

# Ditelosomic Alien Addition Lines in Plant Breeding

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## Abstract

Genetic improvement in crops is essential for addressing agricultural challenges, including increased yields, disease resistance, and adaptability to diverse environments. A notable strategy in cytogenetics and plant breeding involves the transfer of alien genes through wide hybridization and chromosome manipulation, enabling the introduction of genetic material from wild species into cultivated crops. This transfer occurs at various levels, such as whole genome, whole chromosome, alien chromosome arms, and chromosomal segments. Ditelosomic Alien Addition Lines (DAALs) emerge as a powerful method, involving the addition of one or a pair of chromosomes from wild species to the cultivated species, leading to the development of distinct plant lines. The creation and identification of ditelosomic lines, exemplified by the development of barley chromosome ditelosomic addition lines in hexaploid wheat, require meticulous steps, including embryo culture and molecular analyses like Genomic In Situ Hybridization (GISH) and molecular markers. These ditelosomic lines, identified through methods like mitosis, karyotype analysis, and molecular markers, offer applications beyond development. Examples like the wheat-rye line WR56 demonstrate resistance to powdery mildew, showcasing the potential of ditelosomic lines in enhancing crop traits for sustainable and resilient agriculture.

## Introduction

Genetic improvement in crops has been a longstanding goal in agriculture, driven by the need for increased yields, disease resistance, and adaptability to various environmental conditions. One of the remarkable strategies in the field of cytogenetics and plant breeding is the transfer of alien genes through wide hybridization and chromosome manipulation. Alien gene transfer involves the introduction of genetic material from related wild species into cultivated species, providing an avenue for enhancing desirable traits such as hardiness,

earliness, disease resistance, and insect resistance. This transfer can occur at different levels, including whole genome transfer, whole chromosome transfer, alien chromosome arms transfer, and transfer of chromosomal segments.

Among the methods employed for alien gene transfer, Ditelosomic Alien Addition Lines (DAALs) stand out as a powerful tool. DAALs involve the addition of one or one pair of chromosomes from related wild species to the normal chromosomal complement of the cultivated species. In this context, ditelosomic lines, specifically, lack one pair of chromosome arms, leading to the development of plant lines with distinct genetic characteristics.

## Development of Ditelosomic Addition Lines

The process of creating ditelosomic addition lines is intricate and requires a series of carefully orchestrated steps. For example, in the development of barley chromosome ditelosomic addition lines in hexaploid wheat cultivar "Asakaze," the Japanese facultative wheat Asakaze was used as the female parent, and the Ukrainian six-rowed winter barley Manas served as the pollinator. The resulting wheat-barley hybrid underwent embryo culture, tissue culture, and backcrossing to achieve stability and genetic uniformity. Subsequent analyses, including Genomic In Situ Hybridization (GISH) and molecular markers, were employed to identify and select the ditelosomic addition lines.

Friebe *et al.* (1999) production and identification of a complete set of intact *Aegilops geniculata* chromosome and telosome additions to common wheat. It included Thirteen disomic, one monosomic wheat-Ae. *geniculata* chromosome additions, two monotelosomic and nine ditelosomic lines. similarly. Jobba *et al.* (1975) produced durum line with 15 pairs of chromosomes is a 1D-disomic addition line. Seed from the 1D-disomic addition line produced two high-molecular-weight glutenin polypeptide subunits not present in durum

wheats, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

### Identification of Ditelosomic Alien Addition Lines

Several methods can be employed to identify ditelosomic alien addition lines, including mitosis, karyotype analysis, meiotic chromosome pairing, cyto-genetic studies, molecular markers, and morphology.

1. **Mitosis:** Mitotic analysis involves examining root tips from germinating seedlings, where the presence of an extra pair of telochromosomes can be observed in metaphase of mitotic cell division.
2. **Karyotype:** The arrangement of chromosomes in a karyotype reveals the presence of an additional pair of telochromosomes along with the complete set of chromosomes.
3. **Meiotic Chromosome Pairing:** Meiotic analysis, such as GISH, allows for the detection and visualization of rye chromatin in wheat-rye ditelosomic addition lines, confirming genetic stability.
4. **Molecular Markers:** The use of specific molecular markers, such as EST-SSR markers, can further validate the presence of alien chromosome arms in ditelosomic lines.
5. **Morphology:** Variations in plant and spike morphology provide additional insights into the genetic composition of ditelosomic addition lines.

Lin *et al.*, (2006) identified two ditelosomic additions and one disomic substitution line among the offspring of the plant derived from immature hybrid embryos of wheat-*Thinopyrum* intermedium disomic addition line Z6 common wheat variety Zhong8601, a plant with a telocentric chromosome. Two random amplified polymorphic DNA molecular markers were identified among 150 random primers used to detect the different arms of the alien chromosome. These might be useful for developing translocation lines with barley yellow dwarf virus (BYDV) resistance.

Bilgic *et al.* (2007) used the Affymetrix Barley1 GeneChip for comparative transcript analysis of the barley cultivar Betzes, the wheat cultivar Chinese

Spring, and Chinese Spring - Betzes ditelosomic chromosome addition lines to physically map barley genes to their respective chromosome arm locations. They mapped 1257 barley genes to different chromosome arms based on their transcript levels in the ditelosomic addition lines. and also validated the physical locations of the genes through comparison with their previous chromosome-based physical mapping, comparative *in silico* mapping with rice and wheat, and single feature polymorphism (SFP) analysis. *In silico* comparative mapping of barley genes assigned to chromosome arms revealed that the average genomic synteny to wheat and rice chromosome arms was 63.2% and 65.5%, respectively. In the 1257 mapped genes, 924 genes identified were found between the appropriate ditelosomic line and Chinese Spring that supported physical map placements.

### Applications of Ditelosomic Lines

Tan *et al.* (2021) characterized a novel wheat-*P. huashanica* line, DT23, derived from distant hybridization between common wheat and *P. huashanica*. Fluorescence *in situ* hybridization (FISH) and sequential genomic *in situ* hybridization (GISH) analyses indicated that DT23 is a stable wheat-*P. huashanica* ditelosomic addition line. FISH painting and PCR-based landmark unique gene markers analyses further revealed that DT23 is a wheat-*P. huashanica* 7Ns ditelosomic addition line. Observation of spike differentiation and the growth period revealed that DT23 exhibited earlier maturation than the wheat parents. Based on specific locus-amplified fragment sequencing technology, 45 new specific molecular markers and 19 specific FISH probes were developed for the *P. huashanica* 7Ns chromosome. Marker validation analyses revealed that two specific markers distinguished the Ns genome chromosomes of *P. huashanica* and the chromosomes of other wheat-related species. These newly developed FISH probes specifically detected Ns genome chromosomes of *P. huashanica* in the wheat background. The DT23 line will be useful for breeding early maturing wheat. The specific markers and FISH probes developed in this study can be used to detect and trace *P. huashanica*

chromosomes and chromosomal segments carrying elite genes in diverse materials.

Turkosi *et al.* (2016) revealed that six-rowed winter barley cultivar Manas is much better adapted to Central European environmental conditions than the two-rowed spring barley Betzes previously used in wheat-barley crosses. The production of wheat-barley ditelosomic addition lines have a wide range of applications both for breeding (transfer of useful genes to the recipient species) and for basic research (mapping of barley genes, genetic and evolutionary studies and heterologous expression of barley genes in the wheat background).

An *et al.* (2022) produced wheat-rye line WR56 through distant hybridization, embryo rescue culture, chromosome doubling and backcrossing. WR56 was then proved to be a wheat-rye 2RL ditelosomic addition line using GISH (genomic in situ hybridization), mc-FISH (multicolor fluorescence in situ hybridization), ND-FISH (non-denaturing FISH), mc-GISH (multicolor GISH) and rye chromosome arm-specific marker analysis. WR56 exhibited a high level of adult plant resistance to powdery mildew caused by *Blumeria graminis f. sp. tritici* (Bgt). This resistance was carried by the added 2RL telosomes and presumed to be different from Pm7 which is also located on chromosome arm 2RL but confers resistance at the seedling and adult stages. WR56 will be a promising bridging parent for transfer of the resistance to a more stable wheat breeding line.

### Conclusion

Ditelosomic alien addition lines represent a powerful tool in plant breeding, allowing for the transfer of desirable traits from wild species to cultivated varieties. Their applications range from disease resistance to environmental adaptability. The identification, development, and characterization of these lines involve a combination of cytogenetic and molecular techniques. As demonstrated by examples like WR56 and Manas barley chromosome additions, ditelosomic lines hold great promise for the development of improved crop varieties with

enhanced traits, contributing to sustainable and resilient agriculture in the face of evolving challenges.

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