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Introgression of a Terminal Segment From Chromosome 3S of *Festuca mairei* Increases Drought Resistance of *Lolium perenne*

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ABSTRACT

Due to climate change, the requirement for increased resilience by grassland systems to onsets of ever-increasing drought stresses makes the development of drought-resistant forage crops a priority for future food and energy security. Novel *x*Festulolium (*Festuca* $\times$ *Lolium*) cultivars offer unique opportunities to combine complementary traits from both genera, delivering agronomic and ecosystem benefits in sustainable grassland systems. Here, we present a cytological and drought-resistance assessment of *x*Festulolium cultivar “AberRoot” (*L. perenne* $\times$ *F. mairei*), an elite cultivar on the UK National Recommended Grass and Clover List. We show that successive breeding generations lead to progressive elimination of most *F. mairei* chromosomes, whilst terminal *F. mairei* introgressions were retained in 76.7% of genotypes, with up to eight introgressions detected on six *L. perenne* chromosomes. Genotypes that carried *F. mairei* introgressions exhibited substantially lower mortality under drought (11.1%–14.8%) compared with non-introgressed lines and the *L. perenne* cultivar used as control (29.6%–35.7%). GAMM analysis revealed a significant interaction between the genomic group and the experimental phase ($\chi^2 = 14.29$, $p = 0.027$), confirming that introgressed genotypes maintained superior growth trajectories during stress and recovered more rapidly upon re-watering. Most notably, over 46% of genotypes that carried a *F. mairei* introgression on the short arm of *L. perenne* chromosome 3 were associated with grasses with the most statistically robust drought resistance response ($p < 0.001$ during Recovery). This finding is consistent with previous evidence linking chromosome 3 in *Festuca* species to drought tolerance, water-use efficiency, and deep rooting. These results demonstrate, using a high-performing cultivar, how a strategy of targeted chromosomal introgression of genes for drought resistance sourced from related stress-tolerant grass species can provide novel and stable adaptive variation without compromise to agronomic performance. The outcomes provide a practical framework for the breeding of climate-resilient forage grasses.

1 | Introduction

The development of new forage and turf grass cultivars typically requires a decade or more from initial parental selection to commercial registration. Once released, successful cultivars often remain on the market for several decades (Reheul et al. 2010).

However, the accelerating pace of climate change increasingly challenges this longevity. Cultivars bred for historical climatic conditions may progressively lose adaptation as environmental stress intensifies. Breeding strategies must therefore anticipate future climates and prioritize resilience to abiotic stress, particularly drought. Advances in climate modelling, high-throughput

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phenotyping, and genomic tools have strengthened the capacity to design such forward-looking breeding programs (Kopecky and Studer 2014; Trnka et al. 2020; Humphreys et al. 2018).

Across Europe, grasslands are exposed to increasingly frequent and severe climatic extremes, including heatwaves and prolonged droughts. Projections indicate that by 2050, the frequency and intensity of drought events in southern and western Europe may double, with similar conditions expanding northwards (Trnka et al. 2020). Drought is among the most significant constraints on grassland productivity, reducing biomass yield, seed production, persistence, and the overall agronomic performance (Casler and van Santen 2010; Staniak and Kocon 2015). For grassland-based livestock systems, such reductions directly threaten forage supply and production stability. One of the principal drought-avoidance mechanisms in grasses is the development of deep and extensive root systems, enabling access to residual soil moisture (Fu et al. 2004). This adaptive response has been documented across multiple grass species under water deficit conditions (Fry and Butler 1989; Qian and Fry 1996, 1997; Huang et al. 1997; Fu et al. 2007a, 2007b; Qian et al. 1997).

Substantial interspecific variation in drought resistance exists among forage grasses. Perennial and Italian ryegrasses (*Lolium perenne* L. and *L. multiflorum* Lam.) are highly productive, nutritious, and palatable but typically drought-sensitive. In contrast, fescues (*Festuca* spp.) are recognized for superior drought tolerance, attributable in part to their deeper and more extensive root systems (Rognli et al. 2010; Humphreys et al. 2018). Tall fescue (*F. arundinacea* Schreb.) has long been regarded as the most drought-tolerant cultivated species; however, recent evidence suggests that certain wild relatives, notably *F. mairei* St.-Yves and *F. glaucescens* Boiss., may exhibit even greater resilience under water deficit (Cernoch and Kopecky 2020). Additionally, cytotypic variation influences drought performance: while diploid meadow fescue (*F. pratensis* Huds.) generally shows limited drought tolerance, autotetraploid cytotypes can display markedly enhanced resistance, in some cases approaching that of *F. mairei* and *F. glaucescens* (Cernoch and Kopecky 2020).

Despite their adaptive value, direct use of wild fescues in forage systems is constrained by unfavorable agronomic traits. Species such as *F. mairei* and *F. glaucescens* produce coarse, less palatable foliage and form uneven swards. Autotetraploid *F. pratensis*, although drought-tolerant, often exhibits reduced seed set associated with multivalent formation during meiosis (Kopecky et al. 2009). Consequently, the most effective strategy to exploit fescue-derived drought tolerance is introgression breeding, whereby specific chromosomal segments carrying adaptive genes are transferred into agronomically superior ryegrass backgrounds. Such transfer is facilitated by homoeologous chromosome pairing and recombination between *Festuca* and *Lolium* genomes (Humphreys et al. 1995; Kopecky et al. 2008; Majka et al. 2020).

Multiple studies have demonstrated successful introgression of drought-related loci from *Festuca* into *Lolium* species. Quantitative trait loci (QTLs) conferring drought resistance have been transferred from *F. arundinacea* into *L. multiflorum*, with genomic in situ hybridization (GISH) revealing discrete chromatin segments integrated within ryegrass chromosomes (Humphreys and Thomas 1993; Humphreys and Pasakinskiene 1996). Notably,

several independent studies have identified chromosome 3 as a key reservoir of stress-resistance genes. Introgressed segments derived from *F. glaucescens* or *F. pratensis* and localized to chromosome 3 in *Lolium* backgrounds have been associated with enhanced drought tolerance (Humphreys et al. 2005; Barnes et al. 2014). QTL analyses in *F. pratensis* further identified a major locus spanning much of chromosome 3 that contributes to severe drought resistance (Alm et al. 2011). Together, these findings highlight chromosome 3 as a conserved and recurrent source of adaptive variation for water-deficit tolerance across *Festuca* species.

Among wild fescues, the North African xeromorphic species *F. mairei* represents a particularly valuable source of drought-adaptive traits. Although phylogenetically more distant from *Lolium* than other broad-leaved fescues (Catalan et al. 2004), substantial homoeologous pairing between *L. perenne* and *F. mairei* enables effective chromosomal recombination and introgression (Chen and Sleper 1999; Cao et al. 2000, 2003). The ×*Festulolium* cv. “AberRoot”, derived from crosses between autotetraploid *L. perenne* ($2n=4\times=28$) and *F. mairei* ($2n=4\times=28$), was developed through such an approach and is currently listed on the UK National Recommended Grass and Clover List (Humphreys et al. 2014).

In this study, we address several previously unexplored aspects of ×*Festulolium* biology and breeding. Specifically, we assessed the presence and frequency of introgressed *F. mairei* chromosomal segments and tested their association with drought-resilience phenotypes, with particular emphasis on *F. mairei* introgressions located on the terminal region of the short arm of *L. perenne* chromosome 3. To our knowledge, this is the first detailed report documenting the transfer of drought resistance from *Festuca mairei*, a highly drought-adapted North African fescue, into perennial ryegrass, together with a comprehensive cytological characterisation of the resulting introgressions. Furthermore, this represents the largest combined cytological and phenotypic study ever undertaken on a single ×*Festulolium* cultivar, providing unprecedented resolution of the relationship between genome composition and drought response at the individual plant level. Finally, we report for the first time in a *Festulolium* polyploid introgression population that gene dosage does not appear to influence phenotypic outcome. Genotypes carrying a *F. mairei* introgression on chromosome 3 in a single dose exhibit drought resistance equivalent to those carrying it in higher doses, suggesting that even a single copy of the introgressed segment is sufficient to confer a functionally meaningful agronomic advantage. Together, these findings advance our understanding of how targeted chromosomal introgression can stabilise adaptive variation within elite forage germplasm and provide a practical framework for breeding climate-resilient grasses capable of sustaining productivity under increasing environmental stress.

2 | Material and Methods

2.1 | Plant Material

We used seeds of ×*Festulolium* cv. AberRoot ($2n=4\times=28$). This cultivar was developed following the initial interspecific hybridization of autotetraploid *Lolium perenne* HSGs (High Sugar Grass) cultivar with the allotetraploid drought and heat-tolerant

North African species *Festuca mairei* (Atlas fescue), as described in Humphreys et al. (2014). As there is currently no allocation of a specific *Festulolium* section within the UK National Recommended Grass and Clover List (NRGCL; <https://ahdb.org.uk/>), cv. AberRoot was included (2022–2026) with, and compared directly to, intermediate heading and best-performing tetraploid perennial ryegrasses. AberRoot is at F₇-F₈ generation after the initial hybridization. As a control for drought tolerance tests, 14 plants of widely used autotetraploid *L. perenne* cv. Kertak were used.

2.2 | Karyotyping

Seeds were germinated in Petri dishes on wet filter paper and seedlings were planted in 30-mm pots and kept in the growth chamber (14 h/20°C day, 10 h/17°C night). After 3–4 weeks, plantlets were transferred to a hydroponic culture with aerated solution of Hydroponex at 0.9 g/L (Hu-Ben, Čerčany, Czech Republic). After about 1 week, actively growing root tips were collected from individual plants to ice water for 26–28 h, fixed in a 3:1 mixture of absolute ethanol and glacial acetic acid at 37°C for 7 days, and handled according to Kopecky et al. (2005). In situ probing with total genomic DNA (GISH) was done according to Masoudi-Nejad et al. (2002). Total genomic DNA of *F. mairei* was labeled with digoxigenin using DIG-Nick Translation Kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's recommendation and used as a probe. For the 45S rDNA probe, clone pTa71 (Gerlach and Bedbrook 1979) containing a 9 kb EcoRI fragment of wheat ribosomal DNA was labeled with biotin by nick translation. This 9 kb fragment carries the 18S-5.8S-26S cluster of ribosomal RNA genes (here referred to as 45S rDNA). Total genomic DNA of *L. perenne* was sheared to 200–500-bp fragments by boiling for 45 min and used as a block, with the probe to block ratio of ca. 1:100. The sites of probe hybridization were detected using Anti-DIG-FITC (Roche Applied Science) and streptavidin-Cy3 conjugate (Amersham, Piscataway, NJ, USA); counterstaining was with 1.5 µg/mL 4',6-diamidino-2-phenylindole (DAPI) made in Vectashield antifade solution (Vector Laboratories, Newark, CA, USA). Slides were viewed under an Axio Imager Z.2 Zeiss microscope equipped with Cool Cube 1 camera (Metasystems, Altlußheim, Germany). ISIS software v.5.4.7 (Metasystems) and ADOBE PHOTOSHOP software were used for picture processing. In total, 120 plants of cv. AberRoot were karyotyped.

2.3 | Drought Resistance Tests

The drought treatments were divided temporally into three successive experimental phases ["Experimental Phase"]: [1] growth during irrigation prior to the drought treatment (50 days, the first scoring was done after 1 week of growth; the "Establishment" phase), [2] no irrigation (50 days; the "Drought" phase), and [3] regrowth following irrigation after the drought treatment (29 days; the "Recovery" phase). All plants used were randomly selected genotypes drawn from four distinct genomic groups ["Genomic group"]: those with introgressed segment(s) from *F. mairei* in the terminal region of the short arm of chromosome 3 ["3S_int"], plants

with introgression(s) on different chromosomes ["other_int"], plants with no detectable introgressions ["no_int"], and plants of the *L. perenne* cv. Kertak without fescue-derived introgressions ["Kertak"]. All plants were planted in a randomized design on April 16th, 2024, in four experimental rainout shelters (2.5 × 5.5 m) at the Institute of Experimental Botany in Olomouc (Czech Republic). The soil type was Gleyic Fluvisol. A total of 40 plants were established in each rainout shelter, including four plants of *L. perenne* cv. Kertak. A border of tall fescue plants surrounded the experimental plants within each rainout shelter to prevent water entry from the outside (for the design, see Table S1). Initially, plants were regularly irrigated by tap water to ensure moist soil and rapid development (the "Establishment" phase; until June 5th). After 7 weeks of plant establishment, irrigation was withheld and plants were maintained without water for 7 weeks (the "Drought" phase; until July 25th). Subsequently, the irrigation was recommenced ("Recovery" phase). Visual observations were made weekly throughout the trial from the day of planting, through establishment, and the subsequent drought and recovery periods. Visual scores were completed after 4 weeks of the recovery phase. The growth response was assessed visually on an ordinal scale ranging from 1 (plant death) to 9 (vigorous, healthy growth). The temperatures during the drought trial are given in Table S1.

2.4 | Statistical Analysis

The primary response variable consisted of ordinal scores of the plant growth response ("Growth response"; 1 to 9), and was first visualized using boxplots over each assessed time, and for each of the four genomic groups over three experimental phases. The growth response of individual plants was analyzed using a Generalized additive mixed model (gamm). This approach was chosen to account for the nonlinear dynamics of the experimental phases and the hierarchical structure of the repeated measures design (Wood 2017). The model was fitted using the ocat family (ordered categorical) with an identity link function, which is appropriate for cumulative link modeling of ordinal data (Sullivan and Artino, 2013). The fixed effects structure included a full interaction between "Genomic group" (representing the groups of plants described above) and "Experimental Phase" (the three successive phases: "initial", "drought", and "recovery") used to assess baseline differences between groups across phases. To capture the nonlinear temporal dynamics, factor-smooth interactions were employed. Specifically, separate thin-plate regression splines were fitted for each combination of "Genomic group" and "Experimental Phase". Based on preliminary analyses, the basis dimension was set to $k = 5$ and restricted with a cubic shrinkage penalty ($bs = "cs"$) to prevent overfitting while allowing the model to penalize nonlinear trends down to zero (linearity) where supported by the data. To account for the dependency of repeated measurements within the same subjects, random intercepts for genotype ("Genotype") and individual plant ("ID") nested within Genotype were included as random smooth terms ($bs = "re"$) (Pedersen et al. 2019). Model parameters were estimated using Restricted Maximum Likelihood (REML). Convergence was verified via the Hessian matrix and inspection of the eigenvalue range. The basis dimension (k) was

validated using the `gam.check` procedure to ensure that the effective degrees of freedom (edf) did not saturate the basis size. Statistical significance of the smooth terms was assessed using Wald-type tests.

During model refinement, Genotype was found to explain a negligible proportion of the total variance, with the estimated variance component approaching zero. Furthermore, inclusion of Genotype as a random factor did not lead to a lower AIC (Akaike Information Criterion) or improved model performance. Therefore, Genotype was removed from the final specification to ensure model parsimony. The final random effects structure consisted of a random intercept for ID to account for the dependency within repeated measurements. Two gamm models were performed: the first (`gamm1`) included all four genomic groups, including *L. perenne* cv. Kertak, whilst the second (`gamm2`) was restricted to the three *×Festulolium* genomic groups. All other model settings remained identical.

To visualize the temporal dynamics, the estimated smooth functions for each genomic group were plotted alongside their 95% point-wise confidence intervals (CI). Within each experimental phase, the divergence between genomic groups was assessed on the degree of overlap between these 95% confidence intervals. Periods where the confidence intervals of two groups did not overlap were interpreted as intervals of significant difference in response levels. To ensure a fair visual representation of the experiment's timeline, the x-axis for each phase was scaled proportionally to its actual duration using a free-space facet grid layout. Additionally, to compare specific group differences, Estimated Marginal Means (EMMs; Lenth 2024) were used to compare the average response level between treatments within each phase, using both post hoc pairwise comparisons and specific contrasts (`gamm2: no_int` vs. each other genomic group, `no_int` vs. mean of other genomic groups). All statistical analyses were performed in R version 4.3.2 (R Core Team 2023). Generalized additive mixed models were fitted using the `mgcv` package (Wood 2017). Post hoc pairwise comparisons were conducted using the `emmeans` package (Lenth 2024), and model diagnostics and

visualizations were supported by the `gratia` (Simpson 2024) and `ggplot2` (Wickham 2016) packages.

3 | Results

3.1 | Genome Composition

Using GISH, we assessed the genome composition of all 120 plants of *×Festulolium* cv. AberRoot. In all plants, the *L. perenne* genome predominated, whereas *F. mairei* chromosomes had been partially or completely eliminated over successive generations of seed multiplication following the initial development of the F_1 hybrid, in which the chromosome complements of the two species must have been equal.

However, the *F. mairei*-derived chromatin was still evident in the majority (76.7%) of AberRoot plants, only in the form of introgressions located on *L. perenne* chromosomes, resulting from preceding interspecific crossover events. In all cases, *F. mairei* chromatin was present as short terminal introgressions on *L. perenne* chromosomes, with the terminal introgression on the short arm of chromosome 3 being the most frequent (present in 46.7% of plants; Figure 1a).

Three types of *F. mairei* introgressions were distinguished:

- A terminal segment on the short arm of *L. perenne* chromosome 3. This chromosome arm carries a secondary constriction. Although chromosomes 2 and 7 also possess secondary constrictions, chromosome 3 can be readily distinguished from the other two by the position of the 45S rDNA probe signal (Thomas et al. 1996; Kopecky et al. 2010). This type is hereafter referred to as the 3S introgression;
- Terminal segments on the arms of various *L. perenne* chromosomes. Based on chromosome lengths and arm ratios, these are most frequently located on chromosomes

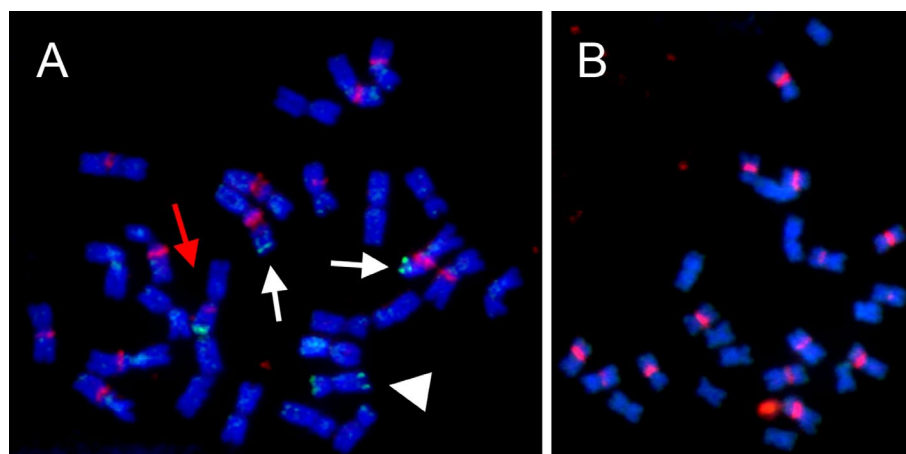


FIGURE 1 | Cytological analysis of different plants of cv. AberRoot. Genomic in situ hybridization was performed using total genomic DNA of *Festuca mairei* (green color) and 45S rDNA (red color) as probes and blocking DNA of *Lolium perenne*. Chromosomes were counterstained by DAPI (blue color). The position of the 45S rDNA locus identifies chromosome 3. Note the variability in genome composition ranging from (A) plants with 3S introgression (red arrow), terminal introgressions on different chromosomes (white arrows) and introgressions on both arms of a single chromosome (white arrowhead) to (B) a plant with no detectable introgression.

1, 5, or 6, according to the published *L. perenne* karyotype (Thomas et al. 1996; Kopecky et al. 2010);

- c. Terminal introgressions on both arms of a single chromosome, other than chromosome 3.

The frequency of alien *F. mairei* introgressions varied among plants, with a maximum of eight introgressions on six *L. perenne* chromosomes (Table 1; Table S1). The *F. mairei* chromatin on the short arm of chromosome 3 was relatively frequent (56 of 120 plants), most commonly present in a single dose, with the remaining homoeologous *L. perenne* chromosome copies intact. Out of these 56 plants, only 19 solely carried *F. mairei* introgressions. Four plants were found to carry this introgression in a double dose, whilst 15 plants carried the 3S introgression in single dose. Chromosomes with *F. mairei* introgressions on both arms were rare; observed in 17 of the 120 plants studied (14.1%). Overall, there were 0.62 3S introgressions per plant and 1.03 introgressions at other chromosomal locations per plant. Among all plants examined, 28 (23.3%) showed no detectable *F. mairei*

chromatin and thus fully resembled the chromosome constitution of the maternal *L. perenne* parent (Figure 1b).

3.2 | Performance Under Drought Stress and During the Recovery Period

The plants were divided into groups based on the type of introgressions: “3S-int” refers to the plants that carried at least one introgression in the distal part of the short arm of chromosome 3 (irrespective of whether this introgression was in single or double dose, or if other introgressions were also present), “other_int” refers to plants that carried at least one introgression other than on the short arm of chromosome 3 and “no_int” refers to plants without any detectable *F. mairei* introgression (which represented controls). Based on descriptive data (Figure 2), these groups differed in their response to drought. In general, plants carrying *F. mairei* introgressions performed better during and after drought stress compared with the *L. perenne* cv. Kertak

TABLE 1 | Number of plants of cv. AberRoot having various *F. mairei* introgressions in different doses (frequencies in parentheses).

Type of introgression	Single dose	Double dose	Triple dose	None
3S	40 (33.3)	14 (11.7)	2 (1.7)	64 (53.3)
Other (single arm)	53 (44.1)	11 (9.2)	2 (1.7)	54 (45.0)
Other (both arms)	13 (10.8)	4 (3.3)	0 (0.0)	103 (85.8)

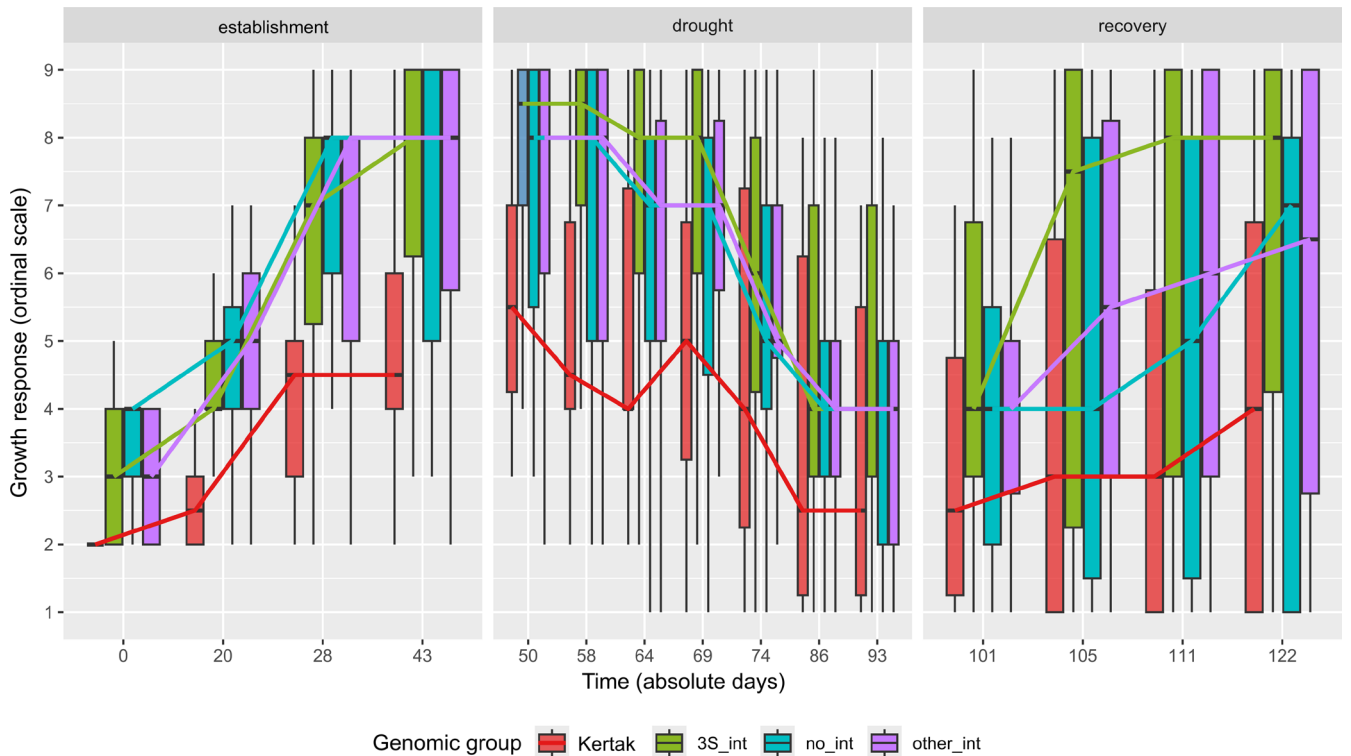


FIGURE 2 | Performance of the plants with different genome composition before, during, and after drought stress. The plant vigour was evaluated weekly using a 9-graded scale for all plants and scored throughout the entire 4 months of the experiment. Plants were scored as 1 = dead plant, 2 = severely stressed, but retaining one green leaf, 9—highly vigorous healthy plant (with no evidence of wilting). In order to illustrate the plant growth responses, boxplots of plant growth were generated for each genomic group across the three experimental phases. Data are presented by recording time within the Establishment, Drought, and Recovery phases, with lines connecting the medians to highlight temporal trends.

and the AberRoot genotypes lacking *F. mairei* chromatin, which served as controls. Approximately one-third of plants without *F. mairei* introgressions (29.6%) and *L. perenne* cv. Kertak plants (35.7%) died during the drought and recovery periods. In contrast, plants containing *F. mairei* introgressions exhibited substantially lower mortality rates: 14.8% in plants with a 3S introgression and 11.1% in plants with alternative *F. mairei* introgressions (Table 2). All groups maintained growth during the first week of drought. Thereafter, plants from all four groups began to show stress symptoms following water withdrawal, leading to a significant decline in plant health after 6 weeks of drought. At the end of the 7-week drought phase, irrigation was restored, and surviving plants reinitiated growth and began recovery, particularly during the first 2 weeks of re-watering.

During the initial pre-drought phase, only minor differences in growth were observed among the four genomic groups. However, water withdrawal had a substantially greater and differential impact across groups. Stress effects were most pronounced in plants lacking *F. mairei* introgressions. Among plants retaining *F. mairei* chromatin, those with a 3S introgression were the least affected (Figure 2). Differences among genomic groups were most evident during the recovery period. Plants containing *F. mairei* chromosomal segments recovered more rapidly and more effectively than those without introgressions (Figure 2). To rigorously evaluate genomic group effects while accounting for repeated measures and subject-specific variability, two gamm models were constructed. Both yielded highly consistent results (Table 3). In both models, the fixed effects (Genomic group, Experimental Phase, and their interaction) and smooth terms (temporal dynamics) explained approximately 7%–8% of total deviance (gamm1: 7.83%; gamm2: 7.24%). When accounting for random effects, the full mixed model explained 45.38% and 44.21% of the total deviance, respectively. Substantial increase of explained deviance indicates that a substantial proportion of the variance is attributable to inter-individual differences. In both models, analysis of deviance (Wald-type tests; Table 3) revealed a significant interaction between Genomic group and Experimental Phase, indicating that genomic

group effects varied across phases (Establishment, Drought, Recovery). While the main effect of the Genomic group was not significant, the Experimental Phase exhibited a strong main effect ($\chi^2=40.34$, $p<0.001$), reflecting substantial physiological shifts across the study period. The analyses further demonstrated that growth dynamics were highly phase-dependent in both models. All smooth terms representing temporal trends within specific genomic group $\times$ experimental phase combinations were highly significant ($p<0.001$ in most cases; Table 3). The fitted smoothing functions for each genomic group were almost identical in both gamm models, confirming a consistent pattern: an increase during Establishment, a decline during Drought, and a secondary increase during Recovery (Figure 3).

3.2.1 | Initial Progression (Establishment Phase)

All genomic groups showed a significant upward trend. All $\times$ Festulolium cultivar genomic groups followed similar non-linear trajectories, with the “3S_int” group showing the most complex and steepest increase (edf=2.84). The *L. perenne* “Kertak” group displayed the most stable profile with the lowest degree of nonlinearity (edf=1.53), indicating a slower and more linear baseline shift.

3.2.2 | Reversal During Stress (Drought Phase)

The Drought phase was characterized by a statistically significant decline in growth scores across all genomic groups. All $\times$ Festulolium genomic groups displayed rapid and highly non-linear decrease (edf $\approx$ 2.16–3.03) in response to the drought. The *L. perenne* “Kertak” group followed a simpler downward trend (edf=1.87).

3.2.3 | Secondary Increase (Recovery Phase)

Re-watering resulted in renewed growth, with recovery patterns differing significantly among genomic groups. The “3S_int” and “Other_int” groups showed robust and significant re-growth (edf $\approx$ 1.8–1.9). The “No_int” group exhibited a more attenuated, nearly linear increase (edf=1.15). The *L. perenne* “Kertak” group showed the slowest increase, which was only marginally significant (edf=0.77, $p=0.049$).

Post hoc pairwise comparisons (Tukey-adjusted) of estimated marginal means (EMMs) for each experimental phase, representing model-predicted responses averaged across phase duration, demonstrated progressive differentiation among genomic groups in both models (Table S2). In the gamm1 model, which included all genomic groups with *L. perenne* “Kertak” as the reference, no significant differences were observed among genomic groups during the establishment phase, confirming a uniform baseline prior to drought. During the Drought phase, the “3S_int” group differed significantly from the *L. perenne* “Kertak” group ($p=0.023$), whereas the “No_int” and “Other_int” groups did not ($p=0.565$ and $p=0.227$, respectively). In the Recovery Phase, divergence among groups increased further. Both “3S_int” ($p<0.001$) and “Other_int” ($p=0.022$) groups exhibited significantly higher growth scores than the *L. perenne*

TABLE 2 | Mortality of plants with different genome composition during drought and recovery period.

Genotype	No. of plants	Mortality during drought period	Mortality during recovery period
3S introgression (3S_int)	56 (54)	14.8%	14.8%
Other introgression (Other_int)	36 (36)	8.3%	11.1%
No introgression (No_int)	28 (27)	18.5%	29.6%
<i>L. perenne</i> cv. Kertak	14 (14)	28.6%	35.7%

Note: Only plants that survived until the beginning of the drought period (in brackets) were taken into account for the mortality assessment.

TABLE 3 | Results of the analysis of deviance for two fitted gamm models (gamm1 and gamm2).

Factor	gamm1: with <i>L. perenne</i> cv. Kertak			gamm2: without <i>L. perenne</i> cv. Kertak		
	df/edf	Chi-square (χ^2)	<i>p</i>	df/edf	Chi-square (χ^2)	<i>p</i>
Parametric terms (fixed effects)						
Genomic group	3	7.28	0.064	2	0.34	0.849
Experimental phase	2	40.34	<0.001	2	92.06	<0.001
Genomic group: Experimental phase	6	14.29	0.027	4	13.29	0.01
Smooth terms (temporal dynamics)						
Phase 1: Establishment						
Kertak	1.53	35.95	<0.001	—	—	—
3S_int	2.84	382.22	<0.001	2.84	376.7	<0.001
no_int	2.7	142.34	<0.001	2.69	140.18	<0.001
other_int	2.52	215.83	<0.001	2.51	213.89	<0.001
Phase 2: Drought						
Kertak	1.87	72.06	<0.001	—	—	—
3S_int	3.03	602.01	<0.001	3.02	590.28	<0.001
no_int	2.16	235.33	<0.001	2.16	230.95	<0.001
other_int	2.48	399.86	<0.001	2.48	393.73	<0.001
Phase 3: Recovery						
Kertak	0.77	3.28	0.049	—	—	—
3S_int	1.89	90.16	<0.001	1.89	89.31	<0.001
no_int	1.15	19.16	<0.001	1.15	19.07	<0.001
other_int	1.82	60.6	<0.001	1.82	60	<0.001
Random effects						
Individual subject (ID)	122.66	3131.76	<0.001	110	2722.54	<0.001

Note: Parametric terms represent the main effects and their interaction, while smooth terms describe the nonlinear temporal trajectories for each group within experimental phases. Significant *p* values ($p \leq 0.05$) are in bold. Abbreviations: χ^2 : Wald-type chi-square statistic; edf: Effective degrees of freedom.

“Kertak” group. The “No_int” group occupied an intermediate position, showing a trend toward higher growth scores that did not reach statistical significance after multiple comparison adjustments ($p = 0.306$).

In the gamm2 model focusing on $\times$ Festulolium genomic groups with “No_int” as the reference group (Table S2), contrast comparisons revealed only one significant difference: between “3S_int” and “No_int” groups during the recovery phase. Similarly, a specific contrast comparing “No_int” against the mean response of the “3S_int” and “Other_int” groups showed a single significant difference in the recovery phase (logit estimate = -1.44 , SE = 0.68 , $t = -2.131$, $p = 0.033$) but not in the Establishment (logit estimate = 0.35 , SE = 0.74 , $t = 0.47$, $p = 0.636$) and Drought phases (logit estimate = -0.83 , SE = 0.61 , $t = -1.36$, $p = 0.174$).

These results are consistent with the predicted 95% confidence intervals of the mean scores for each genomic group across the three experimental phases (Figure 3). Specifically, they

highlight a significantly higher response in plants of the “3S_int” group, and partially also the “Other_int” group during the recovery phase when compared to plants of the “No_int” and “Kertak” genomic groups.

4 | Discussion

This study advances previous work on the development and use of novel $\times$ Festulolium cultivars within a holistic breeding framework aimed at delivering climate-smart and sustainable grassland agriculture. To our knowledge, this is the first detailed report demonstrating the efficacy of a targeted breeding programme designed to enhance drought resistance in *Lolium perenne* through controlled introgression of alien chromatin from the North African fescue *Festuca mairei* (Atlas fescue). Previous studies using the closely related species *F. pratensis* (meadow fescue) have shown that drought resistance, a complex quantitative trait, is distributed widely across the fescue genome

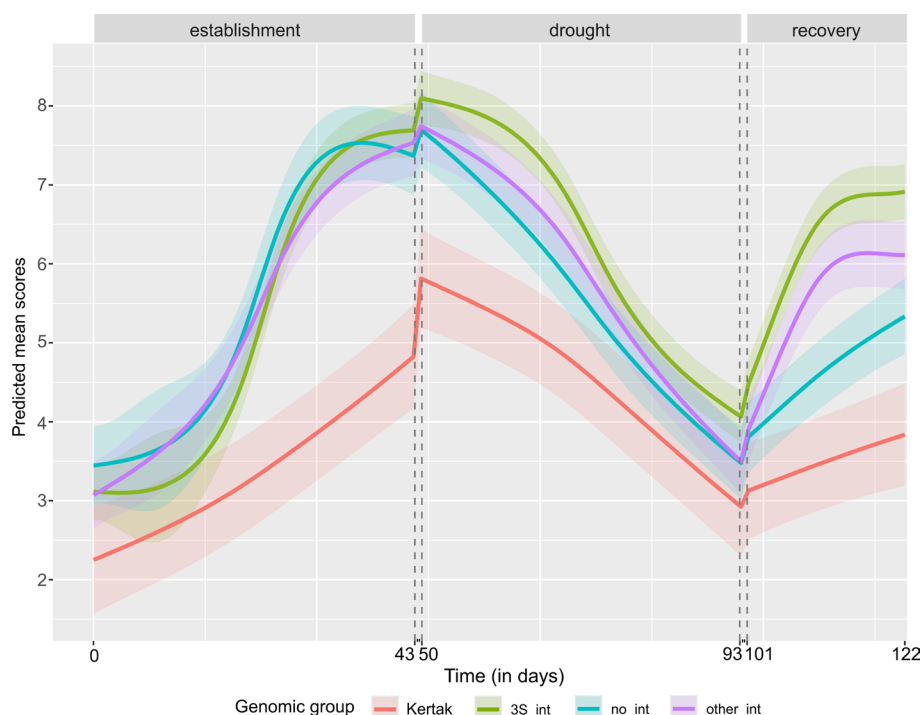


FIGURE 3 | Predicted longitudinal trajectories of growth scores across the experimental timeline. The plot displays the predicted mean scores for each treatment group across the three experimental phases: (1) Establishment, (2) Drought, and (3) Recovery. The solid lines represent the fitted values derived from the Generalized Additive Mixed Model (GAMM), calculated as the weighted average of predicted probabilities for each ordinal category. The shaded areas around each line indicate the 95% confidence intervals, representing the group-level effects after excluding subject-specific random variation (ID). Two vertical dashed lines separate a week period between two consecutive phases (time-compressed), which correspond to the initial changes of environment between phases and hence are not incorporated into the model.

(Alm et al. 2011). A similar genomic architecture would be expected in *F. mairei*. From this perspective, it might appear improbable that introgression of only limited fescue chromosomal segments into *Lolium* could deliver meaningful gains in drought resistance. However, our results show otherwise and are consistent with earlier work on other *Lolium* × *Festuca* hybrids (Humphreys and Thomas 1993; Humphreys et al. 2005).

Three key processes appear to underpin the success of this strategy: (1) interspecific recombination events involving *F. mairei* chromatin that, in combination with the *Lolium* background, enhance drought resistance; (2) their apparent selective advantage across generations, given the high frequency of the same introgression; and (3) near-complete elimination of the remaining fescue genome, thereby minimizing linkage drag and preserving elite agronomic performance. Together, these processes have enabled the development of a climate-resilient, high-quality ryegrass-type cultivar. Cv. AberRoot analysed here is included on the current UK National Recommended Grass and Clover List (NRGCL) and exhibits agronomic performance equivalent to or superior to the best-performing tetraploid intermediate-flowering *L. perenne* cultivars currently available on the UK seed-market (Humphreys et al. 2014).

Cytological screen using GISH to detect *F. mairei* chromatin in AberRoot is perhaps the most extensive effort undertaken in a ×*Festulolium* cultivar. More than 75% of AberRoot individuals screened carried *F. mairei* introgressions. In all cases, individuals with these introgressions showed improved drought

resistance relative to their sister AberRoot genotypes lacking the fescue chromatin. Notably, the introgressions were typically present in a single dose within the tetraploid genome, with three homologous *L. perenne* chromosome copies retaining native (*Lolium*) alleles at the corresponding loci. Despite this numerical disadvantage, the fescue-derived segments must have remained functionally effective, conferring both enhanced tolerance to drought stress and improved recovery following re-watering. Multiple crossovers over several (seven to eight) generations resulted in the retention of only terminal segments of alien (*Festuca*) chromatin. Preferential homoeologous chromosome recombinations in the subtelomeric regions have been observed in other *Festuca* × *Lolium* hybrids (Kopecky et al. 2010). Among these introgressions, a segment translocated to the distal part of the short arm of *L. perenne* chromosome 3 was both the most frequent (~50% of all genotypes) and the most strongly associated with drought resistance ($p < 0.001$). Its preferential retention suggests selective advantage and highlights chromosome 3 as a conserved hotspot for drought-adaptive loci.

These findings align with earlier reports of drought-resistant derivatives of *Lolium* × *Festuca* hybrids (Humphreys and Thomas 1993; Humphreys and Pasakinskiene 1996; Humphreys et al. 2005), in which chromosomal segments from *F. arundinacea* or *F. glaucescens* were similarly translocated to the short arm of *Lolium* chromosome 3. In each case, transfer of fescue chromatin conferred improved drought resistance relative to the ryegrass parent. Importantly, those earlier breeding programmes employed diploid introgression lines, where fescue alleles were

present in a 1:1 ratio with homologous *Lolium* alleles. In contrast, the present study demonstrates that in tetraploid AberRoot, fescue alleles remain effective despite being outnumbered three to one by *L. perenne* homologues. Previous work has also shown that $\times$ Festulolium introgression lines, including AberRoot, possess deeper and more extensive root systems and improved water-use efficiency compared with *Lolium* parental genotypes (Humphreys et al. 2018; Humphreys and Zwierzykowski 2020). Thus, it appears plausible that the effects observed here are related to the root system characteristics.

Cv. AberRoot originated from tetraploid *L. perenne* $\times$ *F. mairei* F_1 hybrids containing equal parental genome complements. The present study demonstrates that successive intermating led to substantial genome restructuring: most of the *F. mairei* chromatin was eliminated, leaving a limited number of introgressions, all in terminal position on otherwise intact *L. perenne* chromosomes. Such genome instability and progressive *Lolium* dominance have been widely reported in amphiploid $\times$ Festulolium hybrids, particularly those involving *F. pratensis* or *F. arundinacea* (Glombik et al. 2020; Humphreys and Zwierzykowski 2020). Our previous work showed that silencing of *Festuca* alleles of several kinetochore genes prior to and during meiosis leads to occasional improper kinetochore assembly on *Festuca* chromosomes, followed by their non-attachment to karyokinetic spindle, lagging in anaphases, micronucleation and loss (Majka et al. 2023). It appears certain that in hybrids with *Lolium*, some *Festuca* chromosomes are more prone to elimination than others (Kopecky et al. 2019). This dominance of *Lolium* parental genome in *Festuca* $\times$ *Lolium* hybrids was observed also at the level of transcription of nuclear and rDNA genes (Glombik et al. 2021; Mahelka et al. 2023). While hampering genome stability in subsequent generations of amphiploid cultivars, it creates a valuable opportunity for effective introgression breeding. AberRoot reverted from the initial amphiploid state to a tetraploid *L. perenne*-like introgression line. The limited number and terminal positioning of fescue segments suggest minimal linkage drag, consistent with the superior fertility, seed production, and agronomic performance of $\times$ Festulolium cultivars, which comprises the desirable attributes of both its *Lolium* and *Festuca* parents.

Increasingly, holistic approaches in forage breeding are becoming more frequently employed that embrace targets additional to food production, and in particular, ecosystem services capable of lowering the “environmental footprint” of livestock agriculture (Humphreys and Zwierzykowski 2020). AberRoot and alternative $\times$ Festulolium cultivars have demonstrated improved efficiency of protein utilization by ruminants compared with *Lolium* cultivars (Humphreys et al. 2014; Kamau et al. 2020; Muhandiram et al. 2023). Kamau et al. (2020) demonstrated using simulated ruminant conditions that proteolysis was lower in the two $\times$ Festulolium cultivars than in *L. perenne* and *L. multiflorum*. Muhandiram et al. (2023) subsequently found similar advantages in protein provision from ensiled plant material using the same two $\times$ Festulolium cultivars. Taken together, these studies indicate a potential environmental advantage and lowering harmful greenhouse gas emissions (GHG) by livestock using $\times$ Festulolium cultivars with more efficient protein retention. Heat-shock proteins, active in drought- and heat-tolerant fescues such as *F. mairei*, have been proposed to potentially

mitigate proteolysis in ruminants and support better protein retention (Shaw, 2006). If confirmed, cultivars such as AberRoot may contribute simultaneously to climate resilience and reduced livestock-associated emissions.

Drought resistance in $\times$ Festulolium is closely associated with deeper and more extensive rooting systems providing access to water reserves inaccessible to shallow rooting grasses like *Lolium* species (Barnes et al. 2014; Cernoch and Kopecky 2020). A detailed phenomics study on the root growth over four consecutive months revealed that both *L. perenne* $\times$ *F. mairei* including cv. AberRoot and *L. perenne* $\times$ *F. glaucescens* hybrids have larger deeper roots than *L. perenne* (Humphreys et al. 2018). The correlation between the presence of *Festuca* 3S introgression, deep rooting and increased drought resistance was revealed also in other studies (Humphreys et al. 2005; Barnes et al. 2014). An association between the presence of fescue genes at the terminal region of *L. perenne* chromosome arm 3S, leaf extension and root growth under drought stress has been observed also in chromosome substitution lines (Turner et al. 2010). The drought resistance reported here in AberRoot combined with its known deep and extensive rooting properties is likely to be interconnected. Kell (2011) promoted the development of deep rooting grasses for improved carbon-sequestration in soils. Through their large root systems, $\times$ Festulolium cultivars like AberRoot have indeed demonstrated their positive impact on soil C acquisition (Gregory et al. 2013; Li et al. 2017). The use of $\times$ Festulolium cultivars has also improved soil structure and helped reduce soil compaction (Muhandiram et al. 2020). Compacted soils caused by drought periods reduce aeration, water transport, access to soil nutrients, and root growth. Heavily compacted soils also encourage surface flooding after the rains return. $\times$ Festulolium cultivars have been shown to improve soil structure, increasing soil porosity and after heavy rains, reduce surplus overland flows of water that can lead to flooding (Macleod et al. 2013). In addition to their potential for improved water-use-efficiency (WUE), $\times$ Festulolium varieties including AberRoot demonstrate improved nitrogen-use-efficiency (NUE) when compared to *Lolium* cultivars (Muhandiram et al. 2024).

We are aware of the limitations of our study, most notably that the phenotypic evaluation was conducted over a single growing season, and that replication across multiple years and environments would strengthen the robustness of our conclusions. Future work should also incorporate direct assessment of root architecture and physiological traits to further elucidate the mechanisms underlying the enhanced drought tolerance conferred by *F. mairei* introgressions. Despite this, our pilot study provides a clear rationale for expanding cytogenetically informed introgression breeding as a practical strategy for developing climate-resilient forage grasses. Future breeding programmes should prioritise the targeted retention of chromosome 3 introgressions from drought-adapted *Festuca* species, given the consistent and robust association with drought resistance demonstrated here and in previous studies. From a genetic perspective, a key future research direction is the identification of genes and their regulatory elements present in *Festuca* but absent in *Lolium* that may be responsible for the observed drought response, particularly those located within the chromosome 3 introgression region. Complementing this, physiological investigations aimed at elucidating which specific drought-resistance mechanisms (such as osmotic adjustment,

stomatal regulation, or root hydraulic conductance) are deployed by *Festuca* species will be essential for fully understanding the basis of the enhanced resistance. Marker-assisted selection targeting the chromosome 3 region could subsequently accelerate cultivar development and improve the precision with which adaptive variation is captured within elite ryegrass-type germplasm. Beyond drought resistance, the broader portfolio of beneficial traits documented in $\times$ *Festulolium* cultivars including deeper rooting, improved carbon sequestration, reduced soil compaction, enhanced nitrogen- and water-use efficiency, and more efficient protein retention in ruminants suggests that future varieties could simultaneously address multiple sustainability challenges facing grassland agriculture. Crucially, AberRoot demonstrates that these attributes can be assembled without compromise to forage yield or quality, as evidenced by its continued inclusion on the UK National Recommended Grass and Clover List. As climate projections indicate increasingly frequent and severe summer droughts across temperate grassland regions, deploying $\times$ *Festulolium* cultivars combining the agronomic performance of *Lolium* with the stress resilience of *Festuca* represents one of the most immediately practical and scalable responses available to grassland farmers and breeders.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available in Tables S1 and S2 of this article.

References

- Alm, V., C. Busso, Å. Ergon, et al. 2011. "QTL Analyses and Comparative Genetic Mapping of Frost Tolerance, Winter Survival and Drought Tolerance in Meadow Fescue (*Festuca pratensis* Huds.)." *Theoretical and Applied Genetics* 123, no. 3: 369–382. <https://doi.org/10.1007/s00122-011-1590-z>.
- Barnes, B. D., D. Kopecky, A. J. Lukaszewski, and J. H. Baird. 2014. "Evaluation of Turf-Type Interspecific Hybrids of Meadow Fescue With Perennial Ryegrass for Improved Stress Tolerance." *Crop Science* 54, no. 1: 355–365. <https://doi.org/10.2135/cropsci2013.03.0198>.
- Cao, M., S. Bughrara, and D. Sleper. 2003. "Cytogenetic Analysis of *Festuca* Species and Amphiploids Between *Festuca mairei* and *Lolium perenne*." *Crop Science* 43, no. 5: 1659–1662. <https://doi.org/10.2135/cropsci2003.1659>.
- Cao, M., D. Sleper, F. Dong, and J. Jiang. 2000. "Genomic In Situ Hybridization (GISH) Reveals High Chromosome Pairing Affinity

Between *Lolium perenne* and *Festuca mairei*." *Genome* 43, no. 2: 398–403. <https://doi.org/10.1139/gen-43-2-398>.

Casler, M., and E. van Santen. 2010. "Breeding Objectives in Forages." In *Fodder Crops and Amenity Grasses*, vol. 5. Springer. https://doi.org/10.1007/978-1-4419-0760-8_5.

Catalan, P., P. Torrecilla, J. A. L. Rodriguez, and R. G. Olmstead. 2004. "Phylogeny of the Festucoid Grasses of Subtribe Loliinae and Allies (Poeae, Pooideae) Inferred From ITS and trnL-F Sequences." *Molecular Phylogenetics and Evolution* 31, no. 2: 517–541. <https://doi.org/10.1016/j.ympev.2003.08.025>.

Cernoch, V., and D. Kopecky. 2020. "Drought Tolerance and Regrowth Capacity Revealed in the *Festuca-Lolium* Complex." *Biologia Plantarum* 64: 561–568. <https://doi.org/10.32615/bp.2020.093>.

Chen, C., and D. Sleper. 1999. "FISH and RFLP Marker-Assisted Introgression of *Festuca mairei* Chromosomes Into *Lolium perenne*." *Crop Science* 39, no. 6: 1676–1679. <https://doi.org/10.2135/cropsci1999.3961676x>.

Fry, J., and J. Butler. 1989. "Responses of Tall and Hard Fescue to Deficit Irrigation." *Crop Science* 29, no. 6: 1536–1541. <https://doi.org/10.2135/cropsci1989.0011183X002900060045x>.

Fu, J., J. Fry, and B. Huang. 2004. "Minimum Water Requirements of Four Turfgrasses in the Transition Zone." *HortScience* 39, no. 7: 1740–1744. <https://doi.org/10.21273/HORTSCI.39.7.1740>.

Fu, J., J. Fry, and B. Huang. 2007a. "Growth and Carbon Metabolism of Tall Fescue and Zoysiagrass as Affected by Deficit Irrigation." *HortScience* 42, no. 2: 378–381. <https://doi.org/10.21273/HORTSCI.42.2.378>.

Fu, J., J. Fry, and B. Huang. 2007b. "Tall Fescue Rooting as Affected by Deficit Irrigation." *HortScience* 42, no. 3: 688–691. <https://doi.org/10.21273/HORTSCI.42.3.688>.

Gerlach, W., and J. Bedbrook. 1979. "Cloning and Characterization of Ribosomal RNA Genes From Wheat and Barley." *Nucleic Acids Research* 7, no. 7: 1869–1885. <https://doi.org/10.1093/nar/7.7.1869>.

Glombik, M., V. Bacovsky, R. Hobza, and D. Kopecky. 2020. "Competition of Parental Genomes in Plant Hybrids." *Frontiers in Plant Science* 11: 200. <https://doi.org/10.3389/fpls.2020.00200>.

Glombik, M., D. Copetti, J. Bartos, et al. 2021. "Reciprocal Allopolyploid Grasses (*Festuca* $\times$ *Lolium*) Display Stable Patterns of Genome Dominance." *Plant Journal* 107, no. 4: 1166–1182. <https://doi.org/10.1111/tpj.15375>.

Gregory, P., C. Atkinson, A. Bengough, et al. 2013. "Contributions of Roots and Rootstocks to Sustainable, Intensified Crop Production." *Journal of Experimental Botany* 64, no. 5: 1209–1222. <https://doi.org/10.1093/jxb/ers385>.

Huang, B., R. Duncan, and R. Carrow. 1997. "Drought-Resistance Mechanisms of Seven Warm-Season Turfgrasses Under Surface Soil Drying. 1. Shoot Response." *Crop Science* 37, no. 6: 1858–1863. <https://doi.org/10.2135/cropsci1997.0011183X003700060032x>.

Humphreys, J., J. A. Harper, I. P. Armstead, and M. W. Humphreys. 2005. "Introgression-Mapping of Genes for Drought Resistance Transferred From *Festuca arundinacea* var. *glaucescens* Into *Lolium multiflorum*." *Theoretical and Applied Genetics* 110, no. 3: 579–587. <https://doi.org/10.1007/s00122-004-1879-2>.

Humphreys, M., J. Doonan, R. Boyle, et al. 2018. "Root Imaging Showing Comparisons in Root Distribution and Ontogeny in Novel *Festulolium* Populations and Closely Related Perennial Ryegrass Varieties." *Food and Energy Security* 7, no. 4: e00145. <https://doi.org/10.1002/fes3.145>.

Humphreys, M., S. O'Donovan, M. Farrell, A. Gay, and A. Kingston-Smith. 2014. "The Potential of Novel *Festulolium* (2n=4x=28) Hybrids as Productive, Nutrient-Use-Efficient Fodder for Ruminants."

- Food and Energy Security* 3, no. 2: 98–110. <https://doi.org/10.1002/fes3.50>.
- Humphreys, M., and Z. Zwierzykowski. 2020. “*Festulolium*, a Century of Research and Breeding and Its Increased Relevance in Meeting the Requirements for Multifunctional Grassland Agriculture.” *Biologia Plantarum* 64: 578–590. <https://doi.org/10.32615/bp.2020.108>.
- Humphreys, M. W., and I. Pasakinskiene. 1996. “Chromosome Painting to Locate Genes for Drought Resistance Transferred From *Festuca arundinacea* Into *Lolium multiflorum*.” *Heredity* 77: 530–534. <https://doi.org/10.1038/hdy.1996.180>.
- Humphreys, M. W., and H. Thomas. 1993. “Improved Drought Resistance in Introgression Lines Derived From *Lolium multiflorum* × *Festuca arundinacea* Hybrids.” *Plant Breeding* 111, no. 2: 155–161. <https://doi.org/10.1111/j.1439-0523.1993.tb00622.x>.
- Humphreys, M. W., H. M. Thomas, W. G. Morgan, et al. 1995. “Discriminating the Ancestral Progenitors of Hexaploid *Festuca arundinacea* Using Genomic In Situ Hybridization.” *Heredity* 75: 171–174. <https://doi.org/10.1038/hdy.1995.120>.
- Kamau, S., A. Belanche, T. Davies, P. Rees Stevens, M. Humphreys, and A. H. Kingston-Smith. 2020. “A Route to Decreasing N Pollution From Livestock: Use of *Festulolium* Hybrids Improves Efficiency of N Flows in Rumen Simulation Fermenters.” *Food and Energy Security* 9, no. 3: e209. <https://doi.org/10.1002/fes3.209>.
- Kell, D. 2011. “Breeding Crop Plants With Deep Roots: Their Role in Sustainable Carbon, Nutrient and Water Sequestration.” *Annals of Botany* 108, no. 3: 407–418. <https://doi.org/10.1093/aob/mcr175>.
- Kopecky, D., J. Bartos, Z. Zwierzykowski, and J. Dolezel. 2009. “Chromosome Pairing of Individual Genomes in Tall Fescue (*Festuca arundinacea* Schreb.), Its Progenitors, and Hybrids With Italian Ryegrass (*Lolium multiflorum* Lam.).” *Cytogenetic and Genome Research* 124, no. 2: 170–178. <https://doi.org/10.1159/000207525>.
- Kopecky, D., V. Cernoch, R. Capka, J. Dolezel, and M. Humphreys. 2005. *Genomic Constitution of Festulolium Varieties*. Wageningen Academic.
- Kopecky, D., M. Havráňková, J. Loureiro, et al. 2010. “Physical Distribution of Homoeologous Recombination in Individual Chromosomes of *Festuca pratensis* in *Lolium multiflorum*.” *Cytogenetic and Genome Research* 129, no. 1–3: 162–172. <https://doi.org/10.1159/000313379>.
- Kopecky, D., L. Horáková, M. Duchoslav, and J. Dolezel. 2019. “Selective Elimination of Parental Chromatin From Introgression Cultivars of x*Festulolium* (*Festuca* × *Lolium*).” *Sustainability* 11, no. 11: 3153. <https://doi.org/10.3390/su11113153>.
- Kopecky, D., A. J. Lukaszewski, and J. Dolezel. 2008. “Meiotic Behaviour of Individual Chromosomes of *Festuca pratensis* in Tetraploid *Lolium multiflorum*.” *Chromosome Research* 16, no. 7: 987–998. <https://doi.org/10.1007/s10577-008-1256-0>.
- Kopecky, D., and B. Studer. 2014. “Emerging Technologies Advancing Forage and Turf Grass Genomics.” *Biotechnology Advances* 32, no. 1: 190–199. <https://doi.org/10.1016/j.biotechadv.2013.11.010>.
- Lenth, R. V. 2024. “emmeans: Estimated Marginal Means, aka Least-Squares Means.” R Package Version 1.10.x. <https://CRAN.R-project.org/package=emmeans>.
- Li, Y., Y. Liu, P. Harris, et al. 2017. “Assessment of Soil Water, Carbon and Nitrogen Cycling in Reseeded Grassland on the NorthWyke Farm Platform Using a Process-Based Model.” *Science of the Total Environment* 603: 27–37. <https://doi.org/10.1016/j.scitotenv.2017.06.012>.
- Macleod, C., M. Humphreys, W. Whalley, et al. 2013. “A Novel Grass Hybrid to Reduce Flood Generation in Temperate Regions.” *Scientific Reports* 3: 1683. <https://doi.org/10.1038/srep01683>.
- Mahelka, V., D. Kopecky, J. Majka, and K. Krak. 2023. “Uniparental Expression of Ribosomal RNA in x*Festulolium* Grasses: A Link Between the Genome and Nucleolar Dominance.” *Frontiers in Plant Science* 14: 1276252. <https://doi.org/10.3389/fpls.2023.1276252>.
- Majka, J., M. Glombik, A. Dolezalová, et al. 2023. “Both Male and Female Meiosis Contribute to Non-Mendelian Inheritance of Parental Chromosomes in Interspecific Plant Hybrids (*Lolium* × *Festuca*).” *New Phytologist* 238, no. 2: 624–636. <https://doi.org/10.1111/nph.18753>.
- Majka, J., M. Majka, D. Kopecky, and J. Dolezel. 2020. “Cytogenetic Insights Into *Festulolium*.” *Biologia Plantarum* 64: 598–603. <https://doi.org/10.32615/bp.2020.095>.
- Masoudi-Nejad, A., S. Nasuda, R. A. McIntosh, and T. R. Endo. 2002. “Transfer of Rye Chromosome Segments to Wheat by a Gametocidal System.” *Chromosome Research* 10, no. 5: 349–357. <https://doi.org/10.1023/a:1016845200960>.
- Muhandiram, N., M. Humphreys, R. Fychan, J. W. Davies, R. Sanderson, and C. L. Marley. 2023. “Designing Agricultural Grasses to Help Mitigate Proteolysis During Ensiling to Optimize Protein Feed Provisions for Livestock.” *Food and Energy Security* 12, no. 4: e475. <https://doi.org/10.1002/fes3.475>.
- Muhandiram, N. P., M. W. Humphreys, R. Fychan, J. W. Davies, R. Sanderson, and C. L. Marley. 2020. “Do Agricultural Grasses Bred for Improved Root Systems Provide Resilience to Machinery-Derived Soil Compaction?” *Food and Energy Security* 9: e227.
- Muhandiram, N., M. Humphreys, R. Fychan, et al. 2024. “Scoping Opportunities for Nitrogen Use Efficiency Among Productive Agricultural Forage Grasses With Diverse Rooting Systems.” *Food and Energy Security* 13: e70023.
- Pedersen, E., D. Miller, G. Simpson, and N. Ross. 2019. “Hierarchical Generalized Additive Models in Ecology: An Introduction With mgcv.” *PeerJ* 7: e6876. <https://doi.org/10.7717/peerj.6876>.
- Qian, Y., and J. Fry. 1996. “Irrigation Frequency Affects Zoysiagrass Rooting and Plant Water Status.” *HortScience* 31, no. 2: 234–237. <https://doi.org/10.21273/HORTSCI.31.2.234>.
- Qian, Y., and J. Fry. 1997. “Water Relations and Drought Tolerance of Four Turfgrasses.” *Journal of the American Society for Horticultural Science* 122, no. 1: 129–133. <https://doi.org/10.21273/JASHS.122.1.129>.
- Qian, Y., J. Fry, and W. Upham. 1997. “Rooting and Drought Avoidance of Warm-Season Turfgrasses and Tall Fescue in Kansas.” *Crop Science* 37, no. 3: 905–910. <https://doi.org/10.2135/cropsci1997.0011183X003700030034x>.
- R Core Team. 2023. “R: A Language and Environment for Statistical Computing.” R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reheul, D., B. De Cauwer, and M. Cougnon. 2010. *The Role of Forage Crops in Multifunctional Agriculture*. Vol. 5. Springer New York. https://doi.org/10.1007/978-1-4419-0760-8_1.
- Rognli, O. A., M. C. Saha, S. Bhamidimarri, and S. van der Heijden. 2010. “Fescues.” *Fodder Crops and Amenity Grasses* 5: 261–292. https://doi.org/10.1007/978-1-4419-0760-8_11.
- Shaw, R. K. 2006. “Effect of Gene Transfer From *Festuca* to *Lolium* on plant-Mediated Proteolysis.” PhD Thesis, Aberystwyth University, Wales, UK.
- Simpson, G. L. 2024. “gratia: Graceful ggplot-Based Graphics and Utility Functions for GAMs Fitted Using mgcv.” R Package Version 0.9.x. <https://CRAN.R-project.org/package=gratia>.
- Staniak, M., and A. Kocon. 2015. “Forage Grasses Under Drought Stress in Conditions of Poland.” *Acta Physiologiae Plantarum* 37, no. 6: 116. <https://doi.org/10.1007/s11738-015-1864-1>.
- Sullivan, G. M., and A. R. Artino Jr. 2013. “Analyzing and Interpreting Data From Likert-Type Scales.” *Journal of Graduate Medical Education* 5, no. 4: 541–542.

- Thomas, H., J. Harper, M. Meredith, et al. 1996. "Comparison of Ribosomal DNA Sites in *Lolium* Species by Fluorescence In Situ Hybridization." *Chromosome Research* 4, no. 7: 486–490. <https://doi.org/10.1007/BF02261775>.
- Trnka, M., J. Balek, M. A. Semenov, et al. 2020. "Future Agroclimatic Conditions and Implications for European Grasslands." *Biologia Plantarum* 64: 865–880. <https://doi.org/10.32615/bp.2021.005>.
- Turner, L., K. Macleod, C. Watts, et al. 2010. "Dissecting Festulolium Chromosome 3 to Locate Rooting and Drought Resistance Traits." In *Sustainable Use of Genetic Diversity in Forage and Turf Breeding*, edited by C. Huyghe, 471–477. Springer Science+Business Media.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Wood, S. N. 2017. *Generalized Additive Models: An Introduction With R*. 2nd ed. Chapman and Hall/CRC.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Genome composition of individual plants, phenotypic data for each plant across monitored period and meteorological data for the period of plant cultivation in Olomouc, Czech Republic. **Table S2:** Results of the post hoc multiple comparison tests or specific contrast for genomic groups from two gamm models (gamm1, gamm2). The estimates from the gamm models represent changes on the latent logit scale. Post hoc pairwise comparisons were performed using the emmeans package, with *p* values adjusted for multiple comparisons via the Tukey method. Significant *p* values ($p \leq 0.05$) are in bold.