



OPEN Genetic variation and heritability of agronomic traits in a native perennial forage species from drylands: breeding potential of *Festuca pallescens*

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Festuca pallescens is a native forage grass species of Patagonia, playing a crucial role in supporting sheep production in arid and semi-arid ecosystems. This study assessed genetic differentiation among populations and estimated the heritability of traits linked to biomass production and phenological development as part of its domestication effort. A common garden trial was established with ten half-sib families from four preselected populations, and phenological and morphological traits were measured over three seasons. Trait correlations were analyzed, and a mixed model approach was employed to estimate Wright's Q_{ST} and narrow-sense heritability. Q_{ST} estimates ranged from 0.18 to 0.47 for phenological traits and from 0.086 to 0.093 for morphological traits; heritability values ranged from 0.33 to 0.78 for phenological traits and from 0.27 to 0.50 for morphological traits. Results indicate strong genetic structure for most phenological traits, suggesting diversifying selection. A population effect and within-population variability were also observed for most traits, highlighting potential for genetic improvement. We propose selection strategies to establish a breeding program for this species, aiming to develop adapted synthetic varieties with greater fitness. These new varieties could enhance forage productivity and potentially be applied in regions with similar climatic and environmental conditions.

Keywords Native perennial species, Plant breeding and genetics, Common garden, Selection, Patagonia, Arid and semi-arid regions

Dryland ecosystems provide extensive rangeland territory that sustains diverse low input farming systems around the world¹. Approximately 75% of the global population resides in these regions, with 90% in developing countries. These marginal lands have historically been used for husbandry, mainly focused on raising beef cattle and sheep, which represents the main economic income of most rural families^{2–4}. In addition to the historical overgrazing, desertification of arid and semiarid regions limits the productivity of natural rangelands, reducing their receptivity, and therefore the stocking rate, which challenges rural economies^{5,6}. Developing elite germplasm for native plant species to sustain productivity under challenging conditions is usually not the aim of commercial plant breeding programs. Breeding efforts typically prioritize highly productive areas and commercial crops, instead of developing suitable germplasm for agricultural systems in marginal regions^{7,8}. Conversely, the anticipated effects of climate change and its consequences place agriculture and traditional breeding programs in a challenging position⁹. Under this scenario, developing technology based on native species can bring extensive benefits related to their adaptation to regional environments, maintenance of natural

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diversity while providing ecosystem services, and the possibility of becoming a reservoir of useful genetic resources that could be transferred to crop species^{10–13}.

In Argentina, there are several breeding programs dedicated to improving crops and commercial forage grass species^{14,15–18}. The primary source of germplasm for these programs usually comes from exotic sources, which poses a disadvantage for adaptation to the harsh conditions of drylands. Only a few of these programs focus on native species, especially those from marginal environments like the Patagonian steppe (e.g. Refs.^{19,20}). Rangelands of arid and semiarid ecosystems of Patagonia, are intensively used for beef cattle and sheep production on extensive grazing systems since early nineteenth century^{21,22}. Populations of native species that are preferred by livestock are diminishing due primarily to overgrazing and the outcomes of global change. Bare soil gives way to desertification processes that lead to a loss of net primary productivity and botanical diversity²³. Other consequences include a fierce competition between local farmers and herbivores, as well as an increase in colonization by species that are not palatable or have a lower nutritional value^{24,25}. In this context, domesticating native forage grasses could become a productive resource for farmers while also supporting restoration purposes.

Festuca pallescens (St. Yves) Parodi is a native forage grass species of the rangelands of the Patagonian steppe. This cool-season perennial grass produces abundant forage of moderate quality well into the colder months and covers up to 20% of the diet of domestic livestock^{26,27}. Its extensive natural distribution across a variety of environments could facilitate the local adaptation of populations²⁸. This cross-pollinated allohexaploid bunch grass ($2n = 6x = 42$) has been well studied morphologically and physiologically as an essential component of Patagonian production systems^{29–33}. However, while it has been genetically described^{34–36}, research on the population genetics of this species has only recently been addressed^{37–40}. As a key species of the Patagonian steppe, it has been included in a domestication program where different populations are being evaluated for important agronomic traits, including biomass production through tillering and germination traits under diverse temperature and water availability conditions^{28,39,40}. There are still knowledge gaps on other important characteristics regarding its reproductive cycle or seed production, and how genetic variation of these traits is structured both between and within populations.

We aim to maximize the survival and productivity of this widely distributed species, with a secondary focus on developing germplasm tailored to regional climates where natural populations are heavily utilized for livestock production. To achieve this goal, knowledge on the extent of genetic variability across populations, differentiation between populations and genetic variation of agronomic traits within populations is essential. These are key steps to characterize the response to selection⁴¹, and therefore the plausible results of the domestication efforts. Different parameters can be estimated to describe population differentiation and transmissibility of phenotypic characteristics to progeny. One of these parameters, named Q_{ST} by Spitze⁴², quantifies genetic divergence among populations based on quantitative traits. Within populations, the heritability specifies the proportion of the total variance that results from genetic variance under specific conditions⁴³. This parameter can be calculated in a narrow or in a broad sense. *Narrow sense heritability* (h^2) refers to the fraction of additive genetic variance that contributes to the overall phenotypic variance, while *broad sense heritability* (H^2) encompasses both additive and non-additive genetic variance^{44,46,49}. By estimating the h^2 of traits, we can elucidate the role of additive effects of genes, which would let us estimate the component of a phenotype that can be transmitted to the offspring generation within a population⁴⁵. Phenotypic assessments of a trait of interest on half-sib families established on a common garden trial would allow us to infer the different variance components used to quantify Q_{ST} and estimate h^2 , since these designs help separate the additive effects from environmental effects and phenotypic plasticity^{45,46}). Data collected from this type of trial would also be a useful source of information to compare the populations and families involved in the program and delineate the selection strategies.

The aims of this study were to: i) detect differences between preselected populations for phenological and morphological traits ii) evaluate the genetic differentiation among the populations through the estimation of a Q_{ST} parameter per variable iii) estimate a narrow sense heritability for each trait. By estimating the degree of differentiation among these populations, we aimed to establish a baseline for monitoring the extent of crossing and genetic variance structuring in the advanced generations of our mixed breeding population. Additionally, we sought to infer the action of natural selection by comparing the Q_{ST} parameter with a measure for neutral genetic differentiation calculated for *F. pallescens*, to identify traits that might be undergoing selection. Furthermore, by estimating the heritability of agronomic traits, we sought to enhance our understanding of the transmissibility of phenotypic values in this species, providing insight into its overall response to selection.

Material and methods

Experimental design and plant resources

We conducted a common garden trial with *F. pallescens* seedlings at the INTA Bariloche Experimental Station in Bariloche, Argentina (41° 07' S, 71° 15' W, 810 m a.s.l.) (Fig. 1). The experimental site is located within the Andean Patagonian region, which is characterized by soils developed on volcanic ash and glacioclastic deposits. The predominant soil types in this area are Andisols, specifically Hapludands, known for their high organic matter content, low bulk density, and the capacity to retain water effectively⁴⁷. These soils are typically low in phosphorus, as indicated by the site's soil analysis (<2.82 ppm). Additionally, the region experiences an alternation of freezing and thawing periods, which can contribute to reduced water availability for plants despite the soil's inherent water retention capacity⁴⁸. These seedlings were sourced from a multi-site provenance trial (MST) from which we selected four populations that excelled in forage and seed production: Pilcaniyeu (Pa), Jacobacci (Jb), Cronómetro (Cr) and Yagüe (Ya) (see Table 1)³⁹. Each of the four selected populations was represented by ten open-pollinated families whose mother plants shared the same geographic origin. Hereafter, we refer to these four groups as populations. We selected the ten mother plants per provenance encompassing homogeneously the range of phenotypic variation for forage and seed yield in the MST, intending not to skew the sampling. The seeds we used in seedling production were harvested in 2018 from two-year-old plants.

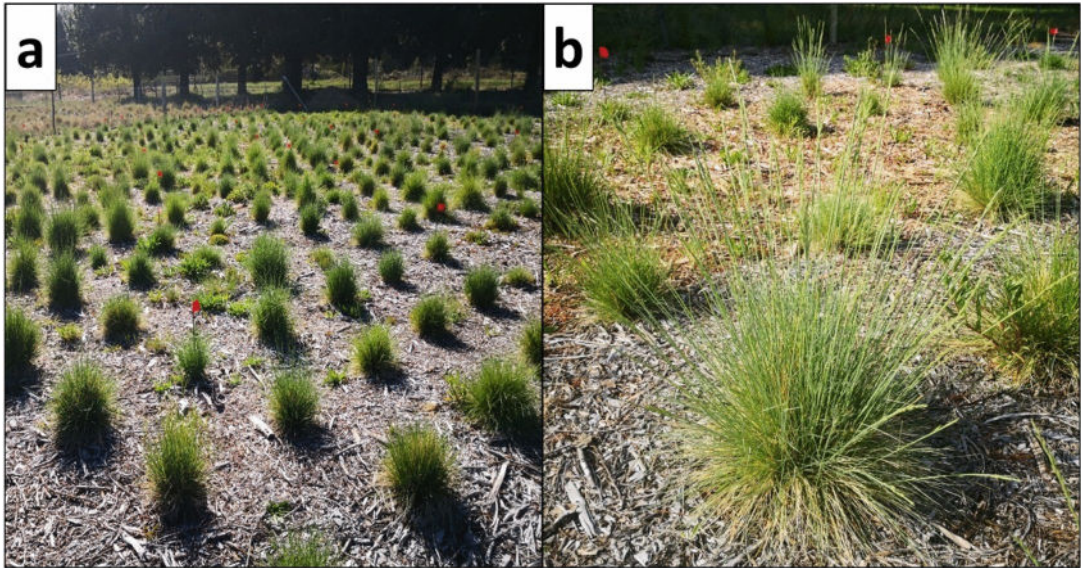


Fig. 1. Half-sib trial of *Festuca pallescens* at the INTA Bariloche Experimental Station. The image on the left (a) shows the plants in a vegetative state and image on the right (b) shows the plants in a reproductive state, both pictures were taken during the first growing season.

Populations	Province	Latitude S	Longitude W	Altitude m a.s.l	Mean annual precipitation (mm)	Mean annual temperature (T°)
Pilcaniyeu (Pa)	Río Negro	41°30'12"	70°34'48"	1260	209*	7.8
Jacobacci (Jb)	Río Negro	41°55'80"	69°12'58"	1141	125	8.2
Cronómetro (Cr)	Chubut	43°14'55"	71°40'12"	875	497	8.6
Yagüe (Ya)	Chubut	42°57'00"	71°12'00"	748	666	7.6

Table 1. Geographic location of four preselected populations of *Festuca pallescens* established in a common garden trial. Mean annual precipitation and mean annual temperature (2007–2023) from CRU TS v. 4.08⁵¹. *Mean annual precipitation (2007–2023) from INTA experimental field station located in Pilcaniyeu.

Seedlings were grown in individual pots under greenhouse-controlled conditions and transplanted to the field in November 2019. The trial had five replicates per family, established in plots of three individuals for a total of 600 plants. The five plots representing each of the 40 families were set in a completely randomized design (see Fig. S1). At planting, each plant was set with 1.5 g of Basacote® 6 M fertilizer (13-6-16) and the whole trial was irrigated with ~220 m³/ha three times a week until March 2020 to help with establishment. The planting scheme was 0.7 m X 0.7 m spacing between plants, and the interline was covered with chipped wood for weed control.

Data collection

Phenological and morphological traits were measured on each plant during the maximum growing period between 2020 to 2023, encompassing three growing seasons from July 1st to June 30th of the following year: GS1 (2020–2021), GS2 (2021–2022) and GS3 (2022–2023). Phenological traits focused on the reproductive cycle, while morphological traits related to biomass production and vegetative growth of the bunch. For phenological traits, five variables were measured: the starting dates of three different reproductive pheno-phases (F1, F2 and F5), the duration of the reproductive cycle (RC), and total seed production per plant (SP). The pheno-phases were registered every three days according to a subjective ordinal scale of five phases, adjusted by observations of inflorescence development for *F. pallescens* (see Fig. 2 for the description of each pheno-phase). The variables F1, F2 and F5 are expressed as the number of days from July 1st of each year to avoid dividing the growth cycle. The RC (in days) represents the duration between F1 and F5. These reproductive variables were registered over two growing seasons (GS2 and GS3). Total seed production (SP) was measured as the weight of seeds harvested per individual (in grams) over three consecutive reproductive seasons (2021–2023). As for the morphological traits, we evaluated crown volume (V) and tiller production (T). The crown volume (cm³) was estimated as a half ellipsoid using measurements of height and average basal diameter of the bunch (Fig. 2a and b). This non-destructive method, adapted from Oñatibia et al.⁴⁹ for bunch grasses, allows reliable biomass estimation while maintaining trial integrity and continuity⁵⁰. As tussocks grow asymmetrically, an average crown diameter was calculated using cross-measurements of the basal circumference. Crown volume represents accumulated biomass production and was measured at the end of two seasons (April 2021 and 2022). Tiller production was assessed by marking individual tillers at three cardinal points of each bunch with colored wire rings at ground

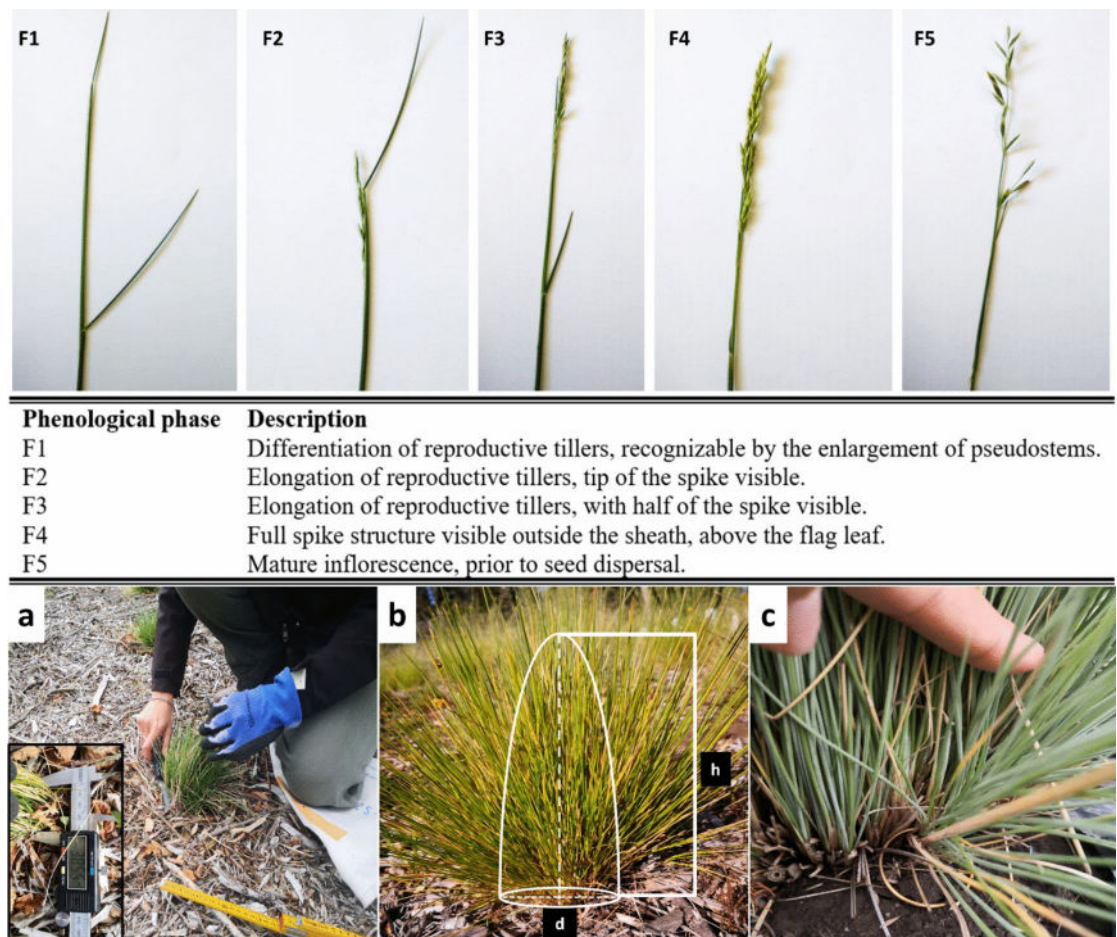


Fig. 2. Phenological phases and measurement methodology in *Festuca pallescens*. The top panel displays the five phenological phases (F1–F5) used to evaluate the reproductive phenological development of *Festuca pallescens*. Descriptions for each phase are provided below the corresponding images. The bottom panel shows the methodology used for plant measurements in the half-sib trial. In (a), the method for measuring the basal diameter of each bunch is illustrated, using a digital caliper and a ruler to capture approximately 80% of the plant's height. Image (b) presents a schematic representation of the half-ellipsoid model used to estimate crown volume. Image (c) shows one of three colored rings placed around the plant to track tiller division over time.

level in November 2020 and recording tiller division at the end of the season (April 2021), following López et al.²⁸ (see Fig. 2c). In later-developing populations, vegetative tillers may differentiate into reproductive tillers after marking, halting further division. To prevent bias toward lower production, rings with a single tiller that transitioned to reproductive growth were excluded from the analysis. As a summary, Table 2 details the traits measured in each growing season. Climatic data was obtained from the CRU TS v. 4.08 online database⁵¹, to characterize each season of the trial. The CRU database was selected as it has the lowest bias in North Patagonia⁵². The reference climatology period was 2007–2023, as a climate shift occurred in 2007 that affected precipitation and temperature in the region^{53–55}.

Statistical Analysis and parameter estimation

Variance component analysis

Under the univariate analysis approach, we fitted linear mixed models (LMM) for all the traits using the following general form:

$$y_{ijklm} = \mu + year_i + pop_j + year \times pop_{ij} + fam_{k(j)} + e_{ijkl} + d_{ijklm} \quad (1)$$

where y_{ijklm} is the response variable for the m th genotype, from the k th half-sib family, nested within the j th population, located in the l th plot in the i th year, μ is the overall mean of the response variable; $year_i$ is the fixed effect of the year, $year \times pop_{ij}$ is the fixed effect of the interaction between year and population, pop_j is the fixed effect of the j th population ($j = 1-4$); $fam_{k(j)}$ is the random effect of the k th family ($k = 1-10$) nested within the j th population; e_{ijkl} is the experimental error; and d_{ijklm} is the subsampling error (except for the variable T where the experimental unit corresponds to one plant per plot). We assumed that $fam_{k(j)} \sim N(0, \sigma_f^2)$, $e_{ijkl} \sim N(0, \sigma_e^2)$ and $d_{ijklm} \sim N(0, \sigma_d^2)$, where all random effects were independently distributed with zero covariance.

Measured trait	GS1	GS2	GS3
SP (g)	✓	✓	✓
F1 (days)		✓	✓
F2 (days)		✓	✓
F5 (days)		✓	✓
RC (days)		✓	✓
V (cm³)	✓	✓	
T (count)	✓		

Table 2. Morphological and phenological traits measured in each growing season in the half-sib trial of *Festuca pallescens*. GS=growing season (from July 1st to June 30th of the following year). SP=seed production; F1, F2, F5=phenological phases of the reproductive cycle; RC=duration of the reproductive cycle; V=estimated crown volume; T=tiller production.

For seed production (SP), we used a first-order autoregressive correlation structure for heterogeneous variance (AR1h) to model the variance and covariance of the random effects, since it is expected that each individual yield is affected by the previous year’s production. This structure accounts for the temporal dependency between measurements, with stronger covariances between consecutive years and weaker covariances for measurements further apart. After verifying the assumptions of the LMMs, data transformation was considered necessary for two variables due to the lack of normality of the residuals: T and SP, which were transformed using the logarithmic and square root functions respectively. We used restricted maximum likelihood for all analyses. An incremental Wald test was applied to assess the significance of the fixed factors on the complete model (Eq. 1). All the analyses were carried out in *RStudio* (version 4.2.2) using the package *Asreml* (version 4.2.0.276, The VSNi Team 2023). For the estimation of Q_{ST} , the variables were analyzed on a per-year basis, whereas heritability (h^2) was estimated both per year and within populations. Consequently, model (Eq. 1) was simplified by removing unnecessary terms to accommodate specific analyses.

Q_{ST}: genetic differentiation among populations for quantitative traits

For each trait, we estimated Wright’s Q_{ST} parameter using annual datasets separately as the year effect was significant in all cases. This parameter describes a relation of variances and allowed us to assess the degree of genetic differentiation among populations for quantitative traits. It was calculated following the procedure described by Spitze⁴²:

$$Q_{ST} = \frac{V_{pop}}{V_{pop} + 2 \cdot V_A}$$

where V_{pop} represents the among-population variance component and V_A is the additive genetic variance, derived as four times the family variance component extracted from the linear mixed models (LMMs). To estimate variance components, we used a simpler model derived from (Eq. 1) with no *year* or *year x population* terms, with *population* treated as a random effect. We performed likelihood ratio tests (LRTs) to assess the contribution of the population effect and its significance.

As *Festuca pallescens* is an allohexaploid species, the additive genetic variance may be confounded with dominance or epistatic components, likely resulting in an underestimation of Q_{ST} ^{56,57}. It is worth noting that since the populations were not entirely isolated due to one cycle of cross-pollination within the multi-site trial, the Q_{ST} we estimated is not strictly classical. Instead, it reflects a combination of genetic differentiation due to both maternal origin and the effects of cross-pollination among populations. Nevertheless, a significant coefficient of genetic differentiation among populations is informative since it indicates that there is genetic variability among the groups defined according to their maternal origin (populations). Therefore, a significant Q_{ST} could suggest that: (1) despite the open-pollination, the populations have retained distinct genetic characteristics, possibly due to their maternal lineage (2) the differentiation might reflect adaptive differences among populations, which could be due to natural selection or other ecological pressures (3) cross-pollination within the multi-site trial (MST) was not entirely random, this is, if crossings were completely random, the genetic differentiation between the selected populations (Q_{ST}) would be expected to be close to zero for all traits. Significant differentiation may suggest that there are non-random factors influencing mating or gene flow. We validated the obtained Q_{ST} values reported in this study (based on the proposed population structure), to make sure that they were not a result of chance. To achieve this, we conducted a non-parametric bootstrap analysis⁵⁸ to generate random distributions of Q_{ST} estimates. This involved resampling the original data at the family level 1,000 times with replacement, randomly assigning different groupings of open-pollinated families within the population groups each time. We calculated the 95% confidence intervals for these distributions using the ‘quantile’ (Type-8) function in *RStudio*; this quantile type is approximately median unbiased, regardless of the variable’s distribution⁵⁹. After validation, the empiric Q_{ST} parameters was compared to a Φ_{PT} for the species obtained from bibliography and based on

codominant SSR markers ($\Phi_{PT}=0.12$)²⁸. The Φ_{PT} parameter is an analog and good estimator of Wright's F_{ST} which measures neutral genetic differentiation among populations, and is well-suited to polyploid species⁶⁰. This comparison enabled us to infer the influence of natural selection by contrasting neutral differentiation with differentiation driven by potential adaptive processes.

Narrow sense heritability

Narrow sense heritability (h^2) quantifies the proportion of phenotypic variation among individuals or families that is attributable to additive genetic variance. In the context of a domestication program, it serves as a foundation for estimating breeding values and predicting genetic gains over time^{61,62}. Given its dependency on specific environmental and temporal conditions⁶³, we partitioned the dataset by year and population. Therefore, we reduced the model (Eq. 1) by removing the terms for these factors and their interaction (*year x population*). For each trait, we initially conducted likelihood ratio tests (LRTs) to determine whether the family variance component significantly contributed to the overall variance. This was achieved by comparing a model that included the family term against one that did not. When family variance components were found to be significant, we estimated h^2 on a mean family basis, following the methodology outlined by Nguyen and Slepner⁶⁴:

$$h^2 = \frac{\sigma_{fam}^2}{\sigma_{fam}^2 + \frac{\sigma_e^2}{r} + \frac{\sigma_d^2}{r \cdot n}}$$

where σ_{fam}^2 represents the family variance component, which corresponds to the genetic variance among half-sib families; σ_e^2 is the error variance component (the variance between plots); σ_d^2 is the variance among individual plants within plots, r indicates the number of replicates, and n indicates the number of individual plants per plot.. Heritability estimates were calculated separately for each population and year, as h^2 can vary among populations. Estimating h^2 per population could also be useful for identifying a base population where selection would be most effective⁶⁵. The standard errors (SE) for the h^2 were calculated according to Falconer and Mackay⁶⁶:

$$SE_{h^2} = 4 \cdot \sqrt{\frac{2 \cdot \left(1 - \frac{1}{4} \cdot h^2\right)^2 \cdot [1 + (n - 1) \cdot \frac{1}{4} \cdot h^2]^2}{1 \cdot (n - 1) \cdot (N - 1)}}$$

where N is the number of families per population, and n is the number of half siblings per family. In cases where family sizes varied, N was calculated as the harmonic mean.

Finally, Pearson correlations were calculated in *RStudio* for all pairwise combinations of traits to assess their degree of integration. Potential correlations would be useful for identifying traits that can be jointly targeted for selection within the domestication program.

Results

Climatic conditions during the growing seasons

The climatic characterization of the three growing seasons in which this trial was conducted revealed a trend of lower accumulated precipitation for the first growing season (July 2020–June 2021) compared to the subsequent seasons and to the historical values from the 2007–2023 time series used as a reference. This trend was especially pronounced in the second half of the growing season, where differences exceeded 100 mm compared to historical values and over 300 mm compared to the other seasons (Table 3, Fig. S2). In the second growing season (GS2), although the total accumulated precipitation was close to the reference value, the first half of the season received over 200 mm less precipitation than the historical average (Fig. S2). In contrast, we did not detect differences in temperature across growing seasons or when compared to the reference values (Table 3).

Genetic variation among populations

The ANOVA model using the complete model (Eq. 1) showed interaction between population and year, and both main effects for all the traits (Table 4).

Period	Ppt (mm)	Tmean (°C)	Tmin (°C)	Tmax (°C)
GS1	1039	7.3	-2.5	20.1
GS2	1263	6.9	-3.0	20.6
GS3	1485	7.7	-2.3	21.8
TS 2007–2023	1348	7.1	-1.9	21.1

Table 3. Climatic conditions during the three growing seasons for the half-sib trial of *F. palleescens* at the INTA Bariloche Experimental Station. GS=growing season (from July 1st to June 30th of the following year); TS=time series covering the years 2007–2023. Ppt=accumulated precipitation; Tmean=mean temperature; Tmin=mean minimum temperature of the coldest month; Tmax=mean maximum temperature of the warmest month.

Phenological traits

Differences among populations were observed for the starting date of the pheno-phases (F1, F2 and F5), as well as for seed production. We also found an effect of the population for the duration of the reproductive cycle ($p=0.014$). The phenotypic mean values, standard deviations, and range of every trait measured are shown in Table 5.

For the variables F1, F2 and F5, the populations clustered into two different groups: one with an earlier phenology (Yagüe and Cronómetro) and a second group with a later phenology (Jacobacci and Pilcaniyeu) (Fig. 3b–d). The starting date of each one of these pheno-phases strongly and positively correlated with the starting date of the same phase on the next flowering season ($r=0.59$, $p<0.001$; $r=0.83$, $p<0.001$ and $r=0.82$, $p<0.001$ for F1, F2 and F5 respectively) (Fig. 4). The F1 of both growing seasons had a moderately negative correlation with the duration of the reproductive cycle ($r=-0.38$, $p<0.001$ and $r=-0.73$, $p<0.001$). All four populations had a significantly higher seed production in the first year of harvest (Fig. 3a), which dropped in the following seasons, being at its lowest in the second year. During the first season, population Jb had a significantly higher yield than the other three populations. The genetic effect of the population was lower for the second season with minimal differences and increased again by the third season. During the third harvest season Ya and Cr presented the highest yield, and Pa showed the lowest. The population Jb presented an intermediate behavior.

Seed production of the second and third season highly and positively correlated with the SP of previous years ($r=0.45$, $p<0.001$ and $r=0.45$, $p<0.001$ for SP of 2021 with the SP of 2022 and 2023; and $r=0.49$, $p<0.001$ for SP of 2022 with SP of 2023). Regarding the duration of the reproductive cycle, Jb presented a significantly longer cycle during the first year of measurements, which mostly explains the whole population effect. All populations presented a similar behavior during the second year (Fig. 3e).

Morphological traits

A population effect was observed for tiller production (T) and estimated crown volume (V) ($p=0.004$ and $p=0.013$ respectively). These results are mostly explained by a lower production of tillers in population Jb, and a smaller crown volume in population Pa in the second year. Additionally, T presented a low and negative correlation with the starting date of each phenological phase of the following growing season ($r=-0.27$, $p<0.001$; $r=-0.29$, $p<0.001$ and $r=-0.37$, $p=0.001$ for F1, F2 and F5 respectively). The estimated crown volume also showed to be negatively correlated to the F1 in both seasons ($r=-0.15$, $p=0.002$ and $r=-0.17$, $p<0.001$ for the first and second season respectively).

As expected for any growth variable, the effect of the year was highly significant for V ($p<0.001$), since plant sizes inevitably alter. On average, the plants exhibited approximately an 85% change in size, but growth rates varied among populations. The year \times population interaction effect is mostly explained by the higher growth rate observed in population Ya (Fig. 3f, Table 4). At the end of the second season, the highest V was observed for Ya and Jb while Pa presented a significantly lower value (see Table 5). For the first growing season, the estimated crown volume had a moderate and positive correlation with the seed production, which was lower on the second growth season ($r=0.70$, $p<0.001$; $r=0.26$, $p>0.001$ for V-SP of the first season and V-SP of the second season respectively). The number of tillers per ring ranged from 0 (loss of the tiller) in all populations to 14 for Pa, with Jb reaching only 7 tillers as maximum value (Table 5).

Comparison between Q_{ST} and Φ_{PT}

Since Q_{ST} were estimated over trial material, validation of this statistic was performed by simulating a distribution of Q_{ST} values assuming a random family structure per population. The empiric Q_{ST} was compared to the values of this distribution (Fig. 5).

The Q_{ST} estimates for the starting date of the different pheno-phases (F1, F2 and F5) resulted higher than the Φ_{PT} of 0.12²⁸ in both growing seasons. The duration of the reproductive cycle presented a slightly higher Q_{ST} in the first year of measurements as well, but this value decreased drastically in the second year. Regarding the seed production, Q_{ST} values remained higher than the Φ_{PT} in all seasons, but it gradually decreased each year (0.38–0.18). For the morphological variables (V and T), the degree of differentiation was in all cases significantly lower than the Φ_{PT} (Table 6).

Narrow sense heritability

Narrow sense heritability (h^2) was estimated for all traits, per population and per year only when the family variance component resulted to be significant (Table 7). In general, we found moderate to high heritabilities for the phenological traits in all the populations and across seasons, particularly for the F2 and F5 pheno-phases.

Effect	SP (g)	F1 (days)	F2 (days)	F5 (days)	RC (days)	V (cm ³)	T (count)
Population	30.12 <0.001	82 <0.001	77 <0.001	88 <0.001	10.50 0.014	10.84 0.013	13.15 0.004
Year	1139.72 <0.001	14 <0.001	26 <0.001	101 <0.001	5.00 0.025	440.71 <0.001	-
Population x year	54.61 <0.001	46 <0.001	15 0.002	33 <0.001	18.10 <0.001	11.52 0.009	-

Table 4. Wald statistic and p values for each effect for phenological and morphological traits. SP = seed production; F1, F2, F5 = pheno-phases of the reproductive cycle; RC = duration of the reproductive cycle; V = estimated crown volume; T = tiller production.

	SP (g)			F1 (days)			F2 (days)			F5 (days)			RC (days)			V (cm ³)			T (count)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
GS1																					
Pa	10.6 ^{bcd}	8.8	0.1–46.9	119 ^a	6.6	107–142	142 ^{ab}	8.8	117–159	166 ^a	5.6	149–180	46.8 ^{ab}	7.0	24–62	1152 ^d	665	12–3080	3.1 ^{ab}	2.4	0–14
Jb	19.8 ^a	11.7	0.2–46.1	118 ^a	6.4	107–152	146 ^a	7.6	124–163	168 ^a	5.1	152–180	51.0 ^a	5.1	33–59	1480 ^{cd}	659	143–3542	2.2 ^b	1.6	0–7
Cr	12.9 ^{bc}	10.5	0.2–47.1	112 ^{cd}	4.9	107–133	130 ^d	8.0	114–147	158 ^{bc}	5.7	147–179	46.2 ^b	5.8	30–72	1312 ^d	726	12–3303	3.1 ^a	2.1	0–13
Ya	13.9 ^b	11.9	0.2–57.0	114 ^{bc}	7.0	107–152	130 ^d	9.0	110–159	159 ^b	6.0	147–180	45.0 ^b	5.8	18–61	1371 ^d	789	47–3670	3.3 ^a	2.1	0–12
GS2																					
Pa	1.5 ^h	1.9	0.1–11.1	120 ^a	10.5	106–145	139 ^c	7.5	124–166	165 ^a	6.4	152–180	45.0 ^b	8.9	28–63	1991 ^{bc}	1113	23–5106			
Jb	4.0 ^h	3.9	0.2–18.5	120 ^a	10.6	91–152	143 ^{bc}	7.3	124–158	167 ^a	5.7	152–187	47.2 ^b	9.4	17–78	2536 ^a	1095	310–5423			
Cr	3.2 ^{gh}	4.3	0.1–24.4	106 ^c	10.7	91–152	129 ^d	7.3	111–145	153 ^d	5.7	141–169	47.1 ^{ab}	9.1	21–75	2469 ^{ab}	1290	210–6423			
Ya	5.2 ^{fg}	7.1	0.1–36.7	109 ^{de}	10.9	91–145	130 ^d	6.6	111–152	155 ^{cd}	5.8	141–172	45.7 ^b	9.8	20–67	2696 ^a	1387	40–6028			
GS3																					
Pa	3.8 ^{fg}	5.0	0.1–28.7																		
Jb	6.5 ^{ef}	6.3	0.2–31.6																		
Cr	8.8 ^{de}	8.3	0.2–38.0																		
Ya	10.2 ^{cde}	10.5	0.1–55.4																		

Table 5. Phenotypic mean values, standard deviation and range of the traits measured in four preselected populations of *E. pallescens*. GS = growing season from the 1st of July to the 30th of June of the following year; SP = seed production; F1, F2, F5 = pheno-phases of the reproductive cycle; RC = duration of the reproductive cycle; V = estimated crown volume; T = tiller production; SD = standard deviation. Means with different letters for each variable are different at p < 0.05.

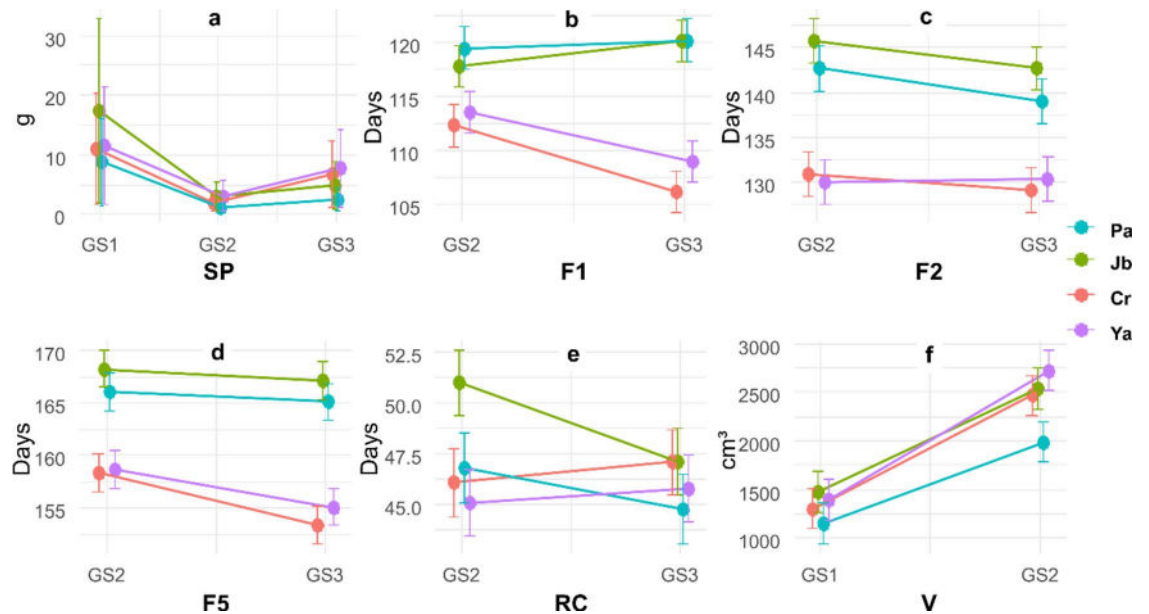


Fig. 3. Predicted values per trait and per year. Each graph shows the behavior of the measured traits in the four populations of *F. pallescens*. SP= seed production in grams (g); F1, F2 and F5= pheno-phases of the reproductive cycle in days from the 1st of July; RC= duration of the reproductive cycle in days and V= estimated crown volume in cm³. GS= growing season.

For the morphological traits, the h^2 was high for estimated crown volume (V) and moderately low for tiller production (T).

Discussion

We evaluated four preselected populations of *Festuca pallescens* in a common garden trial to obtain information on the extent of the among and within population genetic structuring and the heritability of important agronomic traits. This study is the first to evaluate the structuring of genetic variance in a breeding population of the species. Our results show differentiation among populations for most traits, strong genetic structuring of populations for phenological characters, and moderate to high heritability for some of the measured traits. Here we want to assess future selection strategies based on the results of our study, to outline how to better proceed with the establishment of a breeding program for *F. pallescens*. Additionally, we will discuss the possible paths to move forward into developing the first synthetic variety for this species.

Among population genetic differentiation

The two southern populations, Cronómetro and Yagüe, were precocious in all flowering phenology traits consistently across seasons. Nevertheless, this did not affect the duration of the reproductive cycle after three years of establishment in the trial, since these populations also ended the flowering process earlier, regarding their two northern counterparts, Pilcaniyeu and Jacobacci. In the first year of measurements (i.e. second growing season of the trial), the plants from Jacobacci displayed a longer flowering cycle than the other three populations. Coming from an extremely arid environment, it is possible that they benefited from the higher water availability at the location of the trial. This aligns with results from Munson and Long⁶⁷ who suggest that perennial C₃ grasses can extend the flowering period with increased mean annual precipitation. Nevertheless, this effect did not prevail in the next season. All four populations presented a higher seed production (SP) during the first year, which declined significantly by the second and third years of harvest. The reduction in seed yield is a normal behavior for perennial grasses as nutrient allocation to storage and belowground tissues is a priority for survival⁶⁸. Similar results are described for other bunchgrass species⁶⁹, other cultivated perennial grasses like intermediate wheatgrass⁷⁰, and long-lived plants in general⁷¹. It is also noteworthy that accumulated precipitation during the first half (July to December) of the second growing season was approximately 250 mm lower than the reference period based on the average for the location, as well as lower than that of the first and third growing seasons. Moreover, the second half of the first growing season (January to June) was also drier than the average by about 180 mm. This reduction in rainfall may help explain the sharp decline in seed yield observed during the second season.

Although our trial material came from an experimental setup that allowed free mating among different populations, we found evidence suggesting the retention of variation between populations for most phenological traits, which could be due to local adaptation (i.e. $Q_{ST} > \Phi_{PT}$). Seed production, measured in three consecutive years, showed Q_{ST} values that decreased over time. Populations could have manifested a strong genetic influence of their maternal lineage (natural population) by initially presenting contrasting behaviors that do not prevail over the following seasons. While differences among populations diminished over time, family variance for

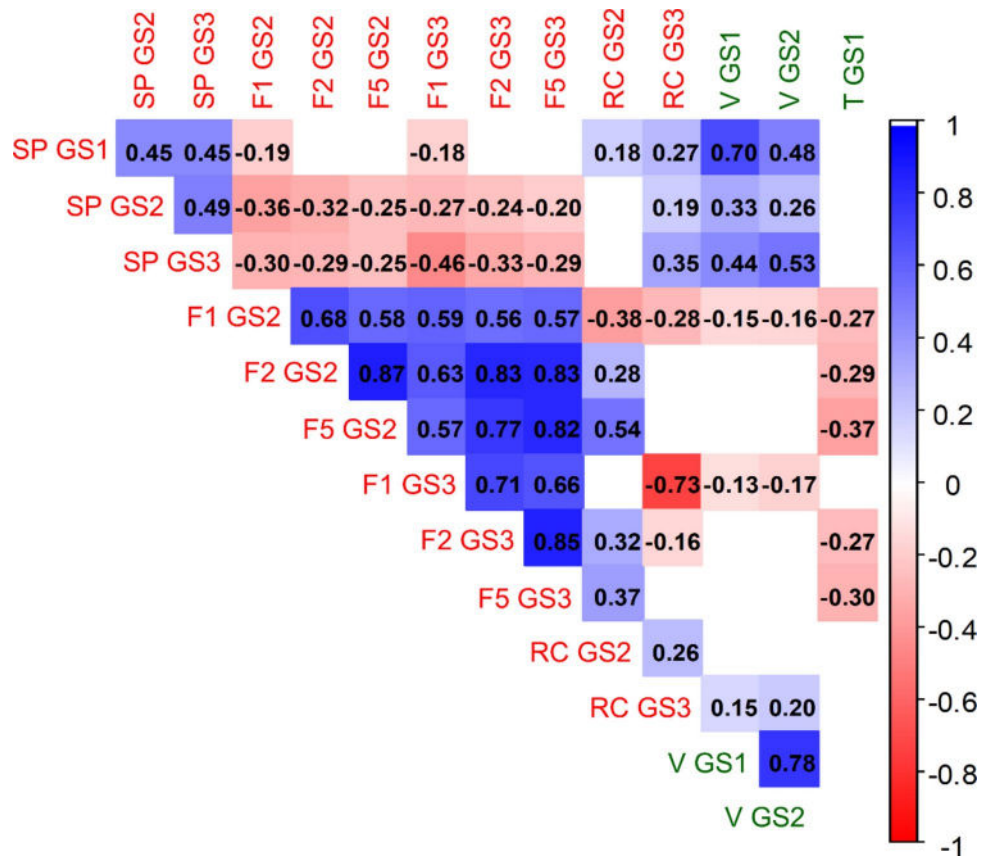


Fig. 4. Correlation plot showing only significant relationships among the analyzed variables, with p -values < 0.01 . Correlations are represented with a color scale ranging from blue (strong positive correlations) to red (strong negative correlations). Red labels represent phenological variables, while green labels correspond to morphological variables. Pearson's r coefficients are displayed in bold inside the plot. Non-significant correlations ($p \geq 0.01$) are excluded from the plot. SP=seed production; F1, F2 and F5=pheno-phases of the reproductive cycle in days from the 1st of July; RC=duration of the reproductive cycle; V=estimated crown volume and T=tiller production. GS=growing season.

this trait increased. This suggests that as plants mature, populations express the genetic variance they harbor, leading to a homogenizing effect among them. Nevertheless, Q_{ST} estimates for seed production in all three years remained higher than the Φ_{PT} value, suggesting an effect of diversifying selection that is still evident in our breeding population. Regarding the starting date of each reproductive phase (F1, F2 and F5), there was a trend towards differentiation among the northern and southern populations, with Q_{ST} estimates for both the beginning and the end of the reproductive cycle increasing over time. For all three pheno-phases, the Q_{ST} values were consistently higher than Φ_{PT} every year, reflecting possible selection processes acting over these traits. The timing of flowering and the speed of inflorescence development is highly influenced by temperature, day length, their interaction and elevation (Heide, 1994, Ref.⁷²) which differ among these four locations. Local adaptation of the populations to their original provenance at different latitudes and altitudes, explained by different day lengths, nutrient and water availability, could be a plausible reason for why these traits showed high genetic differentiation in a common environment ($Q_{ST} > 0.25$). Adaptation of phenology to regional climate and light regimes has been described for other plant species along latitudinal gradients⁷³, and a precocious start of flowering in response to warmer temperatures has been stated as an important adaptation of plants from higher latitudes to completing their reproductive cycle before unfavorable conditions⁷⁴. The duration of the reproductive cycle showed a different behavior, with a Q_{ST} value similar to Φ_{PT} in the first year, possibly reflecting a genetic effect of maternal lineages related to environmental response, and almost nil in the second year. The duration of the flowering period has been suggested to be species specific, and it would not necessarily change in relation to different climatic variables⁷².

For the morphological traits, we found a population effect for both estimated crown volume (V) and tiller production (T), which was mainly explained by the differential behavior of one population in each case, Pilcaniyeu and Jacobacci, respectively. The Jacobacci population exhibited a lower capacity for tiller multiplication compared to the other three populations. Despite the reduced tiller production, this population maintained a high seed yield, which may suggest a reproductive strategy that balances sexual and asexual propagation. Plants from Pilcaniyeu produced approximately 22% less aboveground volume compared to the other populations by the end of the second season. Further studies, including root biomass observations, could

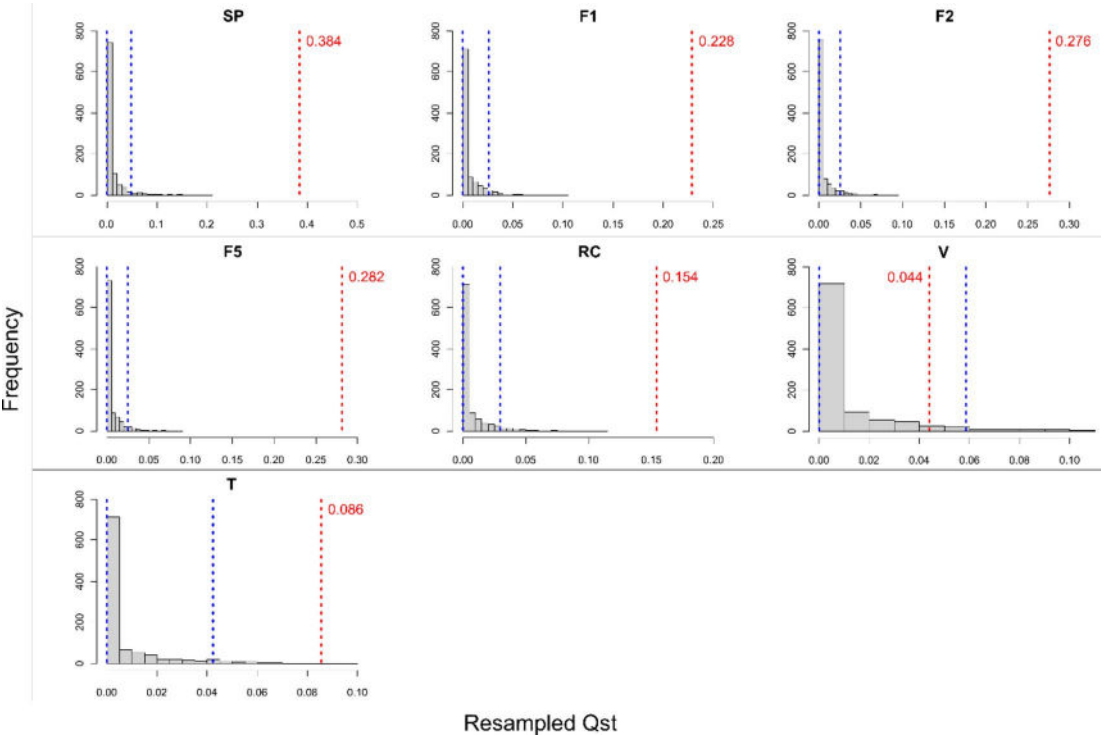


Fig. 5. Q_{ST} Distributions and confidence intervals for *F. pallescens* traits. Each plot shows a simulated Q_{ST} distribution with a 95% confidence interval (blue dotted line) and observed Q_{ST} (red dotted line) for all variables measured on the half-sib trial of *F. pallescens*. SP=seed production; F1, F2 and F5=pheno-phases of the reproductive cycle; RC=duration of the reproductive cycle; V=estimated crown volume and T= tiller production. Only the first year of measurements is shown, following years behaved similarly.

	Source of variation	SP (g)	F1 (days)	F2 (days)	F5 (days)	RC (days)	V (cm ³)	T (count)
GS1	Family	1.95					5.72	6.35
	Population	9.76					2.10 ^{ns}	4.76
	Plot	12.74					13.87	–
	Residual	75.55					78.30	88.89
	Q_{ST}	0.38					–	0.086
GS2	Family	4.71	9.26	15.29	14.02	9.85	5.55	
	Population	9.84	21.93	46.53	44.00	14.39	4.56	
	Plot	12.07	0.43	3.22	3.26	1.04	11.22	
	Residual	73.39	68.38	34.96	38.72	74.73	78.67	
	Q_{ST}	0.21	0.23	0.28	0.28	0.15	0.093	
GS3	Family	6.96	4.45	15.26	12.90	5.12		
	Population	12.04	31.00	43.51	57.13	<0.01 ^{ns}		
	Plot	4.54	6.25	4.82	1.72	7.30		
	Residual	1.49	58.31	36.42	28.26	87.57		
	Q_{ST}	0.18	0.47	0.26	0.36	–		

Table 6. Partitioning of the phenotypic variance (in percentages) for different agronomic traits of *Festuca pallescens*, and genetic differentiation among four sampled populations (Q_{ST}). GS= growing season from the 1st of July to the 30th of June of the following year; SP seed production; F1, F2, F5= pheno-phases of the reproductive cycle; RC= duration of the reproductive cycle; V= estimated crown volume; T= tiller production.^{ns}Not significant. As reference, Φ_{pT} value for neutral genetic differentiation among populations=0.12²⁸.

	Population	SP (g)	F1 (days)	F2 (days)	F5 (days)	RC (days)	V (cm ³)	T (count)
GS1	Pa							
	V _{fam}	ns					ns	ns
	h ²	–					–	–
	SE _h ²	–					–	–
	Jb							
	V _{fam}	13.13					ns	ns
	h ²	0.47					–	–
	SE _h ²	0.33					–	–
	Cr							
	V _{fam}	ns					ns	9.69
	h ²	–					–	0.35
	SE _h ²	–					–	0.27
	Ya							
	V _{fam}	ns					ns	6.96
	h ²	–					–	0.27
	SE _h ²	–					–	0.24
GS2	Pa							
	V _{fam}	ns	18.61	37.50	25.72	ns	ns	
	h ²	–	0.53	0.77	0.67	–	–	
	SE _h ²	–	0.39	0.45	0.43	–	–	
	Jb							
	V _{fam}	ns	12.37	35.67	20.97	ns	ns	
	h ²	–	0.41	0.74	0.59	–	–	
	SE _h ²	–	0.30	0.40	0.35	–	–	
	Cr							
	V _{fam}	13.74	ns	26.48	36.72	25.74	ns	
	h ²	0.46	–	0.64	0.74	0.63	–	
	SE _h ²	0.37	–	0.40	0.43	0.40	–	
	Ya							
	V _{fam}	ns	ns	15.80	16.19	ns	16.48	
	h ²	–	–	0.51	0.51	–	0.50	
	SE _h ²	–	–	0.35	0.35	–	0.33	
GS3	Pa							
	V _{fam}	ns	ns	22.73	35.77	ns		
	h ²	–	–	0.63	0.74	–		
	SE _h ²	–	–	0.41	0.43	–		
	Jb							
	V _{fam}	17.60	8.85	32.53	27.52	ns		
	h ²	0.52	0.33	0.71	0.66	–		
	SE _h ²	0.35	0.28	0.40	0.38	–		
	Cr							
	V _{fam}	ns	12.99	32.31	41.78	ns		
	h ²	–	0.46	0.74	0.78	–		
	SE _h ²	–	0.32	0.40	0.41	–		
	Ya							
	V _{fam}	ns	ns	16.05	14.52	ns		
	h ²	–	–	0.50	0.48	–		
	SE _h ²	–	–	0.34	0.33	–		

Table 7. Narrow sense heritability (h^2) and standard errors (SE_h^2) for agronomic traits in four preselected populations of *F. pallescens*. Estimates of h^2 are only shown in cases where the family component was significant ($p < 0.05$). GS = growing season from the 1st of July to the 30th of June of the following year; SP = seed production; F1, F2, F5 = pheno-phases of the reproductive cycle; RC = duration of the reproductive cycle; V = estimated crown volume; T = tiller production; V_{fam} = among-family variance as percentage of the total phenotypic; h^2 = narrow sense heritability; SE_h^2 = standard error of h^2 ; ns not significant.

provide insights into a potential survival strategy where trade-offs favor root development in this population. For both morphological variables, the estimates of genetic differentiation were very low, which contrasts with other scientific reports describing the effects of diversifying selection on morphological traits such as plant height and rosette diameter^{74–76}. The lack of genetic differentiation among populations for these traits could be attributed to the survival strategy of *F. pallescens*, which may not rely on maintaining genetic variation in traits related to plant size under the harsh conditions of the Patagonian steppe. Instead, this species might prioritize traits linked to resilience, such as a deep root system (which has yet to be studied) or traits that ensure perpetuation, such as those related to seed production and phenological development, which could be subject to local adaptation, as suggested by the Q_{ST} found in this study.

Trait heritability

Heritability estimates for seed production (SP) were calculated for two populations, Cronómetro and Jacobacci, with the highest values of h^2 for the latter in the third year. Aiming for seed production was not the main goal of breeders when it came to forage grasses⁶², but it has received increasing attention as an important trait for commercial purposes⁷⁷. Burton and De Vane⁷⁸ reported a broad sense heritability for seed yield of 0.34–0.76 on tall fescue from measurements on individual plants, which aligns with our results (0.46–0.52). Although the starting date of flowering (F1) showed variation due to the family effect in two populations each year, the family variance component was not as high as for the starting date of the other two pheno-phases of the reproductive cycle, internode elongation (F2) and maturity (F5). For F2, h^2 estimates resulted high for all populations in both years ($h^2 > 0.45$) as well as for F5 ($h^2 > 0.48$). As a reference, Elgersma⁸⁷ reported comparable h^2 values based on family means for the starting date of two phenological stages in perennial ryegrass (*Lolium perenne*). In that study, narrow sense heritabilities for heading date and first anthesis date ranged from 0.35 to 0.46. For the duration of the reproductive cycle (RC) we could only capture considerable and significant variation among half-sib families for one population (Cronómetro) in the first year, which presented a high heritability ($h^2 = 0.63$).

As for morphological traits, we found a significant family variance component and calculated heritabilities only for the two southern populations. For crown volume (V), only population Yagüe in the second year showed a significant family variance component, and an heritability of $h^2 = 0.50$. This result aligns with findings from other studies on *Festuca* species on traits related to vegetative growth. For instance, h^2 values of 0.46–0.60 were reported for dry matter yield and plant height in tall fescue^{62,79}. For tiller production, both Cronómetro and Yagüe presented moderate to low heritabilities. In general, morphological traits are expected to present higher estimates of heritability, which probably, we were not able to estimate here on individual plants on a family basis, possibly due to high environmental within family effects. This could also be a consequence of not working with clonal material, but in cases where species reproduce by seed dispersal, narrow sense heritability can give more realistic results (Elgersma⁸⁷). Our findings, while preliminary, underscore the genetic potential of these populations and the importance of continued research to validate and expand these results. We acknowledge that our heritability estimates are based on a limited sample size and should be interpreted with caution, emphasizing the need for larger sample sizes and multi-environment trials to provide more robust validation.

Implications for breeding

The breeding program for this native species of the Patagonian steppe will be structured around two main objectives: improving forage production and maintaining seed reproductive capacity (i.e., maintaining high seed yield). Seed production (SP) resulted positively correlated with the estimated crown volume (V) of previous years. This result might only indicate that bigger plants produce more seeds. However, this correlation was lower in the second year, suggesting an increasing investment in root development, typical of perennial species, where belowground growth may be prioritized over aboveground growth⁸⁰. An important consideration is that selection for seed yield can have a toll on perenniality, and although this is not always the case, it is one of the biggest challenges when working with perennial grass species^{81,88}. Selection for seed yield should focus on maintaining high productivity across seasons, while balancing longevity. Therefore, future selection efforts must include root development traits and analyze the correlations with seed and aboveground biomass growth.

In general, seed yield and most of its components are not promising for selection when measured over spaced plants on half-sib trials, since narrow sense heritability tends to be low (Elgersma⁸⁷). Based on our heritability estimates, selecting for seed production would only show promising outcomes in two of our breeding populations, Cronómetro and Jacobacci. In both cases, the family variance component was considerable, which would let us aim for specific lines within these populations to develop a synthetic variety based on seed yield. Selection of families over higher seed production could start as early as the first harvest, since seed yield in every season resulted positively correlated to previous years yields.

The phenology traits were highly consistent across years. This could potentially facilitate the planning of practices related to fertilization, harvesting or animal management, with the focus on forage quality. Moreover, genetic differentiation in phenology may lead towards selection for specific environments based on regional climate characterization, since a later development could be advantageous in areas prone to late-season frosts. The starting date of reproductive differentiation (F1) showed to be negatively correlated to the duration of the reproductive cycle, meaning that an earlier start of flowering relates to longer reproductive cycles. Even though we did not find significant differences between populations for the duration of the reproductive cycle, this information could be useful in future selection efforts, by allowing an earlier selection of families with a shorter flowering period. The heritability of the starting date of internode elongation (F2) resulted high for all populations in both years ($h^2 > 0.45$). Selection for this phenological trait could imply correlated selection for the timing of forage quality decline. Forage quality based on digestibility is closely related to internode elongation, since nutrient allocation shifts to reproductive organs at this stage. Within population variability in this trait is highly useful for a breeding program, as it would allow the development of different synthetic varieties from

elite parents⁶⁴. Similar results were obtained for the seed maturity phase (F5), which can lead to an analogous approach.

Selection of families for future varieties should target phenological timing for optimal pasture management. High h^2 estimates for the duration of the reproductive cycle (RC) for population Cronómetro may allow selection for shorter flowering periods. Shorter or late flowering cycles could mean better quality forage availability for beef cattle and sheep during the colder months, when food sources can diminish (Horrocs and Valentine, 1999). It is important to keep in mind that for the third year of our trial, this trait did not exhibit genetic variation within populations, meaning that earlier selection would be a better strategy.

Regarding the morphological traits, crown volume and tiller production, the among-population genetic differentiation found in our trial indicates that the program can benefit from retaining or infusing materials from populations with a higher productivity. Tiller productivity can also play an important role in restoration efforts since *F. pallescentis* lacks rhizomes, and therefore, tiller production is essential for vegetative growth and persistence. We recommend that selection efforts for biomass production traits, such as crown volume and tiller production, focus on the populations where a significant family variance component was observed. In our study, these were the two southern populations, Cronómetro and Yagüe. For future experimental designs, we must advance to the inclusion of clonal material. This could be a way of minimizing environmental effects within families and increase the number of replicates to improve the accuracy of heritability estimates for these and other target traits⁸².

It is worth noting that tiller production presented a low and negative correlation with all flowering traits. This implies that constant selection for higher tiller productivity might indirectly lead to earlier phenological development. On the other hand, the estimated crown volume of each season showed a low and negative correlation with the starting date of the reproductive cycle (F1), a trait that has been suggested for indirect selection aiming to increase biomass yield (Prince and Casler, 2014). In future trials, it will be important to assess the correlations between all productive traits measured on individual plants and those same traits measured in dense stand trials, as the response to selection may be lower in the latter.

Lastly, our study highlights evidence of genetic structuring among populations in phenological traits. This suggests that the development of synthetic varieties should be tailored to specific regions. With this in mind, we recommend retrieving genetic materials from the original native populations and incorporating them into future breeding programs for this species to capture a broader genetic variation suited for regional selection. Failure to consider this genetic differentiation may result in genetic material that is not well adapted to local climatic conditions, which could bring negative consequences to natural populations.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

V.G. wrote the main manuscript text, performed the formal analysis, and applied the methodology; A.G.A. and I.B. assisted with the application of the methodology, formal analysis, and conceptualization; M.M.A. contributed to funding acquisition, writing, conceptualization, and investigation; A.S.L. was involved in conceptualization

and writing; J.A.A-R. participated in investigation, writing, and review and editing; V.P. engaged in investigation, review, and editing; L.G. supported the formal analysis, methodology, and provided software licensing; and P.M. contributed to funding acquisition, writing, conceptualization, and investigation. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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