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Divergent Selection for Anthesis of Annual Ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot)

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ABSTRACT

Annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) is a prolific species expanding into winter-fallow row crop fields of the southeastern US. While its dense growth makes it an excellent cover crop, late senescence, and herbicide resistance pose challenges for spring eradication. This study aims to develop an annual ryegrass population that naturally senescences early by employing recurrent phenotypic selection to alter anthesis date. In 2022, a base population was collected with anthesis dates from 21 March to 2 April, (mean anthesis date of 28 March). The base population was allowed to polycross in isolation. To obtain $Cycle_0$, 903 seedlings of this population were grown under ambient winter conditions at Starkville, MS. Upon anthesis, the $Cycle_0$ individuals were segregated into three groups: the "NV_0" group consisting of 11 non-vernalizing progenies, the "E_0" group consisting of the 70 progenies earliest to anthesis, and the "L_0" group, comprising the last 70 individuals to reach anthesis. Isolated groups were allowed to polycross within each group to give three new populations of $Cycle_1$: NV_1, E_1, and L_1 in 2023. Genetic variation was used to predict a gain due to selection for the anthesis date. The results observed exceeded the predicted gain of 6 days. Groups NV_1, E_1, and L_1 had a gain of 18, 14, and 10 days, respectively, and heritability (h^2) of 0.39, 0.61, and 0.45, respectively, for anthesis. The NV and E populations will be assessed for usefulness as a cover crop and L as an enhanced forage variety.

KEYWORDS: gain; heritability; selection differential; phenotypic selection

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ABBREVIATIONS

E, early; L, late; NV, non-vernalizing

INTRODUCTION

Annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot), also known as Italian ryegrass, is a popular cool-season species in the southeastern US used for livestock feed, landscaping, and ecosystem services due to its high productivity, palatability, and adaptability [1,2].

Producers at this location typically grow soybean (*Glycine max* (L.) Merr.), corn (*Zea mays* (L.)), and cotton (*Gossypium hirsutum* (L.)) in a continuous system, where the field is planted in the spring and harvested in fall. After the harvest of the main crop, throughout the fall, winter, and early spring, the field should be ideally occupied by cover crops and terminated before the subsequent crop is planted. Cover crops with high winter survival are preferred for over-winter cover, nutrient retention, weed suppression, and soil structure improvement. Annual ryegrass has gained popularity as a cover crop, primarily because of its contribution to the agroecosystem by adding organic matter from the decomposition of plant biomass and establishing ground cover to protect against sediment loss from wind and water. However, its late senescence and multiple herbicide resistances make it challenging to kill prior to and during spring planting [3]. Additionally, lack of cold tolerance is a serious problem in many commercial annual ryegrass cultivars [4].

Annual ryegrass as a species is self-incompatible, allowing the development of synthetics or open-pollinated, random-mating populations from ecotype selection or phenotypic recurrent selection [5,6]. Annual ryegrass populations exhibit high genetic variability, and cross-pollination leads to significant variation within individual populations, fostering the plant's adaptability in a wide range of areas. Recurrent selection is used to divide the populations into smaller selection units, necessarily safeguarding against potential environmental effects and improving realized genetic gains from selection [7]. The most effective breeding method to maximize additive gain for cross-pollinated grasses is bi-directional selection. The bi-directional selection changes population means in opposite directions. A population improved through selection has a greater frequency of favorable alleles over the base population [7].

Anthesis date is a complex trait that is affected by environmental variations, especially day length and temperature [8–10]. Annual ryegrass has a photoperiod-induction period that is less than 12 h (before 21 March), with most domestic crop species being photoperiod insensitive [11]. In annual ryegrass, the ability of the shoot apex to react to photoperiod is achieved without necessarily requiring low temperatures, although this can be accelerated by cold treatment whether naturally occurring or induced artificially [12]. The establishment and reproductive date also influence winter survival [13].

Accurate prediction of anthesis date is required to guide crop management decisions and to form a developmental system of ecological and adaptive importance. The objective of this study was to develop annual ryegrass populations with altered anthesis dates: early and late. Developing annual ryegrass that senescences early in the spring would be highly beneficial as a self-terminating cover crop. If this were the case, it would be possible to exploit the weed suppression, soil stabilization, and nutrient scavenging benefits of annual ryegrass as a cover crop

while eliminating the need for costly control measures at or near the planting window of a row crop. Annual ryegrass is a high-quality forage that can be established without extensive seedbed preparation, grows on a wide range of soil types, and can maintain its integrity even after repeated grazing by livestock. However, forage quality decreases as the plant approaches reproductive maturity, with a rapid decline in quality beyond the heading stage. Hence, a late-maturing population may be an excellent forage variety. While early maturing cultivars have been associated with a high biomass yield in early spring [14], farmers are increasingly inclined to incorporate a higher proportion of later-maturing cultivars over early to mid-maturing types due to increased pasture quality and associated livestock productivity benefits [15]. With this in mind we wished to test equations that predict population changes due to selection, estimate heritability and use Chi square equation to determine if changes due to selection were significant as we progress from a feral annual ryegrass population through two cycles of bi-directional selection.

MATERIALS AND METHODS

Planting Materials and Location

A base population of 27 individuals was collected in 2022. Individuals of the base population were feral (Figure 1). This population had anthesis dates ranging from 21 March 2022 to 2 April 2022, with a mean anthesis date of 28 March 2022 (87th day of 2022) (Figure 2). These individuals arranged as a randomized design were allowed to polycross in isolation. The resulting seed were planted the following fall in media composed of 80:20 (V:V) peat: sand into 50-cell “deep” flats (12.7 × 27.8 × 54.5 cm; T.O. Plastics®—Greenhouse Megastore, Clearwater, MN) in the greenhouse of R.R. Foil Plant Science Research Center, Starkville, MS, US (33.457952, −88.754920). To minimize environmental effects, all plants were grown in a randomized arrangement under uniform conditions and uniform containers, ensuring phenotypic selections predominantly reflect genetic differences. After emergence, approximately two weeks after seeding, seedlings were thinned to a single plant per cell. Irrigation was applied daily unless there was a rainfall event. Fertilization was applied every two weeks as Peters 20-20-20 Water Soluble fertilizer according to label instructions. If predicted temperatures were expected to be significantly below freezing, plants were moved to a cold greenhouse with a minimum temperature of 0 °C.

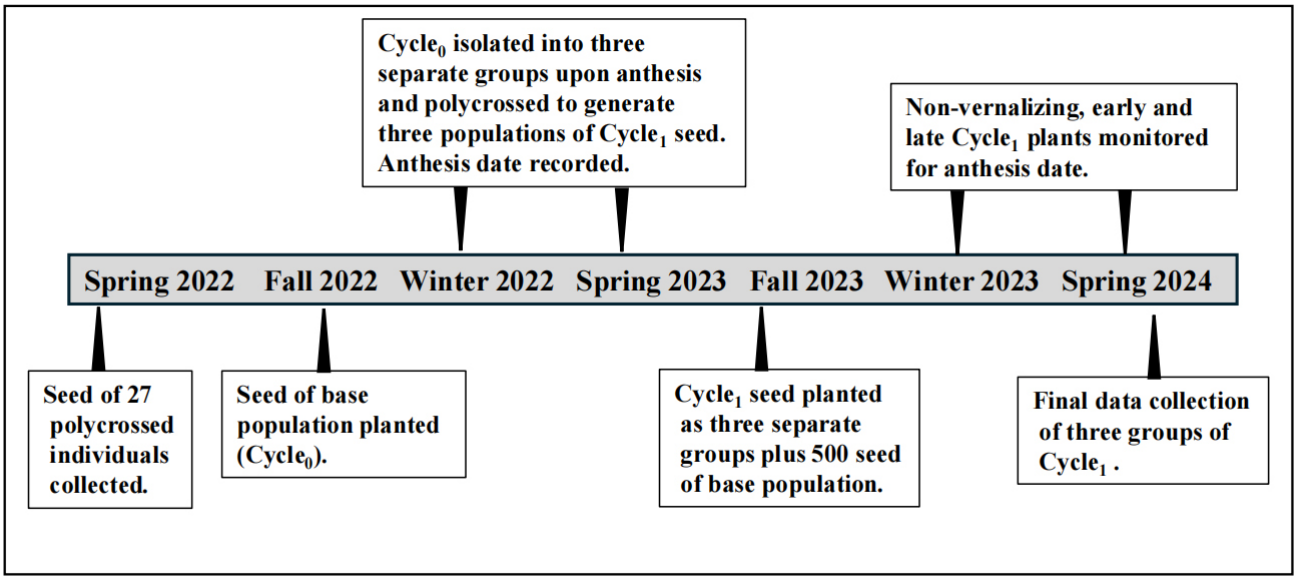

Figure 1. Timeline of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) population selection, segregation and generation.

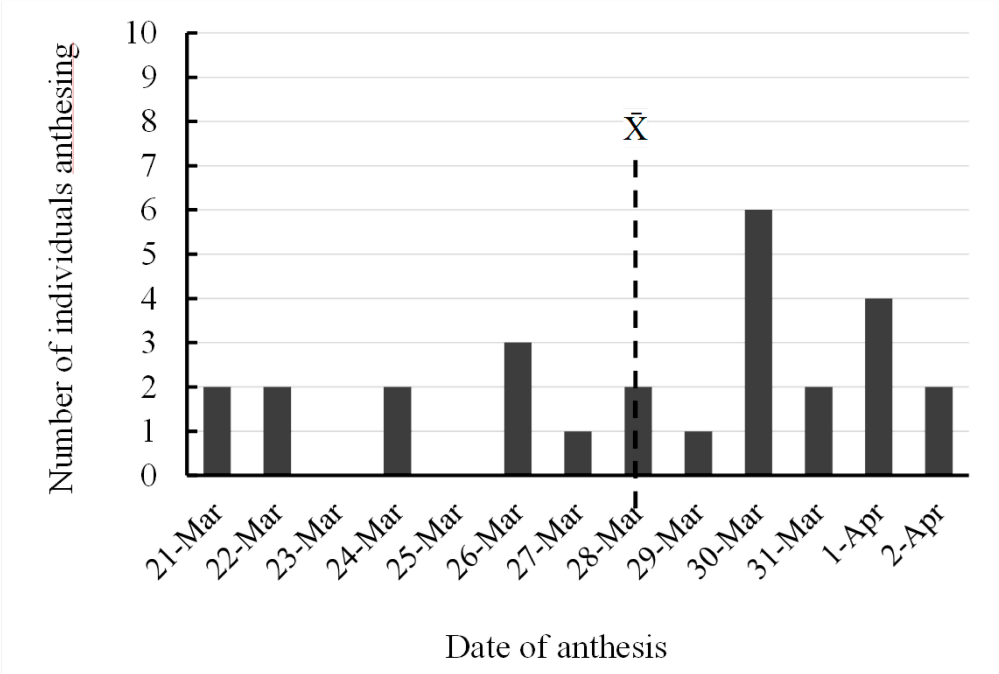

Figure 2. Mean (X̄) and range of anthesis date of the base population of 27 parents of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) collected in spring 2022.

Generation and Data Collection of Cycle$_0$ Populations

A seed increase was made during fall/winter 2022. Nine hundred and three seedlings of the base population shown in Figure 1 were germinated and grown in 50-cell deep trays under ambient outdoor fall conditions until predicted temperatures were significantly below freezing, then plants were moved to a cold greenhouse with a minimum temperature of 0 ℃. This population (Cycle$_0$) was segregated as they matured based on

anthesis. Three distinct parental groups were made during spring 2023: the "NV_0" consisting of 11 non-vernalizing progenies, the "E_0" group consisting of the 70 progenies earliest to anthesis, and the "L_0" group, comprising the last 70 individuals to reach anthesis. The balance of the 903 were discarded because their anthesis occurred between the selection extremes. These select groups were allowed to polycross isolated from one another and other pollen sources giving rise to three $Cycle_1$ seed populations: NV_1, E_1, and L_1 in spring 2023 (Figure 1). $Cycle_1$ seed were planted 3 October 2023 as described previously. Anthesis data were collected on each individual within each population as they matured, thus providing actual mean anthesis dates for comparison using equation (1) to predict gain due to selection.

Prediction for genetic gain

Anthesis date for early and late selected population was predicted by using selection intensity (i), the standard deviation of the base population (σ), and heritability (h^2) to calculate expected genetic gain (ΔG) due to selection (equation (1)).

$$\Delta G = i * \sqrt{(\sigma^2 * h^2)} \quad (1)$$

Where the selection intensity (i) is a selection proportion of 10% measured in standard deviation units (1.909). Standard deviation (σ) was calculated from the mean date of anthesis from the original base population. The heritability (h^2) for the date of anthesis was estimated to be 0.7 [16]. (1) $\Delta G = 1.909 * \sqrt{(4.33^2 * 0.7)}$; (2) $\Delta G = 5.78$ days.

The math from equation (1) suggests that selection under these parameters would result in a genetic change of ± six days in the occurrence of anthesis in the subsequent generation.

Selected parents of the early population (E_0)

The base population had a mean anthesis date of 28 March (the 87th ordinal day of 2022). Equation (1) predicts a mean early anthesis date (87–6 days) to be the 81st ordinal day of the year or 21st March. The 70 individuals of the base population which had an anthesis date earliest than the mean were selected as the parents of our early population (E_0). This group has an anthesis dates ranging from 9 February 2023 to 21 March 2023 with the mean anthesis date of 5 March 2023 (Figure 3).

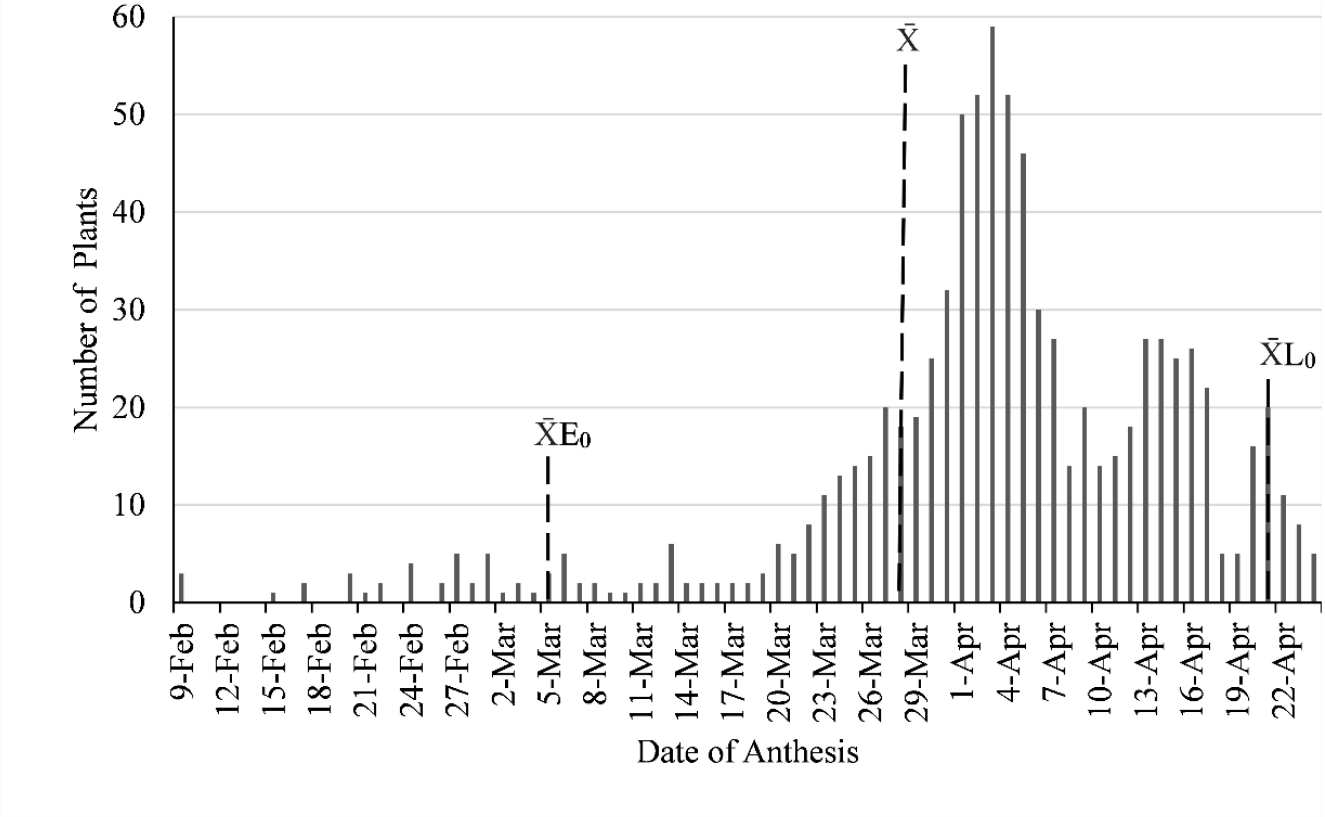

Figure 3. Mean ($\bar{X}$) and range of anthesis date of the $Cycle_0$ population of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) planted in Fall 2022. $\bar{X}E_0$ and $\bar{X}L_0$ are the mean anthesis dates of selected early and late parents, respectively.

Selected parents of the late population (L_0)

The same equation predicts a mean anthesis date of the L_1 population as follows: (87 + 6) 94th ordinal day of the year or 4th April. To make the selection more effective and keep the selection intensity at 10%, the final 70 plants of the population to reach anthesis were isolated and polycrossed as the parents of the late population (L_0). This group had an anthesis date ranging from 18 April 2023 to 28 April 2023 with the mean anthesis date of 21 April 2023 (Figure 3).

Selected parents of the non-vernalizing population (NV_0)

Unexpectantly, 11 plants in $Cycle_0$ flowered exceptionally earlier in the very last weeks of January. These plants were segregated and designated as our non-vernalizing (NV_0) population and were allowed to polycross with each other.

$Cycle_1$

To generate $Cycle_1$, 1000 seed of each of the NV, E, and L populations from $Cycle_0$ along with 500 seed remaining from the original base population were planted in fall 2023 (Figure 1). As before, they were

planted in 50-cell deep trays maintaining a minimum temperature of 0 ℃. Anthesis date was monitored and recorded for each individual of each population.

Statistical Analysis

Analysis of variance

Analysis of variance (ANOVA) was used for mean separation among the anthesis dates of the four populations grown in $Cycle_1$ using R 4.3.0 [17]. The four populations are: non-vernalizing population (NV_1), early population (E_1), base population (B), and late population (L_1). Differences in population groups were analyzed using Tukey's LSD *post hoc* tests after ANOVA. Further analysis was carried out by calculating the χ^2 test and heritability. For the analysis, B was taken as the standard, and the other three populations were compared with the base population to measure the effectiveness of selection.

Determination of heritability

Heritability measures the proportion of phenotypic variance due to genetic factors. Narrow-sense heritability (h^2) can be calculated after the gain is observed by measuring the mean phenotypic similarity of parents to their progeny. For each population, the standard deviation(σ) was calculated. Genetic gains for each population were obtained from equation (1) and h^2 was calculated for each population.

Realized heritability (h^2_R)

The realized heritability(h^2_R) is an estimate of narrow-sense heritability based on the observed response to selection. Narrow-sense heritability includes methods in which pedigrees are constructed by crossing specific males and females, or natural mating for which at least one parent is known [18]. However, these designs are not always applicable as in natural populations pedigrees are not usually known, especially among obligately outcrossing species. Thus, realized heritability is employed by comparing the response to selection, i.e., the actual change achieved in the offspring (mean of the base population minus the mean of the progeny) over the selection differential (mean of the base population minus the mean of the selected parents) [19]. Narrow-sense heritability is always equal to or greater than realized heritability. Equation (2) was used to calculate the realized heritability of the anthesis date and compare it to that found in McLean & Watson [20].

$$h^2_R = (\bar{X}Px - \bar{X}B) \text{ of } Cycle_1 \,/\, (\bar{X}_{selected} - \bar{X}B) \text{ of } Cycle_0 \quad (2)$$

where, $\bar{X}Px$ = Mean anthesis date of polycrossed progeny.
$\bar{X}B$ = Mean anthesis date of base population grown in $Cycle_0$ or $Cycle_1$.
$\bar{X}_{selected}$ = Mean anthesis date of selected population in $Cycle_0$.

Chi-square (χ^2)

Chi-square analysis (χ^2) served as a secondary evaluation tool to assess the differences among the population of the first cycle of selection ($Cycle_1$) for the anthesis date compared to the base population. The χ^2 form is used below.

$$\chi^2 = (\text{observed} - \text{expected})^2 / \text{expected} \quad (3)$$

Where observed is the actual anthesis date of individual plants of each population and expected is the mean anthesis date of the base population plants. The null hypothesis (H_0) is that there was no effect due to selection. These calculated values were then contrasted with the critical value derived from the χ^2 table with degrees of freedom (df) as n−1 and α = 0.05. For instance, with the degrees of freedom of 999 (1000 total plants providing seed), the tabulated critical value of χ^2 was 1074.679 [21].

RESULTS

Genetic Gain

The genetic gain was measured in terms of days gained or lost by comparing the mean anthesis date of NV_1, E_1, and L_1 to that of the B population as the standard using equation (1). When the mean anthesis date of the B population grown in 2023 (Figure 4) was compared with the other three populations, a shift in anthesis date to that of the B population was observed (Figure 2 vs Table 1) compared to the prior year. The NV_1 population, with a mean anthesis date of 14 March 2024 (Figure 5), had the greatest genetic gain of 18 compared to the other populations. The E_1 population, with a mean anthesis date of 18 March 2024 (Figure 6), had an advancement of 14 days compared to B. The genetic gain for E_1 was +14 days. And the lowest was L_1 for which the mean anthesis date was 11 April 2024 (Figure 7). The L_1 population flowered 10 days later than the B population. Hence, it had a genetic gain of −10 days compared to B.

Table 1. Genetic gain for each population grown in $Cycle_1$.

Population	Number of plants	Mean anthesis date	Genetic gain (ΔG)
Base population	500	2 April (92nd day)	-
Non-vernalizing (NV_1)	1000	14 March (73rd day)	+18 days
Early (E_1)	995	18 March (77th day)	+14 days
Late (L_1)	1000	11 April (101st day)	−10 days

The response for the selection of anthesis date was asymmetric, with the E_1 population having a greater response (14 days) than the plant selected for L_1 (10 days).

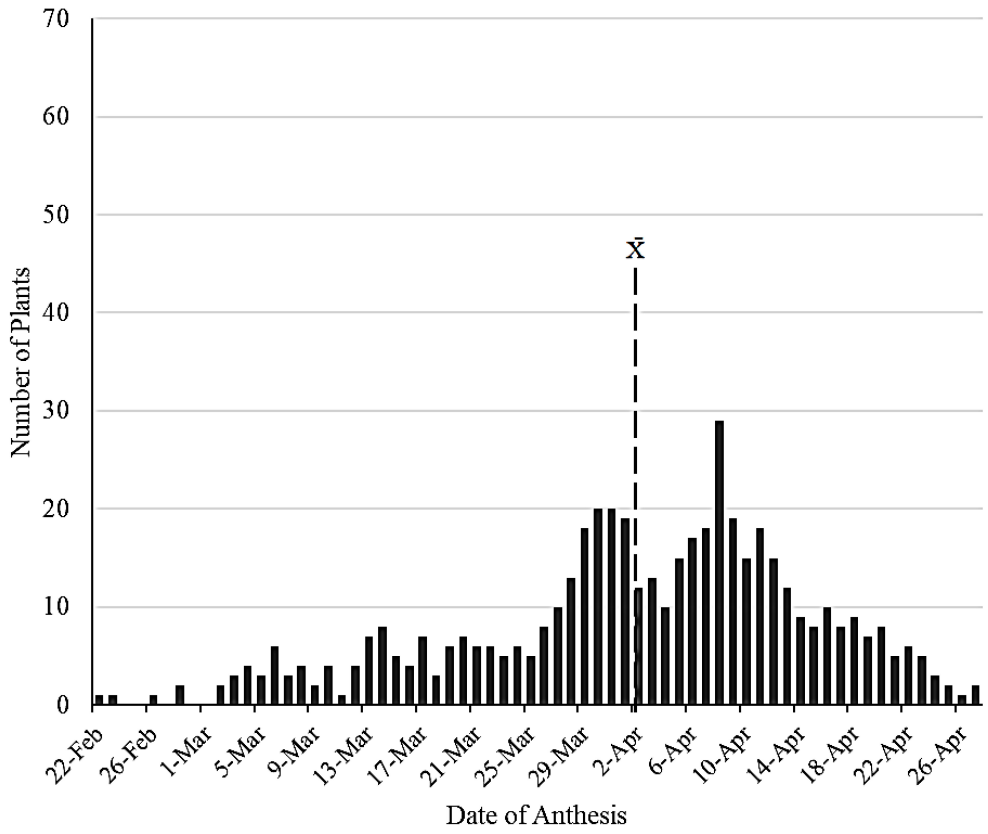

Figure 4. Mean (X̄) and range of anthesis date of the B population of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) collected in 2022 and grown again in $Cycle_1$ (Fall 2023–2024).

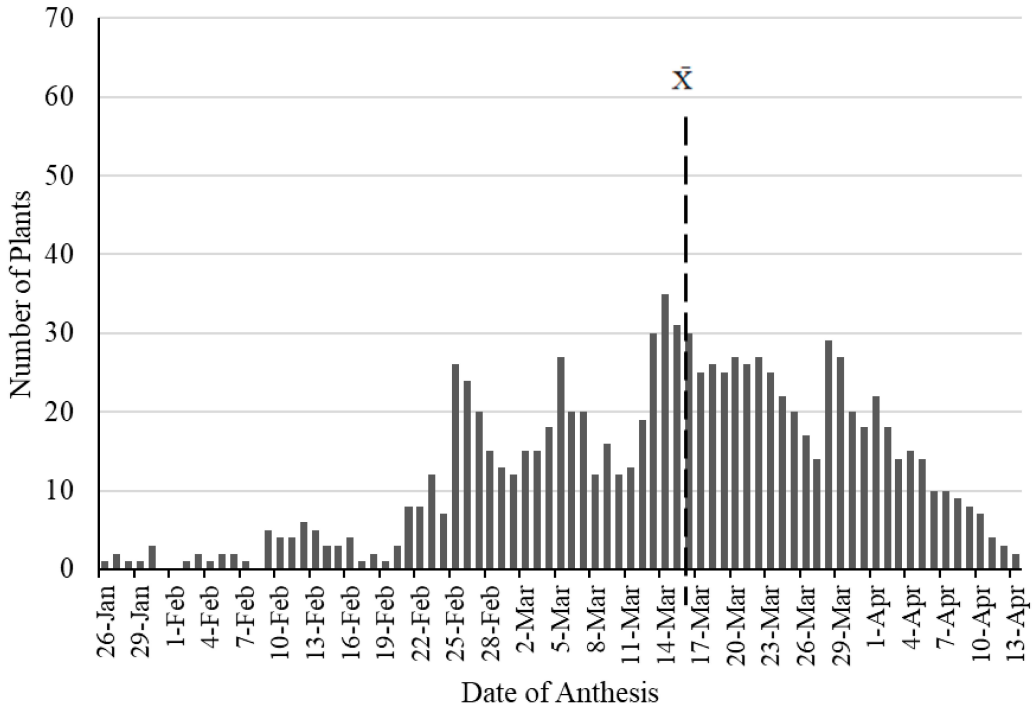

Figure 5. Range and mean (X̄) anthesis date of the NV_1 population of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) grown in $Cycle_1$ (Fall 2023–2024).

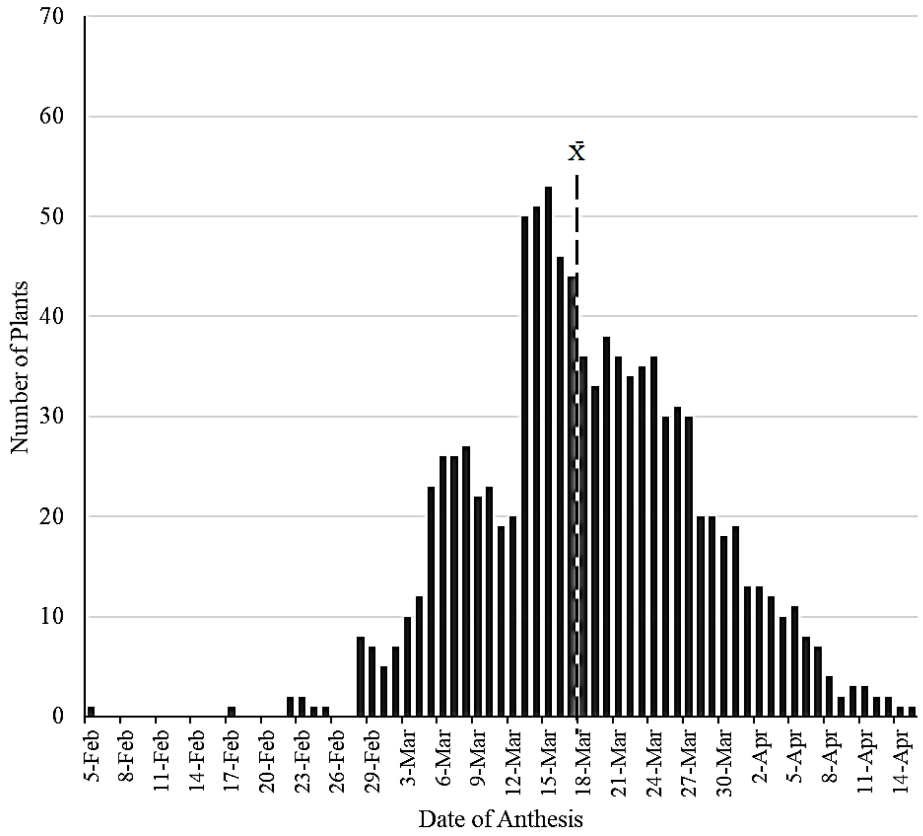

Figure 6. Range and mean (X̄) anthesis date of the E_1 population of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) grown in $Cycle_1$ (Fall 2023–2024).

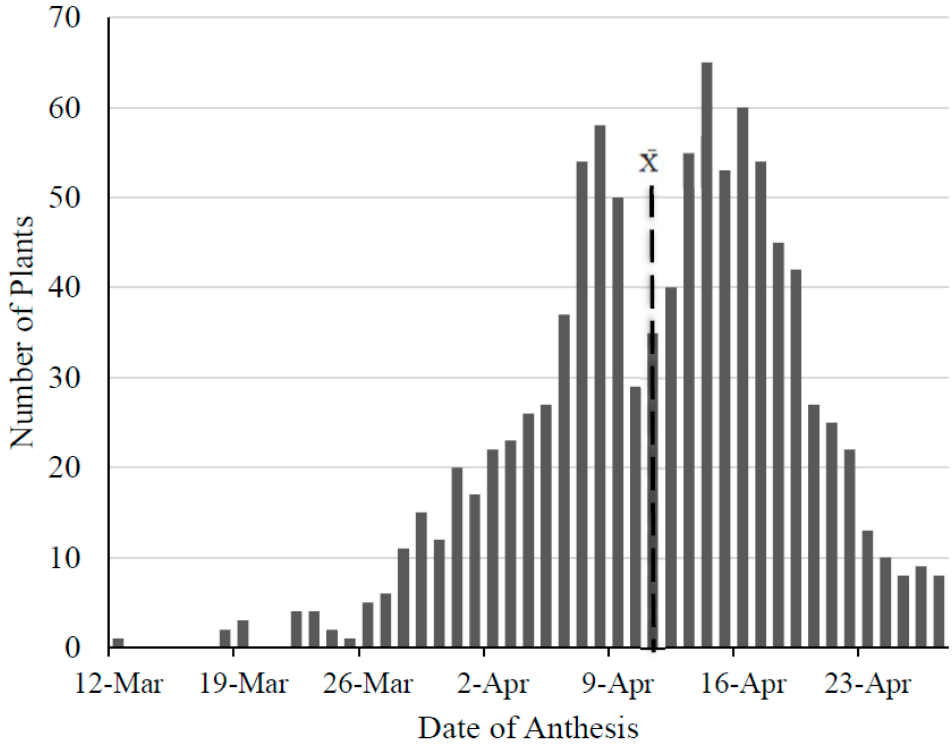

Figure 7. Range and mean (X̄) anthesis date of the L_1 population of annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) grown in $Cycle_1$ (Fall 2023–2024).

Difference in Anthesis Date

Analysis of variance (ANOVA) results indicated that populations in Cycle$_1$ were significantly different (P < 0.01) with respect to the mean anthesis date (Figure 8). Tukey's HSD test shows that all group-wise comparisons were significant (P < 0.01). The NV$_1$ population had the earliest mean anthesis date of 14 March 2024 (73rd day of the year), followed by the E$_1$ population with a mean anthesis date of 18 March 2024 (77th day of the year), B population with a mean anthesis date of 2 April 2024 (92nd day of the year) and the L$_1$ population with a mean anthesis date of 11 April 2024 (101st day of the year).

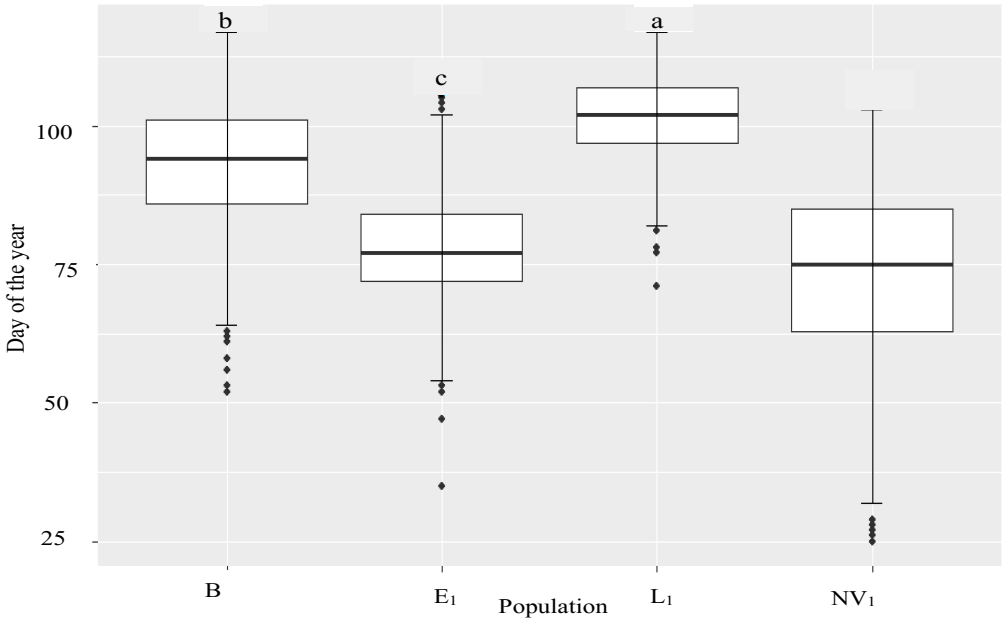

Figure 8. Box plot showing the difference in mean anthesis dates among annual ryegrass (*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot) populations grown in Cycle$_1$ (Fall 2023–2024). Different letters above the boxes indicate statistically significant differences (P < 0.01) among populations. Tukey's LSD *post hoc* test was conducted for mean separation. B, E$_1$, L$_1$, and NV$_1$ denote base population, early population, late population, and non-vernalizing population, respectively.

Narrow-Sense Heritability (h^2)

The NV$_1$ population with +18 days genetic gain had the least h^2 of 0.39 (Table 2). The E$_1$ population with +14 days genetic gain had the greatest h^2 of 0.61. The L$_1$ population with −9 days had a h^2 of 0.45.

Table 2. Narrow-sense heritability for each population in Cycle$_1$, calculated from $\mathbf{\Delta G = i * \sqrt{(\sigma^2 * h^2)}}$ based on the actual gain.

Population	Standard deviation (σ)	Genetic gain	Heritability (h^2)
Non-vernalizing (NV$_1$)	15.058	+18 days	0.39
Early (E$_1$)	9.52	+14 days	0.61
Late (L$_1$)	7.4	−10 days	0.45

Realized Heritability (h^2_R)

The E_1 population had an h^2_R of 0.60 and the L_1 population had an h^2_R of 0.41 (Table 3). In both populations, the h^2 is greater than the h^2_R (Table 3).

Table 3. Realized heritability for E_1 and L_1 of $Cycle_1$, calculated by the genetic gain after selection and estimated reach before selection.

Population	$\bar{X}_{selected}$	Reach ($\bar{X}_{base}$ = 87 (28th March))	Genetic gain	Heritability (h^2_R)
Early (E_1)	64	23	+14 days	0.60
Late (L_1)	111	24	−10 days	0.41

Chi-Square (χ^2)

Chi-square was calculated by using equation 3 for detecting the independence between populations compared to B. The null hypothesis suggests the selected populations were dependent on B (i.e., no difference due to selection). Therefore, the "expected" value is the mean anthesis date of B. The "observed" outcomes were the individual flowering dates of each selected population. The tabulated value for all three populations with (~1000 plants) was 1074.68. The calculated χ^2 for the NV_1 population was 6121.21, the E_1 population was 3269.04, and the L_1 population was 1558.84 (Table 4). For each population, the calculated χ^2 value was greater than the tabulated value at $\alpha = 0.05$). This signifies that the mean date of anthesis of each of the populations were different from that of B.

Table 4. Chi-square test for determining the independence of each population of $Cycle_1$.

Population	Number	χ^2 (calculated)	χ^2 (tabulated)	Decision
Base population	500	-	-	-
Non-vernalizing (NV_1)	1000	6121.206	1074.679	H_0 Rejected
Early (E_1)	995	3269.043	1074.679	H_0 Rejected
Late (L_1)	1000	1558.837	1074.679	H_0 Rejected

p = 0.05, df = 999.

Phenotypic Difference

There was an observable phenotypic difference due to selection between the date of anthesis of the E_1 and L_1 populations grown in $Cycle_1$ (spring 2024). Though both populations originated from the same base population, there was an obvious shift in the mean anthesis date between the E_1 and L_1. The phenotypic difference is such that when all the plants

from E_1 had completed flowering, while the plants from L_1 were still in their late vegetative growth (Figure 9).

Figure 9. Comparison of the Early (E_1) and Late (L_1) populations grown side-by-side showing the E_1 population had already jointed by the15 April (2024) while the L_1 population was still vegetative. Photo by Prakriti Adhikari.

DISCUSSION

With a single cycle of selection, there was a measurable shift in anthesis date in both positive and negative directions. McLean and Watson [20] also found that divergent selection caused a change in the anthesis date of annual ryegrass. The response we observed for the selection of anthesis date was asymmetric, with the E_1 population having a greater response (14 days) than L_1 (10 days). This contradicts the study by McLean and Watson [20] in annual ryegrass, who found that the early cultivars within two cycles of selection showed a greater response to selection for lateness (11 days) than for earliness (6 days), while the late-maturing cultivar showed a greater response to selection for earliness (~5) than for lateness (~3 days).

Rapid response to selection can be attributed to annual ryegrass's wide genetic diversity because of its self-incompatibility and high rate of outcrossing, evolution from diverse wild germplasm, and extremely high gene transfer among different plant populations. About 98% of the total diversity in annual ryegrass is intra-populational and only 2% inter-populational, suggesting a period of gene exchange before separation and no differential selection in different cultivation areas [15]. This high diversity within populations could also be explained by the relatively short period of breeding and selection of this forage species. The population still harbors genetic variation for various traits among its recessive alleles which are maintained by the heterozygous nature of this species [22]. Additionally, the response of the plant to photoperiod is polygenically controlled, with no main gene differences being apparent [12]. This makes possible continuous variation in inflorescence development and hence, anthesis and flowering.

In comparison to the plants from other population groups, flowering in NV_1 occurred before being exposed to sub-zero and January day lengths. This observation is supported by the experimental study by Cooper [12], where 50% of Italian ryegrass plants exhibited obligatory cold and short-day requirements for induction, whereas the rest showed a quantitative response by producing heads in the absence of any inductive treatment. In B population we observed a few individuals jointing in early January completely succumb to subfreezing temperatures. This was also observed by Nelson et al. [4]. It seems cold hardiness is lost once jointing is underway. In areas where cold fronts are common, such as ours, individuals classified as NV would be eliminated by subfreezing events and not normally contribute to the next generation.

The change in mean anthesis date of E_1 (77th day of the year) due to selection in this study was greater compared to the earliest mean anthesis date recorded (101st day of the year) in the study of a divergent selection of annual ryegrass by McLean and Watson [20]. The study by Casler [16], which found that the date of anthesis in switchgrass had a high realized heritability of around 0.7, supports the relatively high realized heritability for E_1 flowering date (0.6). Most plants in outbreeding populations are heterozygous for genes controlling the inductive requirements for flowering. After segregation and recombination, genetic variation is released which provides an important source of variation for long-term response to selection [23]. The earliness in the anthesis date of E_1 can also be attributed to their genetic response to freezing temperatures and the length of the photoperiod. Also, in southern latitudes, selection for earliness of anthesis is more effective [12].

The base population collected in 2022 was feral (Figure 1). Individuals were collected based on their earliness of anthesis. Because of this, even L_1 selected from the base population still had an anthesis date earlier than most other annual ryegrass populations found at this latitude. Also, L_1 flowered rapidly once the first plant in this population initiated jointing and thus, had a low variance. The time of flowering for L_1 started on 12 March 2024 and gained momentum from 17 March 2024 when the temperature was warm (26 ℃ day and 20 ℃ night) and likely accelerated flowering, truncating the latter part of the range [24]. The NV_1 and E_1 populations had greater range than L_1. and thus, had greater genetic diversity for anthesis date than L_1. These findings are supported by Cooper [12] in his study of outbreeding grasses where low-temperature exposure often reveals genetic differences in competence between individuals in the population.

CONCLUSIONS

Anthesis date is an important trait in annual ryegrass because it determines the seasonal distribution and nutritional quality of pastures. This study concludes that the anthesis date of annual ryegrass is highly heritable and responds rapidly to phenotypic selection. Equations tested

in this experiment were not completely accurate, predicting equal change due to selection, but changes were asymmetric. The genetic gain calculated shows that recurrent phenotypic selection was effective for manipulating the anthesis date of annual ryegrass. The Chi-square test also indicated significant population separation through selection. On a genetic level, estimations of h^2 and h^2_R indicate that germplasm at the cycle of selection was accumulating a greater amount of additive gene action for the desired anthesis date. We expect this heritability value to continue to increase with further cycles of selection, as the alleles responsible for the desired flowering date continue to accumulate.

A third, unexpected, population (the non-vernalizing) was discovered because we controlled the minimum temperature to which plants were exposed. Selection among members of the non-vernalizing population (NV_1) resulted in advances flowering date without cold treatment. Such advances would shorten the life cycle of annual ryegrass; however, advances without vernalization result in an early onset of jointing and corresponding loss of cold hardiness resulting in mortality when exposed to severe cold. As such, these individuals are often “self-cleaning” from the general population of annual ryegrass at our location. For the early population (E_1), plants started flowering after the onset of cold temperatures but while the days were still short (February). The was a 14-day advance in anthesis in the early population. While there is a significant advance, it is probably not sufficient to induce complete senesce before land preparation for spring planting at our location; an additional cycle of selection would be necessary to achieve an auto-terminating winter cover crop.

The late population (L_1), however, required both cold temperatures and long-day photoperiodic induction for flowering. One cycle of selection resulted in a 10-day delay in anthesis. A practical application of this population is as a late senescing cultivar. As such it would retain its nutritional quality later in spring than other early cultivars because of delayed fiber accumulation associated with jointing [14,15].

DATA AVAILABILITY

Data generated from this study is available upon request from the lead author.

AUTHOR CONTRIBUTION

Conceptualization, JIM and BSB; Methodology, BSB, JIM and PA; Software, PA and JIM; Validation, JIM, BSB and PA; Formal Analysis, JIM and PA; Investigation, PA; Resources, BSB; Data Curation, PA and BSB; Writing—Original Draft Preparation, PA; Writing—Reviewing & Edition, BSB and JIM; Visualization, BSB; Supervision, BSB; Project Administration, BSB; Funding Acquisition, BSB.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Vieira EA, Castro CM, Oliveira ACD, Carvalho FIFD, Zimmer PD, Martins LF. Genetic structure of annual ryegrass (*Lolium multiflorum*) populations estimated by RAPD. Sci Agric. 2004;61:407-13.
2. Castanheira N, Dourado AC, Kruz S, Alves PIL, Delgado-Rodríguez AI, Pais I, et al. Plant growth promoting *Burkholderia* species isolated from annual ryegrass in Portuguese soils. J Appl Microbiol. 2016;120(3):724-39.
3. Heap I. The international herbicide-resistant weed database. Available from: http://www.weedscience.org. Accessed on 30 Jan 2023.
4. Nelson LR, Phillips TD, Watson CE. Plant breeding for improved production in annual ryegrass. In: Rouquette FM, Nelson LR, editors. Ecology, production, and management of Lolium for forage in the USA. Hoboken (US): Wiley; 1997. p. 1-14. doi: 10.2135/cssaspecpub24.c1
5. Jung HG, Allen MS. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J Anim Sci. 1995;73(9):2774-90.
6. Casler MD, Carlson IT, Berg CC, Sleper DA, Barker RE. Convergent-divergent selection for seed production and forage traits in orchardgrass: I. Direct selection responses. Crop Sci. 1997;37(4):1047-53.
7. Vogel KP, Pedersen JF. Breeding systems for cross-pollinated perennial grasses. Plant Breed Rev. 1993;11:251-74.
8. Hanson AA, Sprague VG. Heading of perennial grasses under greenhouse conditions. Agron J. 1953;45:248-51.
9. Templeton WC Jr, Mott GO, Bula RJ. Some effects of temperature and light on growth and flowering of tall fescue, *Festuca arundinacea Schreb*. II. floral development 1. Crop Sci. 1961;1(4):283-6.
10. Hicks DH, Mitchell KJ. Flowering in pasture grasses: 1. Interactions of day length and temperature on inflorescence emergence for *Festuca arundinacea* Schreb. variety Manade. N Z J Bot. 1968;6(1):86-93.
11. Warnke SE, Barker RE, Jung G, Sim SC, Mian MAR, Saha MC, et al. Genetic linkage mapping of an annual× perennial ryegrass population. Theor Appl Genet. 2004;109(2):294-304.
12. Cooper JP. Studies on growth and development in Lolium: IV. Genetic control of heading responses in local populations. J Ecol. 1954;42(2):521-56.
13. Humphreys MO, Eagles CF. Assessment of perennial ryegrass (*Lolium perenne* L.) for breeding. I. Freezing tolerance. Euphytica. 1988;38(1):75-84.
14. Hunt IV. Comparative productivity of 27 strains of grasses in the west of Scotland. J Br Grassl Soc. 1956;11(4). doi: 10.1111/j.1365-2494.1956.tb00076.x

15. O'Donovan M, Lewis E, O'Kiely P. Requirements of future grass-based ruminant production systems in Ireland. Ir J Agric Food Res. 2011;50(1):1-21.
16. Casler MD. Selection for flowering time as a mechanism to increase biomass yield of upland switchgrass. Bioenerg Res. 2020;13(1):100-8.
17. R Core Team. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2023.
18. Lstibůrek M, Bittner V, Hodge GR, Picek J, Mackay TFC. Estimating realized heritability in panmictic populations. Genetics. 2018;208(1):89-95.
19. Falconer DS. Introduction to Quantitative Genetics. 4th ed. London (UK): Longman; 1996.
20. McLean SD, Watson Jr CE. Divergent selection for anthesis date in annual ryegrass. Crop Sci. 1992;32(4):847-51.
21. Clewer AG, Scarisbrick DH. Practical statistical design for plant and crop science. Hoboken (US): Wiley; 2013.
22. Breese EL, Hayward MD. The genetic basis of present breeding methods in forage crops. Euphytica. 1972;21(2):324-36.
23. Cooper JP. Short-day and low-temperature induction in *Lolium*. Ann Bot. 1960;24(2):232-46.
24. Chen M, Zhang TL, Hu CG, Zhang JZ. The role of drought and temperature stress in the regulation of flowering time in annuals and perennials. Agronomy. 2023;13(12):3034.

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