these crosses can be used to accelerate flowering times in cultivated oats, an important trait for adapting to varying climatic conditions (Smith et al., 2018).

The significant variation in plant height among the derivatives, with values ranging from 58 cm to 95 cm, underscores the potential for introducing height traits from wild species such as *A. strigosa* and *A. sterilis* cv. HFO-508. Taller plants may offer advantages in forage yield and mechanical harvesting efficiency, consistent with findings from previous studies (Semchenko and Zobel, 2005; Rosser, 2014; Coblentz et al., 2014). Similarly, the variation in leaf number and leaf area suggests that wild species can

contribute to improved canopy architecture and productivity, aligning with the observations of leaf trait improvement in other interspecific crosses (Carlson et al., 2021).

The variation in number of tillers per plant and green fodder yield per plant further emphasizes the potential for improving these traits through wild species introgression. The observed superiority of certain plants for these traits, such as those from *A. longiglumis* × *A. sativa* and *A. strigosa* × *A. sativa*, indicates that these wild species can enhance forage yield and quality, which is critical for sustainable oat production (Humphreys, 2005; Kumar et al., 2018; Chand et al., 2022; Sood et al., 2022).

In terms of yield components, the significant differences in biological yield, seed yield, harvest index, thousand-grain weight, and seed length suggest that wild species introgression can positively impact yield and grain quality. The superior values observed in plants from crosses involving *A. orientalis*, *A. byzantina*, and *A. longiglumis* highlight the potential for developing high-yielding oat cultivars with desirable agronomic characteristics (Beavis and Frey, 1987; Sharma et al., 2018). Additionally, the higher protein and beta-glucan content in certain wild accessions, particularly *A. longiglumis* and *A. strigosa*, aligns with findings from previous research that emphasize the nutritional benefits of integrating wild species into cultivated crops (Frison et al., 2011; Kahane et al., 2013; Ebert, 2014).

The promising traits observed in the BC₂F₁ derivatives underscore the value of utilizing wild '*Avena*' species for improving cultivated oat performance. This study's findings support previous research highlighting the benefits of interspecific crosses in enhancing plant traits and emphasize the potential for developing improved oat cultivars through continued exploration of wild species (Murphy, 1981; Johnson et al., 2004).

4.3 Molecular Screening

The molecular characterization of '*Avena*' species accessions using 25 SSR primers provided valuable insights into the genetic introgression from wild species into cultivated oat (*A. sativa* L.). Notably, five SSR primers exhibited polymorphism between the wild species and the cultivated variety, *A. sativa* cv. HJ-8. This finding underscores the potential of SSR markers in distinguishing between wild and cultivated accessions, offering a robust tool for genetic analysis in oat breeding programs (Gunnaiah et al., 2021; Thapa et al., 2021; Liu et al., 2022).

The distinct polymorphic patterns observed in wild species, except for the MAMA_4 primer which was shared between *A. orientalis* and *A. sterilis* cv. HFO-878, highlight the genetic diversity present within the wild '*Avena*' species. Such variability is crucial for the introgression of beneficial traits from wild relatives into cultivated varieties. Previous studies have similarly demonstrated the utility of SSR markers in revealing genetic differences between species and in assessing genetic diversity (Yarvaan et al., 2020; Arora et al., 2021; Koroluk et al., 2022; Kaur et al., 2024).

The confirmation of hybridity in F₁ plants from interploidy crosses, as indicated by the presence of alleles from both parent species, reflects successful genetic transfer. This aligns with findings from

other research where SSR markers were used to validate hybridization and the introgression of alien chromatin (Boutin et al., 2006; Zhang et al., 2009). Specifically, the detection of polymorphic alleles in intraploidy crosses, such as *A. sativa* × *A. byzantina* and *A. sativa* × *A. sterilis*, further supports the effectiveness of these markers in tracking genetic introgression and verifying the presence of alien genes.

However, the cross between *A. longiglumis* and *A. sativa* revealed a notable exception. In this cross, plants 4, 7, and 8 out of 9 lacked the amplicon region from the donor parent (P_1). This observation highlights the challenges associated with introgressing genetic material from certain wild relatives. It suggests that the introgression from *A. longiglumis* may be less efficient or that specific genetic barriers might be present (Jones et al., 2022). Such variability in successful introgression has been reported in other studies, where the presence or absence of donor chromatin in progeny varied significantly (Lee et al., 2020; Kim & Park, 2021).

The differential success in introgression observed in this study underscores the need for a tailored approach when selecting wild relatives for breeding programs and highlights the importance of comprehensive molecular screening to ensure the retention of desirable chromatin segments. The successful integration of traits from wild '*Avena*' species into cultivated oat demonstrates the value of molecular techniques in enhancing the genetic base of crop varieties. This approach aligns with other studies that emphasize the role of wild relatives in broadening genetic diversity and improving traits such as disease resistance and stress tolerance (Perrino et al., 2020; Bohra et al., 2022; Kashyap et al., 2022). Future research should focus on understanding the underlying genetic mechanisms influencing successful introgression and developing strategies to overcome barriers associated with specific wild species.

The overall study's findings emphasize the importance of utilizing wild relatives and landraces to broaden the genetic base of cultivated oat crop. The introgressed lines developed in this study not only possess enhanced agronomic traits but also offer potential for further genetic improvement through conventional breeding and molecular techniques. The observed transgressive segregation and superior performance of certain BC_2F_1 plants underscore the potential for creating new oat cultivars with improved yield, quality, and resilience to biotic and abiotic stresses.

The introgression of desirable alien chromatin from wild '*Avena*' species into cultivated oat has yielded promising results, demonstrating the value of wild species in oat improvement programs (Boczowska et al., 2015; 2016). Several past research studies indicate that genetic diversity within modern oat gene pools is limited. Over time, valuable alleles have been lost through selection, introduction, and domestication making it essential to explore obsolete cultivars, wild ancestors, and landraces for new germplasm to advance oat breeding efforts (Okoń et al., 2014; Koroluk et al., 2022).

The combination of agro-morphological evaluation and molecular characterization has provided a robust framework for selecting superior lines (Ghazy et al., 2024; Bayahi and Rezgui, 2015; Guizani et al., 2024;

khan et al., 2024), paving the way for the development of new oat varieties that meet the challenges of modern agriculture (Kumar et al., 2023; Kebede et al., 2023). Further research should focus on the continued evaluation and utilization of these introgressed lines in breeding programs, with particular attention to their performance under diverse environmental conditions.

5. Conclusion

This study successfully demonstrated the potential of interspecific and interploidy hybridization in 'Avena' species for enhancing cultivated oat (*A. sativa* L.) through the introgression of desirable alien chromatin. A total of 73 F₁ hybrids were generated, and subsequent backcrossing produced BC₁F₁ and BC₂F₁ generations, revealing significant agro-morphological and quality trait variations. Key traits such as plant height, leaf number, green fodder yield, and protein and β-glucan content showed notable improvements in several BC₂F₁ derivatives compared to the recurrent parent. These newly developed interspecific derivatives will be screened for ploidy level and alien introgressions using cytological techniques such as Genomic In situ Hybridization, after two more successive backcrossings to achieve the stable useful introgressions. Molecular screening using SSR markers confirmed the introgression of chromatin from wild species into cultivated oat, validating the hybridity and the presence of alleles from both parent species. This integration of wild genetic material into cultivated oat shows promise for improving yield, stress tolerance, and nutritional quality, aligning with the benefits observed in previous research. The findings highlight the potential for utilizing wild 'Avena' species to enhance the genetic base and performance of cultivated oats.

Declarations

Authors contribution statement:

Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing-original draft- SK, VKS, SKS,

Writing-review and editing, Visualization, Supervision- GS, VR, RK.

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Tables

Tables 3 and 4 are available in the Supplementary Files section.

Plates

Plates 1 and 3 are available in the Supplementary Files section

Figures

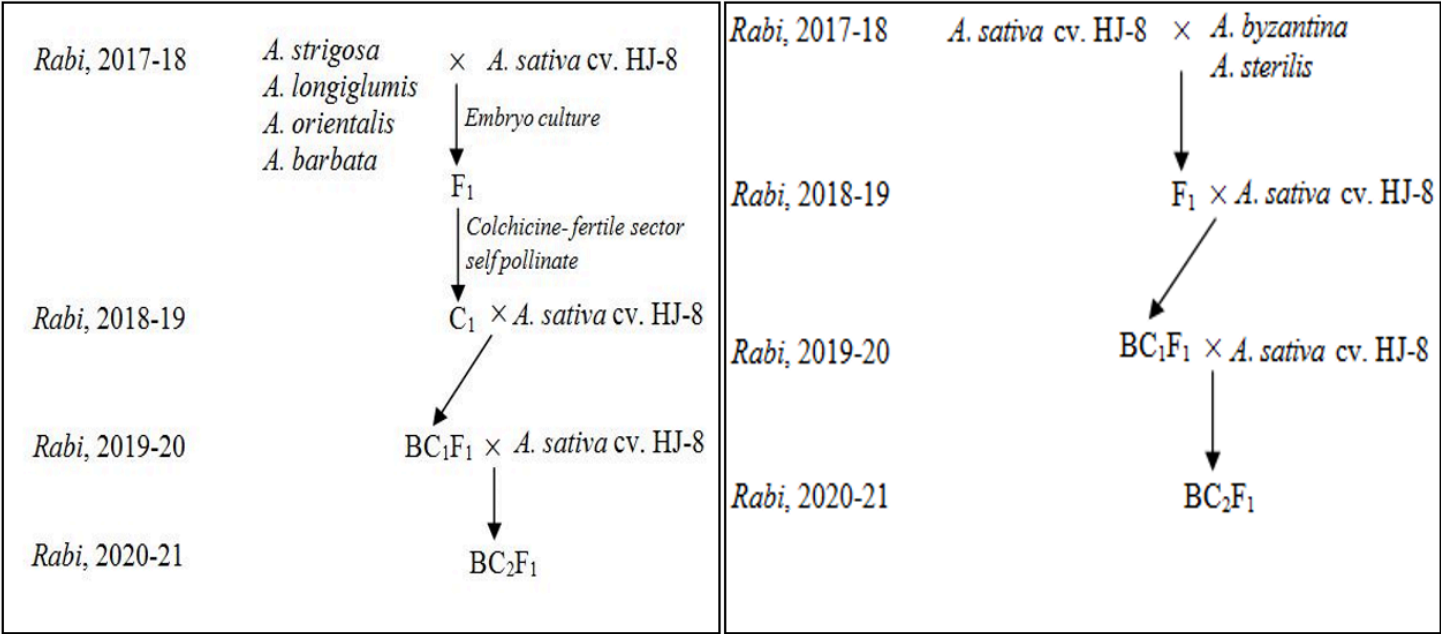


Figure 1

Schematic diagram of crossing scheme for (i) interploidy and (ii) intraploidy hybridization

Supplementary Files

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