Received: 9 March 2023 | Accepted: 19 June 2023 | Published online: 12 July 2023

DOI: 10.1002/csc2.21045

Crop Science

ORIGINAL ARTICLE

Turfgrass Science

Genotypic variation in germination rate, seedling vigor, and seed phenotype of Kentucky bluegrass cultivars

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Assigned to Associate Editor David Jespersen.

Abstract

Kentucky bluegrass (*Poa pratensis* L.) is the most widely used cool-season turfgrass for athletic fields, but its seeds germinate slowly. The objective was to determine the germination rate, seedling vigor, and seed phenotype of Kentucky bluegrass cultivars. Germination and seedling vigor tests were conducted using a seed incubator set at alternating 25/15˚C (day/night) temperatures and an 8 h day^{-1} photoperiod. Twenty-one cultivars were tested using two seed lots for each. Germination was most rapid among the experimental genotype PST-T15-44 and the commercial cultivar "After Midnight, as they took the minimum days to reach 50% germination in only 7.9–8.0 or 8.8 days, respectively. Cultivars Tirem and Volt required >12 days in both lots to reach 50% germination (12.0–13.7 days). There was a difference of 5–6 days in 50% germination between cultivars. Seedling vigor was also measured but few differences between cultivars were noted. Data on seed phenotype including seed length, width, area, circularity, and thousand seed weights (TSW) all varied by cultivar. ''After Midnight'' had the longest seeds (3.065 mm). ''Moonlight SLT'' seeds were the shortest in length (2.366 mm), narrowest in width (0.717 mm), and smallest in area (1.328 cm^2). Across all cultivars, seed length and circularity were correlated with germination rate ($r = 0.62$, $p = 0.0025$; $r = -0.53$, $p = 0.0141$). Seed phenotyping could be an important tool in breeding for improved germination rate. By identifying and using rapidly germinating Kentucky bluegrass cultivars, it may be possible to establish turf more rapidly.

1 | INTRODUCTION

Kentucky bluegrass (*Poa pratensis* L.) is a perennial, cool-season grass used as a forage and turfgrass (Beard & Beard, 2005; Honig et al., 2012). Northern climates of the United States, specifically the cool-humid and cool-arid climate zones, are the preferred areas to grow Kentucky bluegrass (Christians et al., 2017). Due to distinct phenotypes, Kentucky bluegrass cultivars are commonly placed into categories or classifications based on their growth characteristics (Honig et al., 2018; Shortell et al., 2009). Bonos et al. (2000) initially identified six Kentucky bluegrass classification types, including Common, Bellevue, Mid-Atlantic, Aggressive, Compact, and Baron, Victa, Merit, and Gnome (BVMG) based on grass growth and traffic stress results from test plots at Rutgers University. Later, microsatellite markers, known as simple

Abbreviations: AOSA, association of official seed analysts; BVMG, baron, victa, merit, and gnome; D_{50}, days to 50% germination; D_{90}, days to 90% germination; TSW, thousand seed weight; TZT, tetrazolium test.

sequence repeats (SSRs), were used to reclassify Kentucky bluegrass cultivars based on genetics (Honig et al., 2012, 2018).

Kentucky bluegrass is utilized in both high-input and low-input turf areas and may be propagated by seed or vegetatively by sod (Beard, 1973; Beard & Beard, 2005; Christians et al., 2017). Sod is the preferred establishment method for Kentucky bluegrass at these sites as this establishment method provides instant turf (Agnew & Christians, 1992). However, the additional expense of establishing sod means that most choose to plant from seed (Christians et al., 2017; Reicher et al., 2006). Following planting, Kentucky bluegrass may take anywhere from 6 to 30 days to germinate (McCarty, 2018), with more days from planting until germination under cooler conditions. Conversely, tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) and perennial ryegrass (*Lolium perenne* L.) establish more quickly, requiring only 5–9 days to germinate after seeding (Christians et al., 2017).

There are a variety of ways to increase the establishment and growth of the slow germinating Kentucky bluegrass. The seeding date is important for enhancing germination rate and establishment in cool-season grasses as soil temperature influences germination rate (Reicher et al., 2000). Seed mixtures are also used in cool-season turfgrasses to combine species-specific benefits (Beard & Beard, 2005). Kentucky bluegrass is commonly mixed with perennial ryegrass, tall fescue, or fine fescues (*Festuca* spp.) (Brede, 2000). These species improve the establishment rate as a companion species, as they germinate in 3–10 days under ideal conditions (Stier et al., 2005). Seed priming is another way to enhance establishment from seeds by soaking seeds in water prior to planting and seed germination (Campbell et al., 2019; Elias et al., 2012).

Past research on Kentucky bluegrass germination identified the importance of both chilling seeds prior to germination tests (Sprague, 1940) and using alternating rather than constant temperatures to speed germination (Aamlid & Arntsen, 1998; Sprague, 1940). While high temperatures during anthesis can reduce seed production yield of Kentucky bluegrass, total germination is similar among production environments if the seed is prechilled for 7 days prior to germination tests (Maun et al., 1969). Soares et al. (2016) evaluated both a tetrazolium test (TZT) and a standard germination test on the viability of turfgrass seeds and found TZT worked to determine viability on most grasses tested, except for Kentucky bluegrass. Germination tests provide data on the speed of germination (i.e., germination rate) and/or the total germination percent. While germination rate is known to differ among Kentucky bluegrass cultivars (Maguire, 1962), few experiments have explored the potential genetic variability in germination rate among Kentucky bluegrass cultivars (Kim, 2015; Larsen et al., 2004).

Core Ideas

- Germination vigor differs among Kentucky bluegrass cultivars.
- Seed size and shape were variable among Kentucky bluegrass cultivars.
- More rapid Kentucky bluegrass germination was associated with longer and more linear-shaped seed.
- It may be possible to establish turf more quickly through Kentucky bluegrass cultivar selection.

Previous research (Christians et al., 1979; Larsen et al., 2004) assessed the size of Kentucky bluegrass seed through seed counts and weight, but not seed size or shape. Christians et al. (1979) determined that the seed weight varies by Kentucky bluegrass cultivar as some have much larger seeds than others. Additionally, Larsen and Andreasen (2004) found that within a single Kentucky bluegrass cultivar, heavier individual seeds had a shorter mean germination time than light seeds, but there are no studies that have investigated the influence of seed phenotype on germination rate or seedling vigor across a group of Kentucky bluegrass cultivars.

The objectives of this research were to determine (1) the germination rate and seedling vigor of Kentucky bluegrass cultivars and (2) the relationship between seed phenotype (weight, size, and shape) and Kentucky bluegrass germination.

2 | MATERIALS AND METHODS

The experiments were conducted at Purdue University in West Lafayette, Indiana. Kentucky bluegrass cultivars were tested (Table 1) using two seed lots of each cultivar to determine the influence of genetics on germination rate and seedling vigor (Table 2).

2.1 | Germination rate and percent

After 8 months of refrigeration (4°C), the first germination test was initiated on October 13, 2021 using seeds from seed lot 1. Seeds were germinated in 100 × 15 mm Petri dishes with lids (VWR Catalog Number: 25384-342) and 83-mm diameter blue blotter paper (PBB 325, Hoffman Manufacturing). Fifty seeds of each cultivar were placed in each Petri dish with four replications total for each cultivar. The Petri dishes were arranged in a randomized complete block in the germination chamber (Geneva Scientific LLC,

TABLE 1 Name and characteristics of Kentucky bluegrass (KBG) classification and cultivars used in this research as well as the name of the company supplying seed.

Name of KBG classifications	Characteristics of classification[a]	Cultivar[b,c,d,e]	Company
Baron, Victa, Merit, and Gnome (BVMG)	Medium to good turf and growth	Jackpot	Jacklin seeds
		Blue Chip	Jacklin seeds
		Tirem	Pure seed testing
		Kelly	Mountain view seeds
Eurasian	High density	Bolt	Mountain view seeds
Julia	High-quality turf, density, and wear tolerance	Jackrabbit	DLF/Seed Research Oregon
Limousine	High density and wear tolerant	Sombrero	DLF/Seed Research Oregon
Shamrock	Good turf quality, good sod strength, and high strength rhizomes	Fielder[f]	DLF/Seed Research Oregon
		SR 2100	DLF/Seed Research Oregon
		SR 2150[f]	DLF/Seed Research Oregon
		Volt	Mountain view seeds
		Arrowhead	Mountain view seeds
Elite compact	Increase turf quality, decrease compact, and drought stress	Desert Moon[f]	Pure seed testing
		Moonlight SLT	Pure seed testing
Compact-America	Increase density, decrease impact with increased turf	Jumpstart	Pure seed testing
		SR 2284	DLF/Seed Research Oregon
		BlueNote	Mountain view seeds
Compact-midnight	Decrease in compact growth and heat tolerance	After Midnight	Jacklin seeds
		Arcadia	DLF/Seed Research Oregon
		Midnight	Jacklin seeds
		Legend	Mountain view seeds
		Acoustic	Mountain view seeds
Unknown	Categorized with unknown specificity	PST-K17-182[f]	Pure seed testing
		Harvest Moon	Pure seed testing
		PST-T15-44	Pure seed testing

[a]Honig and Brilman (2018); Murphy et al. (2004).
[b]Margaret Childers, personal communication (24 Jan. 2022); Jacklin Seed (n.d.).
[c]Greg Freyermuth, personal communication (2 Feb. 2022).
[d]Honig & Brilman (2018).
[e]Mountain View Seeds (2017); Adam Russell, personal communication (20 Jan. 2022).
[f]Cultivars did not meet initial germination testing minimum of 80% in the first experimental run and were excluded from the first and second experimental run.

I-30BLL). The germination chamber was set to 25˚C in daytime and 15˚C for nighttime with an 8-h photoperiod, following protocols of the Association of Official Seed Analysts (AOSA, 2016).

Blotter paper substratum inside the Petri dishes was wet with 0.2% potassium nitrate (KNO_3) solution prior to the start of the test and throughout the 28 days period as needed. The seeds were checked in individual Petri dishes every day for 28 days. Germinated seeds were counted as one seed when the leaf reached 10 mm in height. Germinated seeds were counted, removed, and recorded daily. On the final counting day (28 days), the seeds that did not germinate were counted. The total percentage of germination and the germination rate (speed of germination) was calculated for each Petri dish (Maguire, 1962). Germination rate was calculated for each Petri dish according to Maguire (1962) using Equation (1):

$$\mathrm{GR} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \cdots + \frac{n_i}{d_i}, \tag{1}$$

where GR is the germination rate, n_i is the number of seeds that germinated in the time i (not the accumulated number, but the number correspondent to the ith observation), and d_i is number of days from the beginning of the germination test to the ith observation. A higher value indicates more rapid germination.

Twenty-one cultivars with ≥80% germination from the first seed lot tested were selected and the experiment was repeated on March 7, 2022 using seeds from a second seed lot (Table 2).

TABLE 2 Cultivars of Kentucky bluegrass and seed lots used in these experiments.

	Seed lot 1		Seed lot 2	
Cultivar	Lot#	Harvest year	Lot#	Harvest year
Acoustic	NBS-JP19-A1131-SS	2019	GBS-DM21-KB1131-1	2021
After Midnight	83.66.17	2018	85-39-19-J	2020
Arcadia	85.79.18-J	2018	85-110-19-K1	2019
Arrowhead	GBS-DM19-AH-1	2019	GBS-DM21-AH-5	2021
Blue Chip	81.131.15-J	2016	85-109-19-JTK	2020
BlueNote	Z1-19-9554	2019	GBS-LHF21-BN-4	2021
Bolt	U21-17-02014-1	2017	GBS-WHB21-BOLT-4	2021
Harvest Moon	C8-20-K17-190AD	2020	C8-19BR-K17-190AD	2019
Jackpot	83.124.17-J	2018	85-135-19D	2020
Jackrabbit	H4-20-94712	2019	FS4-13KBT-2	2020
Jumpstart	Z1-19-5183	2019	C8-19-Jumpstart	2019
Kelly	J6-20-K203	2020	J6-21-K454	2021
Legend	GBS-LH8-LEG-2	2018	GBS-LHF21-LEG-3	2021
Midnight	79.103.13R	2014	85-89-18-J	2020
Moonlight SLT	U21-19-85025-1	2019	Z1-20-1352	2020
PST-T15-44	C8-T15-44	2015	C8-21-T15-44	2021
Sombrero	U21-19-60012-2	2019	U21-21-600K3-3	2021
SR 2100	Z1-18-8232	2018	Z1-20-1243	2020
SR 2284	Z1-19-9772	2019	Z1-20-1250	2020
Tirem	Z1-20-1358	2020	Z1-21-3396-B	2021
Volt	J6-19-VO728	2019	J6-21-VO901	2021

Note: Harvest typically occurs around mid-July in each year for Kentucky bluegrass and varies by location.

2.2 | Seedling vigor

An additional experiment was used to assess seedling vigor. In the AOSA handbook, there is no duration recommendation for germinating Kentucky bluegrass in a paper towel test. The protocol for testing was modified from procedures and guidelines from AOSA (2016) and Elias et al. (2012) based on a preliminary test. The vigor test was conducted four times starting on November 29, 2021, May 4, 2022, May 11, 2022, and May 18, 2022, with one replication on each date. Seed lot 1 was used for the first two replications and seed lot 2 for the last two replications.

Paper towels measuring 25.4 cm × 38.1 cm (PG1015, regular weight germination paper, Hoffman Manufacturing) were moistened with tap water and 25 seeds of each cultivar were tested on a paper towel per replication. Seeds were placed 5 cm from the top of the paper towel and positioned so the radical would grow toward the base of the towel. The towels were rolled, labeled, and placed in plastic bags oriented with towels upright and the seeds positioned at the top. The same germination chamber and settings as the previous experiment were used. The paper towels were placed in the germination chamber for 21 days after which the root length and shoot length of germinated seeds were measured by a digital caliper. Seeds that did not germinate or abnormal seedings were not measured or included in the analysis.

2.3 | Seed phenotyping

Seed morphology was assessed through high-resolution scans of each cultivar similar to Qiu et al. (2020). Fifty seeds per seed lot were measured separately by arranging the seeds on a glass Petri dish. The Petri dish was placed on a scanner (Epson Electronics Co.; Perfection V850 Pro Photo Scanner) and covered with a red background prior to scanning at a resolution of 2400 dpi. After scanning, images were reviewed prior to analysis. After confirmation of a good scan, the 50 seeds were weighed and a TSW was calculated. The images were analyzed using code (Jupyter Lab, Python Software Foundation) that measured length, width, area, and circularity of each seed (Ortiz, 2020). Circularity was calculated using Equation (2):

$$\text{Circularity} = \frac{4\pi \times \text{area}}{\text{Perimeter}^2}. \tag{2}$$

The measurements were then subject to analysis of variance (ANOVA) using SAS (Version 9.4, SAS Institute Inc.).

2.4 | Data analysis

The percent germination data were regressed overtime using the four-parameter, sigmoidal regression model of Equation (3):

$$y = a + \frac{(b-a)}{\left(1+\left(\frac{D_{50}}{x}\right)^{\text{Hillslope}}\right)}, \tag{3}$$

where y is percent germination, a is the y value at the estimated bottom level, b is the y value at the estimated top level, D_{50} is the x value when the response of D_{50}, which is halfway between bottom and top (days until 50% germination), and Hillslope (no units) describes the slope of the curve at D_{50}. Days until 50% germination (D_{50}) were calculated from the regression curves. Data were fit for each Petri dish to the sigmoidal regression model using PROC NLIN (Gauss–Newton method). From these data, days until 90% germination were also calculated using Equation (4):

$$D_f = \left(\frac{f}{100-f}\right)^{\frac{1}{\text{Hillslope}}} \times D_{50}, \tag{4}$$

where D_f is days to a fractional (f) response in germination (fraction of maximum, b in Equation (3) and D_{50} and Hillslope are input from Equation (3). The variable f was set to 90 to calculate days until 90% germination (D_{90}).

For the seed germination data, there was a cultivar × experiment run interaction for the variables, so data were not combined. An ANOVA was conducted by experimental run on D_{50}, D_{90}, the hillslope of the regression curve, the germination rate, and the total germination percent using PROC GLM with means separated using Tukey's HSD test ($\alpha = 0.05$) when F-tests were significant ($p \leq 0.05$). For presentation purposes, GraphPad Prism (v.9.2, GraphPad Software Inc.) was used to generate regression figures in this manuscript using the same equation (Equation 3) and data.

Each of the four vigor tests was considered a replication with two replications for each experiment run within a seed lot. Data analysis of seedling vigor could not be combined because of a cultivar × seed lot interaction for root length. ANOVA was conducted by seed lot using PROC GLM with means separated using Tukey's HSD test ($\alpha = 0.05$) when F-tests were significant ($p \leq 0.05$).

Seed morphological data were analyzed with each cultivar within a seed lot considered an experimental unit, seed lot was used as replication, and the observations about each individual seed were considered a subsample (Bowley, 2015). ANOVA was conducted using PROC MIXED with means separated using Tukey's HSD test ($\alpha = 0.05$) when F-tests were significant ($p \leq 0.05$). Correlation analyses between seed morphological characteristics and seed germination and seedling vigor data were conducted using a correlation matrix in GraphPad Prism.

3 | RESULTS

3.1 | Germination rate and percent

Days to 50% germination (D_{50}) or 90% germination (D_{90}), germination rate, and total germination varied by cultivar in each seed lot (Table 3). The mean D_{50} was 10.1 and 10.7 for seed lots 1 and 2, respectively. Genotype PST-T15-44 had the shortest D_{50} across both seed lots (7.9 days, seed lot 1; 8.0 days, seed lot 2) (Table 3; Figure 1). The mean D_{90} was 12.4 and 13.1 for seed lots 1 and 2, respectively. Similar to D_{50}, genotype PST-T15-44 had the shortest D_{90} across both seed lots (9.9 days, seed lot 1; 9.3 days, seed lot 2) (Table 3). In addition to D_{50} and D_{90}, the germination rate of PST-T15-44 was the highest in seed lot 1 at 5.1 and also in seed lot 2 scoring 5.4 with both lots having a total germination >90% (Table 3). The higher germination rate, as calculated by Maguire (1962), indicates faster seedling emergence. Following the experimental genotype of PST-T15-44, the commercial cultivars with the lowest days to germination were After Midnight, classified as a "Midnight" type, and ''Jackrabbit,'' classified as a "Julia" type (Tables 1 and 3). The D_{50} for After Midnight was 8.8 days for both seed lots (Table 3). Jackrabbit had a D_{50} of 8.9 days in seed lot 1 and 9.8 days in seed lot 2. These two cultivars were also among the commercially available cultivars with the highest germination rate, ranging from 4.1 to 4.8 (Table 3). Overall, several cultivars ranked in the top group with a D_{90} range from 9.9 to 11.9 days in seed lot 1. In seed lot 2, three cultivars were in the top group with a D_{90} from 9.3 to 11.0 days. PST-T15-44, After Midnight, and ''Arcadia'' were the three cultivars that had the quickest D_{90} in both seed lots (Table 3).

''Blue Chip,'' ''Jackpot,'' ''Jumpstart,'' ''Kelly,'' and ''SR2284'' were among a group of cultivars that had intermediate germination rate and D_{50} in both seed lots (Table 3). The D_{50} for these cultivars ranged from 9.4 to 11.2 days with a total percentage of germination ranging from 77.0% to 93.4%. ''Volt'' and ''Tirem'' were cultivars with the slowest D_{50}, D_{90}, and germination rate in both seed lots. There were too few cultivars within each of the classification types to make any firm conclusions, but there was no apparent relationship between classification type and germination rate, D_{50}, or D_{90}.

The hillslope, which is the rate of germination at D_{50}, was variable by cultivar with both slow and fast germinating cultivars having either high or low hillslope values (data

TABLE 3 Total germination percent, days to 50% germination (D_{50}), days to 90% germination (D_{90}), germination rate (speed of germination), and total percent germination among 21 Kentucky bluegrass cultivars for two different seed lots. Sorted descending by days to 50% germination in seed lot 1.

	Experimental run (seed lot 1)				Experimental run (seed lot 2)			
Cultivar	Days until 50% germin-ation[a] (d)	Days until 90% germin-ation[a] (d)	Germin-ation rate[b]	Total germin-ation (%)	Days until 50% germin-ation (d)	Days until 90% germin-ation (d)	Germin-ation rate	Total germin-ation (%)
PST-T15-44	7.9 i	9.9 f	5.1 a	90.1 ab	8.0 L	9.3 h	5.4 a	92.4 a
Harvest Moon	8.5 hi	10.6 def	4.4 abc	85.0 ab	10.2 f–j	13.4 de	4.0 b–e	84.5 a–e
After Midnight	8.8 gh	11.4 b–f	4.6 ab	87.6 ab	8.8 kl	10.8 gh	4.8 ab	89.2 a–d
Jackrabbit	8.9 gh	11.1 c–f	4.7 ab	90.8 ab	9.8 h–k	12.3 d–g	4.1 bc	89.1 a–d
Moonlight SLT	9.0 fgh	10.3 ef	4.3 abc	84.2 ab	10.5 e–i	12.5 d–g	3.3 d–g	72.9 e
Sombrero	9.1 fgh	11.6 b–f	4.7 ab	94.2 a	13.6 a	17.1 a	3.2 efg	91.8 ab
Jackpot	9.4 efg	11.7 b–f	4.1 b–e	82.6 ab	9.6 ijk	12.1 efg	4.0 c–f	79.4 b–e
Midnight	9.6 d-g	12.5 bcd	4.5 ab	92.0 ab	10.1 g–j	12.3 d–g	4.0 b–e	87.7 a–d
Acoustic	9.6 d–g	11.2 c–f	4.2 bcd	95.2 a	11.9 bc	14.1 cde	3.7 c–f	92.2 ab
Blue Chip	9.6 d–g	11.9 b–f	4.0 b–f	82.9 ab	10.0 g–j	12.8 d–g	3.7 c–f	77.0 de
Arcadia	9.8 def	11.6 b–f	4.3 abc	92.8 a	9.3 jk	11.0 fgh	4.1 bcd	83.1 a–e
Legend	9.8 def	11.6 b–f	3.2 fg	78.5 ab	10.8 d–h	14.4 bcd	3.4 c–g	76.8 de
SR 2284	10.1 de	12.5 bcd	3.8 b–f	89.8 ab	10.5 e–i	13.0 def	3.8 c–f	88.1 a–d
Jumpstart	10.2 de	12.7 bc	4.1 b–f	88.7 ab	9.8 h–k	12.8 d–g	3.5 c–f	79.6 b–e
Kelly	10.4 cd	12.2 b–e	4.2 bcd	93.4 a	11.2 c–f	13.3 de	4.0 bcd	93.2 a
Arrowhead	10.4 cd	13.1 bc	3.4 d–g	82.8 ab	10.2 f–j	12.8 d–g	3.7 c–f	78.9 cde
Bolt	11.0 c	13.3 b	3.6 c–g	89.2 ab	11.6 cd	14.3 bcd	3.4 c–g	85.3 a–e
Volt	12.0 b	15.3 a	2.9 g	83.8 ab	13.7 a	16.3 ab	2.6 g	79.4 b–e
SR 2100	12.1 b	15.5 a	3.3 efg	87.8 ab	11.4 cde	13.6 de	3.7 c–f	86.5 a–d
Tirem	12.5 b	16.3 a	3.0 g	90.3 ab	12.9 ab	16.0 abc	3.1 fg	84.3 a–e
BlueNote	13.7 a	17.4 a	2.9 g	92.0 ab	11.0 c–g	13.4 de	3.9 c–f	90.0 abc
Mean	10.1	12.4	3.8	84.3	10.7	13.1	3.8	84.8
p Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: Means within a column with a common letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

[a]Sigmoidal regression model (four-parameter equation) defined by Equation (2). Top (%) is the mean total germination percentage. For both Experiment Runs 1 and 2, $R^2 = 0.99$ for all treatments. Days until 50% germination (D_{50}) was calculated from Equation (2) and days until 90% germination (D_{90}) from Equation (3).

[b]Germination rate, also known as speed of germination, was calculated per Maguire (1962) to distinguish seed lots with a similar total germination but a varying rate of emergence. The calculation is unitless. A higher value indicates a higher germination rate (faster emergence).

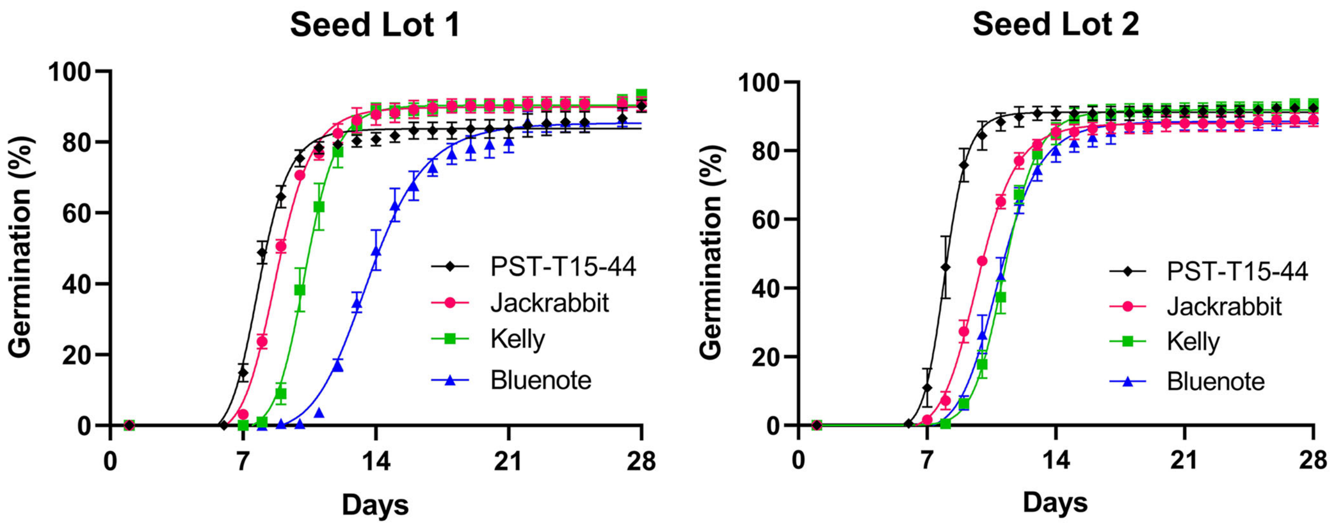

FIGURE 1 Germination of selected Kentucky bluegrass cultivars (PST-T15-44, ''Jackrabbit,'' ''Kelly,'' ''BlueNote'') with >89% total germination and with contrasting germination rates at 25/15˚C, 8/16 h day/night in both years (seed lots). See Table 3 for cultivar specific information. Error bars represent the standard error of the mean.

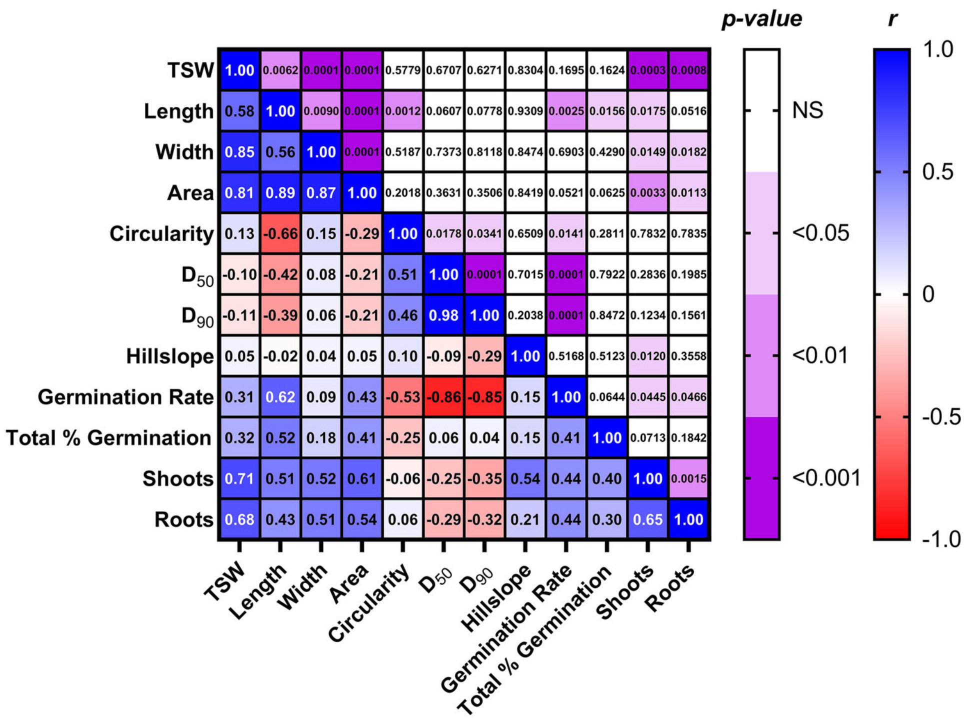

FIGURE 2 Correlation matrix between seed germination calculations and measurements, seedling vigor measures, and seed phenotype data. Correlation (r) (bottom left) and p-values (top right) are shown using a color-coded scales. Means for thousand seed weight (TSW), length, width, and area (Table 5), germination vigor (Table 3), and root and shoot length in the paper towel test (Table 4) were used to determine the relationship between variables.

not shown). Hillslope was not related to D_{50} ($r = -0.09$, $p = 0.7015$), D_{90} ($r = -0.29$, $p = 0.2038$), or germination rate ($r = 0.15$, $p = 0.5168$) (Figure 2) and thus not a good predictor of germination vigor or meaningful cultivar differences.

The total germination percent varied among cultivar ($p < 0.0001$) in both runs and ranged from 78.5% to 95.2% in the first seed lot tested and from 72.9% to 93.2% in the second seed lot (Table 3). The mean total germination

TABLE 4 Shoot and root growth among 21 Kentucky bluegrass cultivars measured 21 days after the start of a paper towel seedling vigor test. Values for two different seed lots are given. Sorted by longest shoot in Experiment 1.

	Experiment run 1 (seed lot 1)		Experiment run 2 (seed lot 2)	
Cultivar	Shoots (mm)	Roots (mm)	Shoots (mm)	Roots (mm)
Kelly	35.49 a	24.43 ab	36.82 a	24.75
Midnight	32.12 ab	21.12 abc	32.49 ab	20.01
Arcadia	31.15 ab	21.14 abc	31.84 ab	20.43
Jackrabbit	30.32 abc	26.46 a	29.19 ab	21.50
After Midnight	29.65 abc	18.62 abc	25.13 ab	16.84
PST-T15-44	28.85 abc	24.89 ab	35.41 ab	22.40
Jackpot	28.52 abc	20.82 abc	28.36 ab	19.35
Jumpstart	28.52 abc	21.34 abc	26.58 ab	16.80
Acoustic	27.51 abc	19.49 abc	31.71 ab	20.28
Sombrero	27.39 abc	26.56 a	29.99 ab	20.33
Legend	26.71 abc	21.41 abc	28.94 ab	21.20
Volt	26.68 abc	20.23 abc	33.30 ab	14.84
Bolt	26.42 abc	19.90 abc	29.85 ab	17.76
Moonlight SLT	26.24 abc	19.45 abc	29.35 ab	17.70
Blue Chip	26.09 abc	21.14 abc	26.57 ab	22.05
SR 2284	26.06 abc	23.18 abc	29.53 ab	19.02
SR 2100	25.10 abc	22.75 abc	24.48 b	15.08
Harvest Moon	24.18 abc	17.93 abc	33.10 ab	23.45
Tirem	23.16 abc	20.33 abc	25.59 ab	18.45
BlueNote	21.90 bc	15.99 bc	30.33 ab	14.69
Arrowhead	18.23 c	14.07 c	29.54 ab	13.72
Mean	27.15	21.01	29.91	19.07
p Value	0.0109	0.0094	0.0247	0.2860

Note: Means within a column with a common letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

was similar between seed lots 1 and 2 at 84.3% and 84.8%, respectively.

3.2 | Seedling vigor

Analysis of variance indicated differences in shoot and root length among 21 cultivars and genotypes tested for seedling vigor (Table 4). All the cultivars and genotypes had longer shoot growth than the root growth (Table 4). An interaction between seed lots and root length prevented a combined analysis across lots. While some cultivars/genotypes such as ''Arcadia,'' Jackrabbit, Kelly, ''Legend,'' ''Midnight,'' ''Acoustic,'' PST-T15-44, and SR 2284 were among the highest statistical grouping for root and shoot length in both seed lots indicating good seedling vigor, the growth of other cultivars was variable (Table 4). For example, "Arrowhead" had 18.23 mm of shoot growth and 14.07 mm root growth in seed lot 1, but more shoot growth (29.54 mm) and less root growth (13.72 mm) in the second seed lot tested (Table 4).

3.3 | Seed phenotyping

The seed phenotype results are presented across seed lots. Seed length, width, area, circularity, and TSW all varied by cultivar (Table 5). Mean length, width, and area were 2.773 mm, 0.792 mm, and 1.713 mm^2, respectively. The average circularity was 0.465, with a value of 1 for a circle and a value of 0 for a line. The average TSW was 0.371 indicating 2,695,418 seeds kg^{-1} (1,222,620 seeds lb^{-1}). ''Moonlight SLT'' had numerically the lowest seed area (1.328 mm^2), in addition to having the shortest width (0.717 mm), length (2.366 mm), and one of the lowest TSW (Table 5; Figure 3). Kelly Kentucky bluegrass had the highest seed area of 2.046 mm^2 and was the highest in TSW at 0.487 g (Table 5; Figure 3). Based on the range in TSW, Kentucky bluegrass cultivars might commonly range from 2,053,388 to 3,367,003 seeds kg^{-1} (931,400–1,527,246 seeds lb^{-1}), a difference of 64% more seed per unit of mass for smaller compared to larger seeded cultivars (Table 5; Figure 3).

TABLE 5 Length, width, area, circularity, and thousand seed weight (TSW) among 21 Kentucky bluegrass cultivars with means presented across two seed lots. Sorted descending by length.

	Year 1 and 2 mean				
Cultivar	**Length (mm)**	**Width (mm)**	**Area (mm^2)**	**Circularity**	**TSW[a] (g)**
After Midnight	3.065a	0.830 abc	1.980 ab	0.425 ab	0.395 abc
PST-T15-44	3.024 ab	0.800 a–d	1.877 abc	0.417 b	0.376 abc
Kelly	2.945 abc	0.892 a	2.046 a	0.478 ab	0.487 a
Jumpstart	2.910 abc	0.770 cd	1.742 a–d	0.421 ab	0.338 ac
Harvest Moon	2.905 abc	0.777 cd	1.753 a–d	0.425 ab	0.394 abc
SR 2100	2.903 abc	0.882 ab	1.997 ab	0.496 ab	0.452 ab
Acoustic	2.902 abc	0.781 bcd	1.769 a–d	0.441 ab	0.335 bc
Midnight	2.847 abc	0.784 bcd	1.745 a–d	0.461 ab	0.405 abc
Jackrabbit	2.843 abc	0.779 cd	1.727 a–d	0.463 ab	0.404 abc
Arcadia	2.828 abc	0.787 bcd	1.741 a–d	0.466 ab	0.376 abc
Jackpot	2.715 a–d	0.843 abc	1.779 abc	0.503 ab	0.409 abc
SR 2284	2.714 a–d	0.754 cd	1.591 bcd	0.474 ab	0.341 bc
Blue Chip	2.707 a–d	0.807 a–d	1.690 a–d	0.471 ab	0.380 abc
Volt	2.704 a–d	0.824 abc	1.739 a–d	0.495 ab	0.395 abc
Bolt	2.674 a–d	0.757 cd	1.574 bcd	0.453 ab	0.356 abc
BlueNote	2.665 a–d	0.776 cd	1.599 a–d	0.465 ab	0.326 bc
Sombrero	2.649 bcd	0.774 cd	1.602 a–d	0.496 ab	0.374 abc
Legend	2.646 bcd	0.793 a–d	1.630 a–d	0.470 ab	0.329 bc
Arrowhead	2.626 bcd	0.743 cd	1.511 cd	0.448 ab	0.297 c
Tirem	2.605 cd	0.774 cd	1.560 bcd	0.488 ab	0.320 bc
Moonlight SLT	2.366 d	0.717 d	1.328 d	0.511 a	0.312 c
Mean	2.773	0.792	1.713	0.465	0.371
p Value	0.0002	0.0001	0.0004	0.0072	0.0013

Note: Means within a column with a common letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).
[a]Thousand seed weight (TSW) was calculated from the mass of 10 total seeds across two seed lots.

A correlation matrix of all the germination rate, seedling vigor, and seed phenotyping data demonstrated several significant correlations (Figure 2). The analysis showed D_{50} and germination rate to be similar to each other ($r = -0.86$, $p = 0.0001$; Figure 4). The seedling vigor paper towel test was correlated with germination rate with shoots ($r = 0.44$, $p = 0.0445$) and roots ($r = 0.44$, $p = 0.0466$). Seed phenotype varied by cultivar (Table 5) with length related to germination rate ($r = 0.62$, $p = 0.0025$) as well as circularity ($r = -0.53$, $p = 0.0141$) (Figures 2 and 4). Circularity was also related to D_{50} ($r = 0.51$, $p = 0.0178$). Seed weight, width, and area were correlated with seedling vigor measurements of shoot growth and root growth. Total percent germination was also correlated with seed length ($r = 0.52$, $p = 0.0516$) (Figures 2, 4). In general, correlation coefficients between D_{50} and D_{90} and other variables were similar with a 0.98 ($p < 0.0001$) correlation coefficient between D_{50} and D_{90}.

4 | DISCUSSION

The results of the germination rate experiment revealed that PST-T15-44 was the fastest to germinate for both seed lots (Table 3). PST-T15-44 and the commercially available cultivar After Midnight, needed only 7.9–8.8 days to reach 50% germination (Table 3; Figure 1). In seed lot 1, there were nine out of 21 cultivars and genotypes that took 10 or more days for germination to reach 50%, but 16 out of 21 cultivars and genotypes took 10 or more days for germination to reach 50% in seed lot 2 (Table 3). A separation of 5.7–5.8 days to reach the 50% germination was documented between the slowest and fastest germinating Kentucky bluegrass cultivars in seed lots 2 and 1, respectively (Table 3). The fewer number of days to 50% germination is both statistically significant and agronomically important as the magnitude of this difference could be critical in achieving faster sward establishment and seedling

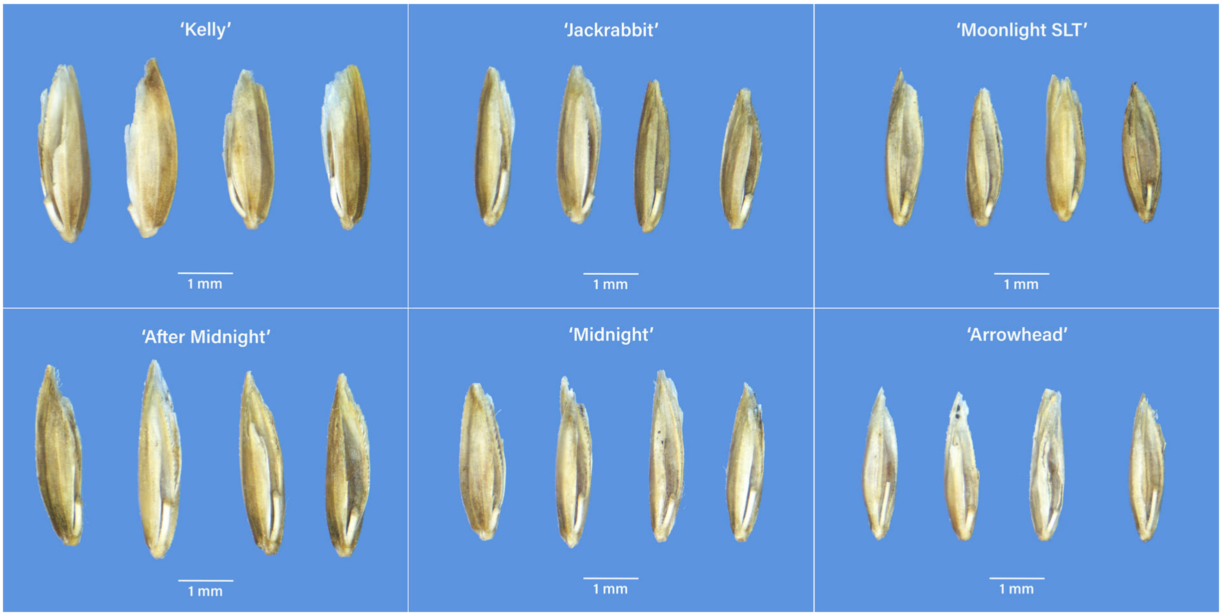

FIGURE 3 Close-up seed images photographed using a digital camera and a stereo microscope of six Kentucky bluegrass cultivars with contrasting phenotypes. All images have the same scale. ''Kelly'' and ''After Midnight'' (left) represent cultivars with a high thousand seed weights (TSW) or length, ''Jackrabbit'' and ''Midnight'' (middle) represent cultivars with a seed phenotype near the mean, and ''Arrowhead'' and ''Moonlight SLT'' (right) are cultivars with the smallest length, width, area, and TSW or largest circularity (Table 5).

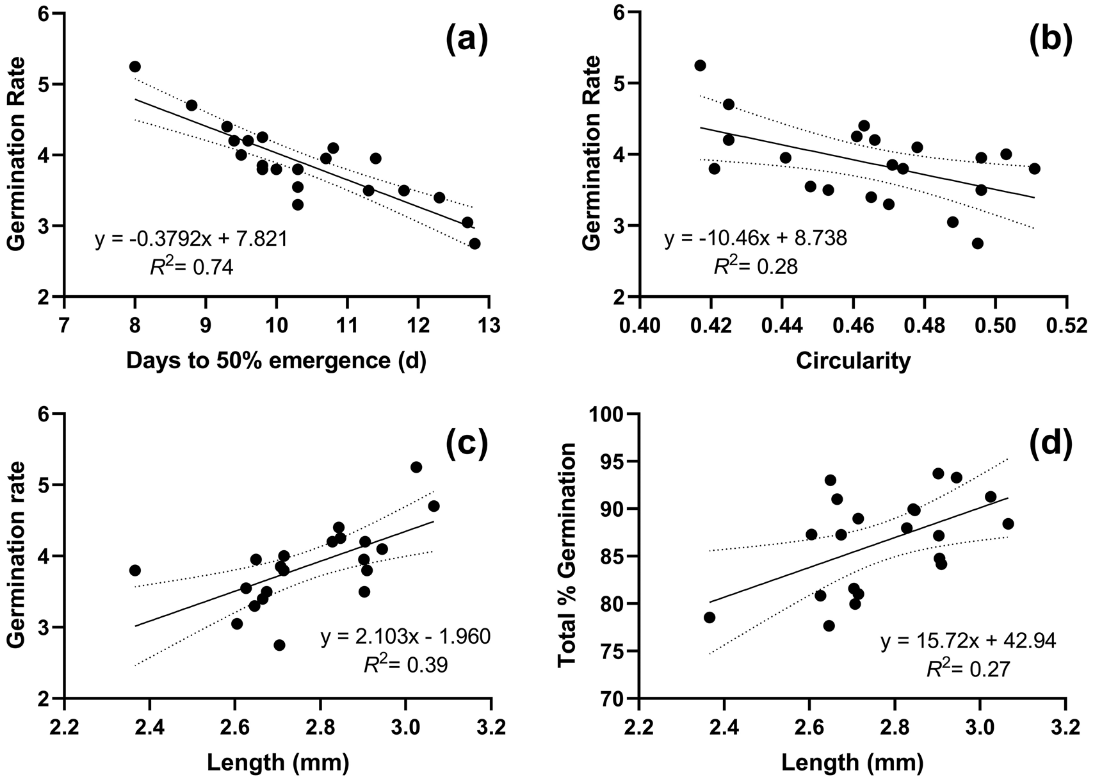

FIGURE 4 Linear regression analysis of significant germination rate, germination vigor, and seed phenotype relationships across 21 Kentucky bluegrass cultivars. A 95% confidence band is shown.

competition with annual weeds like annual bluegrass (*Poa annua* L.).

When examining the days to 90% germination, we come to a similar conclusion. A difference of 7.5–7.8 days, respectively, in seed lots 1 and 2, occurred for D_{90} between the slowest and fastest germinating Kentucky bluegrass cultivars. This weeklong variation between cultivars in 90% germination should result in practical differences in field establishment as Reicher et al. (2006) note that a 1-week delay in autumn seeding will result in 2–4 extra weeks additional time to reach establishment. McCarty (2018) states that Kentucky bluegrass may take 6–30 days to germinate. Under near ideal germination conditions in this study, we concur that germination may occur in as little as 6 days (Figure 1). Although 90% of total percent germination was achieved in 12.4–13.1 days on average in our study using 25/15˚C (day/night) with an 8 h photoperiod, germination may take >28 days under non-ideal conditions such as continuous light, or sub- or supra-optimal temperatures and limited moisture supplies (Aamlid & Arnsten, 1998).

Based on these results and the calculated germination rate from Maguire (1962), there is high possibility for these data to help in selecting cultivars to improve establishment. Also, in the seedling vigor test (Table 4), seven out of the 21 cultivars demonstrated a shoot growth higher than 30 mm in either seed lot 1 or seed lot 2 with two cultivars having >30 mm shoot growth in both seed lots. These data indicate that it is possible to select cultivars with faster root and shoot establishment. To confirm the findings from these germination chamber experiments, field research will be needed to further characterize seeding growth. Reicher et al. (2000) state the importance of seeding in the autumn when the soils are warm due to the slow germination of Kentucky bluegrass and to allow more time for maturity prior to winter. With faster germinating cultivars of Kentucky bluegrass such as PST-T15-44 and After Midnight, a wider seeding window may be possible. However, field testing should be conducted.

Seed lots varied in age between 15 months and 7 years old when tested in the first experimental run with the majority 15–27 months old (Table 2). In the second experimental run, seed lots varied in age between 8 and 32 months old with the majority 8–20 months old. Most of the seed was stored in a warehouse under ambient conditions before being shipped to Purdue University in February 2021 (seed lot 1) or January 2022 (seed lot 2) where it was refrigerated until tested. Kentucky bluegrass seed maintains good viability for 30 months after harvest, regardless of the moisture content of the seed (Bass, 1959). Further, it can be stored in ambient warehouse conditions for 7 years without a loss of viability (Rincker, 1986). Both reports are consistent with our findings on total percent germination.

As grass seed ages, its germination rate (speed of germination) decreases but this is usually coupled with a dramatic decrease in total germination as reported in perennial ryegrass (Naylor, 1989). A more careful examination of age effects on Kentucky bluegrass germination rate may be warranted, but this was not assessed in this experiment, and we lacked sufficient samples of seed >36 months old to make any conclusions. Genetics exerted a large influence on Kentucky bluegrass germination rate in our experiment considering that only seed lots with >80% total germination were included in this study, seeds were stored under similar conditions, and cultivars often performed similarly across lots.

Intraspecific seed number per unit weight often varies among cultivars and this number can have an effect on time to establishment, especially where a planting rate is selected on the low end of recommended seeding rates. Christians et al. (1979) reported how many seeds were counted and weighted in 1g of several Kentucky bluegrass cultivars. The conversion of their results to the units of this paper found an average TSW of 0.364 g (2744 seeds g^{-1}), which was similar to the TSW mean of 0.371 g reported here. More recently, Larsen et al. (2004) reported a TSW range for five Kentucky bluegrass cultivars from 0.217 g to 0.366 g with a mean of 0.298 g and Braun et al. (2023) reported a TSW mean of 0.40 g for two cultivars tested. Comparing our results with other citations on seed weight (Braun et al., 2023; Christians et al., 1979; Larsen et al., 2004), the weights of Kentucky bluegrass are similar to most reports except Larsen et al. (2004). While there was overlap in the TSW to Larsen et al. (2004) and our research, Larsen et al. (2004) found a lower mean TSW. This difference could be due to the cultivars selected for the experiments or the climate and management where the seeds were produced. Larsen et al. (2004) tested Kentucky bluegrass harvested in Denmark, whereas the current study and the research of Christians et al. (1979) and Braun et al. (2023) tested seed harvested in the United States. Also, in our research there was no sorting of “lighter seeds” that could have affected the results of the TSW, as it was done in Larsen and Andreasen (2004). For our study, the variability of seed weight within a cultivar and within a seed lot was not determined. Larsen and Andreasen (2004) found that within a single Kentucky bluegrass cultivar, heavier individual seeds had a shorter mean germination time than lighter seeds, but our research reports no relationship between TSW and germination rate ($r = 0.31$, $p = 0.1695$) or D_{50} ($r = -0.10$, $p = 0.6707$).

Based on the TSW results, the seeding rates chosen by turf managers or recommended by seed companies should vary by cultivar, depending on the size of the Kentucky bluegrass seed. Christians et al. (2017) recommend planting Kentucky bluegrass at 48.8–73.2 kg ha^{-1} (1.0–1.5 lbs per 1000 ft^2). Braun et al. (2021) recommended seeding as little as 1 pure live seed (PLS) cm^{-2}, which is equivalent to 43.6 kg ha^{-1}, assuming 2,695,418 seeds kg^{-1} and an 85% germination rate. Comparing the smallest seeded Kentucky bluegrass (Arrowhead) to the largest (Kelly), these two cultivars would result in a range of seeding rates from 34.9 (Arrowhead) to 57.3 (Kelly) kg ha^{-1} using a 1 PLS cm^{-2} seeding rate and assuming an 85% total germination.

With the variability in seed weight and the amount of seeds needed, the costs associated with planting could vary significantly by cultivar. Ebdon and DeCosta (2021) showed that larger seed (weight) of colonial bentgrass (*Agrostis capillaris* L.), creeping bentgrass (*A. stolonifera* L.), and velvet bentgrass (*A. canina* L.) had a positive correlation with increased establishment. Ebdon and DeCosta (2021) noted that their experiment utilized the same number of viable seeds per area (3.1 seeds cm^{-2}) using PLS information (purity at total percent germination) and seed counts for each cultivar compared to previous work by Christians et al. (1979) whose establishment experiment used a weight per area seeding rate common among practitioners. Further investigation on how seed weight may practically impact the field establishment of Kentucky bluegrass cultivars from seed is warranted.

Regression analysis indicated that seed length and circularity influenced germination rate (Figure 4b,c). Longer and less circular-shaped (more linear) seeds had increased germination rates. Further, total percent germination was greater among cultivars with a longer seed length (Figure 4d). Possibly because seed phenotyping via digital image analysis is a relatively new tool (Williams et al., 2013), few studies have reported any relationship between seed shape and germination. Past work in *Soliva* spp. found that cypsela (achene-like fruit) morphology was linked with germination rate (Lovell et al., 1986). Braun et al. (2023) reported a relationship between seed phenotype and germination within fine fescue taxa, but the relationships were not as strong as those reported here. Seed size is influenced by environmental effects, but maternal genetic inheritance has a strong influence on germination rate (Galloway et al., 2009), seedling vigor (Singh et al., 2017), and seed shape (Cober et al., 1997). While the physiological cause for enhanced germination rate amongst Kentucky bluegrass cultivars with longer, less circular seeds is unclear, this experiment illuminates the potential for seed phenotyping as a useful tool in breeding programs to improve germination characteristics.

5 | CONCLUSIONS

The results of the seed phenotypic analysis found potentially important correlations between length and circularity in the germination of Kentucky bluegrass. The cultivars with longer length and a less circular shape had a lower D_{50}, D_{90}, or a higher germination rate or both. Further, longer seeds were associated with a higher germination percentage. Additionally, seeds with a larger weight, width, and area had improved shoot and root seedling vigor. In combination, these results indicate that turfgrass breeders should use phenotyping as a tool to select for genotypes with a high viability and increased germination rate. Further, research examining the physiological reasons for increased germination rate should also be explored as well as the germination of Kentucky bluegrass under cooler conditions to mimic a later seeding date. It is unclear whether the relative germination performance of these cultivars would remain consistent, though possibly changing in magnitude, under non-optimum conditions (i.e., cooler temperatures). Overall, the data from these experiments demonstrate that germination rate differs among Kentucky bluegrass cultivars and should be an important selection criteria where rapid establishment of Kentucky bluegrass from seed is desired.

AUTHOR CONTRIBUTIONS

Amanda J. Folck: Conceptualization, data curation, formal analysis, investigation, validation, writing—original draft, writing—review and editing. **Cale A. Bigelow** and **Yiwei Jiang**: Supervision, writing—review and editing. **Aaron J. Patton**: Conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—review and editing.

ACKNOWLEDGMENTS

The authors wish to thank Barenbrug USA (Tangent, OR), DLF Pickseed (Halsey, OR), Mountain View Seeds (Salem, OR), and Pure-Seed Testing (Hubbard, OR) for supplying seed for this research. The authors also thank Lauren Courtney, Vera Vukovic, and Brandon McNally for their assistance with the research and the Office of Indiana State Chemist—Seed Section for their support and information sharing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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How to cite this article: Folck, A. J., Bigelow, C. A., Jiang, Y., & Patton, A. J. (2023). Genotypic variation in germination rate, seedling vigor, and seed phenotype of Kentucky bluegrass cultivars. *Crop Science*, *63,* 3065–3078. https://doi.org/10.1002/csc2.21045