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Seed Dormancy & Germination in Three Annual Canarygrass (Phalaris canariensis L.) Cultivars Relative to Spring Wheat (Triticum aestivum L.)

M. Matus-Cádiz, P. Hucl, and G. Munasinghe

Plant Sciences Dept./Crop Development Centre, Univ. of Saskatchewan, 51 Campus Dr., Saskatoon, SK, Canada S7N 5A8.

Key words: *Phalaris canariensis,* Annual canarygrass, CDC Maria, dormancy, potassium nitrate pre-treatment.

Abstract

Seed dormancy in annual canarygrass may lead to unsatisfactory germination in seed tests. The objectives of this study were (i) to quantify the levels of seed dormancy in three morphologically diverse annual canarygrass cultivars ('Keet', 'CDC Maria', & 'CY 184') relative to spring wheat & (ii) to determine the effectiveness of three treatments (GA_3, KNO_3, & chilling) & two temperature regimes (15/25°C & 15°C) in promoting germination of dormant annual canarygrass seeds. The hard red spring wheat cultivar 'Katepwa' control was included as a representative of a cereal crop that has been extensively characterized with regards to seed dormancy. In 1998 & 1999, the four cultivars were grown at Saskatoon, Canada. At maturity, panicles & spikes were hand harvested & stored at –20°C. Four replications of 50 seeds per cultivar were used in each experiment. Three experiments were conducted: (i) seeds were germinated at 10, 15, 20, & 25°C for one week, (ii) seeds were stored at 24°C for zero to eight weeks prior to germination at 22°C for one week, & (iii) seeds were treated with GA_3, KNO_3, & chilling prior to germination at 15/25°C (16/8h) or 15°C for two weeks. For experiment one & three, a split-plot analysis was used to analyze arc sin transformed percentage germination data. Average percentage germination data in experiment two were tested to be significantly different from 98% germination (P=0.05) based on one-tailed t-tests. Annual canarygrass developed deeper dormancy than the wheat cultivar in both years, particularly when germinated at 20 & 25°C. The highest percentage germination was observed at 15°C. Two (1998) & four weeks (1999) of storage at 24°C were required to overcome dormancy in annual canarygrass. Pre-chilling or KNO_3 treatment prior to germination at 15/25°C (16/8h in darkness) resulted in average germination levels of 94% (1998) & 66% (1999). Potassium nitrate treatment prior to incubation at 15°C in darkness was the most effective method of promoting germination in dormant seeds, resulting in 99% (1998) & 97% (1999) germination. Thus, we recommend the use of the latter method, instead of the former or currently recommended method (pre-chilling or KNO_3 treatment prior to germination at 15/25°C [16/8h] in darkness), for testing germination levels of dormant seed of annual canarygrass.

Introduction

Pedigreed seed of *Phalaris* L. crops must be guaranteed for a high level of germination. Dormant seeds can erroneously be confused with non-viable seeds in seed tests leading to unsatisfactory germination levels. The genus *Phalaris* is comprised of 10 annual & five perennial species (Anderson, 1961). Annual canarygrass, commonly known as canaryseed, is grown for feeding caged birds & is the only annual species within *Phalaris* that has gained commercial importance (Putman et al., 1996). Seed dormancy refers to the temporary failure of a viable seed to germinate, after a specified length of time, in a particular set of environmental conditions that later evoke germination when the restrictive state has been terminated by either natural or artificial means (Simpson, 1990). For example, dry, viable, & non-dormant seeds germinate readily when hydrated, but dormant seeds will not germinate. Jimenez-Hidalgo et al. (1995) reported that *P. brachystachys* Link., a direct ancestor of annual canarygrass (Carlson et al., 1996), has strong dormancy levels in the mature seed. Germination in *P. brachystachys* was faster at 10°C than at 20°C during a three-week incubation period & germination was 13% lower using dark versus light incubation conditions. Light has been reported to both inhibit & promote germination in *Phalaris* (Nakamura, 1962; Landgraff & Juntilla, 1979). Difficulty has been experienced by our annual canarygrass breeding program in obtaining satisfactory germination levels from freshly harvested samples of annual canarygrass, particularly in years when grain filling occurs under cool growing conditions. These observations have led us to suspect that seed dormancy in annual canarygrass, instead of non-viable seeds, may be leading to these unsatisfactory germination levels. The Canadian methods & procedures manual outlines the currently recommended seed test for testing fresh or dormant samples of annual canarygrass (Canadian Food Inspection Agency, 1997); however, its use in our laboratory has yielded unsatisfactory germination levels. The recommended seed test involves the use of pre-chilling or potassium nitrate (0.1% w/v solution) treatment prior to exposing the seed to alternating temperatures (15/25°C for 16/8h during each 24h cycle) for 10 days. A recent literature review suggested that studies assessing seed dormancy levels & dormancy-breaking methods in annual canarygrass are lacking. The objectives of this study were (i) to quantify the levels of seed dormancy in three annual canarygrass cultivars (Keet, CDC Maria, & CY 184) relative to spring wheat & (ii) to determine the effectiveness of three treatments (GA_3, KNO_3, & chilling) & two temperature regimes (15/25°C & 15°C) in promoting germination of dormant annual canarygrass seeds.

Materials & methods

Two annual canarygrass cultivars, Keet (pubescent hulls & brown caryopses) & CDC Maria (glabrous hulls & brown caryopses), & experimental line CY 184 (pubescent hulls & yellow caryopses) were obtained from the Crop Development Center (University of Saskatchewan) for use in this study. The Canadian Western Red Spring (CWRS) wheat cultivar Katepwa was included as a control in this study. The CWRS wheat class has been extensively studied with regards to seed dormancy & sprouting tolerance (Derera, 1989). The seed dormancy level in Katepwa is comparable to the level found in many CWRS wheat cultivars. Katepwa has an intermediate level of sprouting tolerance (Hucl, 1994). In 1998 & 1999, each of the four cultivars were grown at the Seed Farm (University of Saskatchewan, Saskatoon, SK) in 1.2m x 7.5m strips. The cultivars were seeded on 12 May 1998 & on 07 May 1999 at a rate of 250

seeds/m^2 on fallow land. Fertilizer (11-51-0, N-P-K) was drilled in with the seed at a rate of 50 kg ha^{-1}. The soil type was a Vertic Haploborall silty clay. At Zadoks growth stage 92 (Zadoks et al., 1974), 1000 panicles & 500 spikes were harvested from the upper canopies, air-dried at 24°C (40% relative humidity) in darkness for one week, & then stored at –20°C until needed. Panicles & spikes were hand-threshed to avoid split hulls & damaged seeds. Germination tests followed standard protocols (Canadian Food Inspection Agency, 1997). Germination tests were conducted within four months of harvesting. Structures (palea & lemma) enclosing the seed were retained. Seeds were considered germinated if the radicle had emerged by 2 mm from the seed coat. Percentage germination data were calculated as follows: (number of germinated seeds/50 imbibed seeds) x 100. Seeds were placed in 8 cm petri-dishes each containing one filter paper (Whatman No.1) & filters were moistened with distilled water (5mL), unless otherwise specified. Seeds were uniformly spaced on the filters so that contact of adjacent seeds was avoided & petri-dishes were placed in a large plastic container containing water-saturated towels before placing into incubators (Hotpack Corp., Philadelphia, PA; Model No. 352632). The viability of the three annual canarygrass & Katepwa wheat seed lots, used over the two year study, were deemed acceptable (germination levels of 98-100%) based on germination (%) at 22°C for one-week following storage of the seed lots at 24°C for eight weeks.

Experiment 1 & 2: Level of dormancy

To assess the level of seed dormancy in annual canarygrass relative to spring wheat the following experiments were conducted. Four replications of 50 seeds per cultivar were used in each experiment. In experiment one, seeds were incubated at 10, 15, 20, or 25°C in darkness for one week & counts were taken at seven days. In experiment two, seeds were placed into petri-dishes (containing a filter) & stored at 24°C in darkness (40% relative humidity) for zero, one, two, three, four, five, six, seven, or eight weeks to assess loss of dormancy over time. Following storage at 24°C, seeds were incubated at 22°C in darkness for one week & counts were taken at seven days.

Experiment 3: Dormancy breaking treatment & incubation temperature regime

A combination of dormancy breaking methods, including three dormancy breaking treatments (chilling, KNO_3, & GA_3) & two temperature regimes (15/25°C & 15°C), were assessed for their effectiveness in breaking dormancy in freshly harvested seed of annual canarygrass. Dormancy breaking treatments, including pre-chilling (24h at 24°C followed by 72h at 4°C), potassium nitrate (KNO_3; Sigma Cat No. P-6083), & gibberellic acid (GA_3; Sigma Cat. No. G-7645), followed standard protocols & were used in our study because they are commonly used for promoting germination of dormant seeds (Canadian Food Inspection Agency, 1997). Four replications of 50 seeds from CDC Maria & Katepwa were used for each treatment. For the pre-chilling treatment, seeds were allowed to imbibe water (5 mL per petri-dish) for 24h at 24°C in darkness followed by 72h at 4°C in darkness. For the KNO_3 treatment, 5 mL of a 0.1% (w/v) solution of KNO_3 (1g KNO_3 in 1L water) was used per petri-dish. For the GA_3 treatment, 5 mL of a 0.05% (w/v) solution of GA_3 (0.5g GA_3 in 1L of water) was used per petri-dish. Distilled water (5 mL) was used for subsequent moistening of filter papers within petri-dishes. Following pre-treatments, seeds were incubated for two weeks at an alternating temperature regime of 15/25°C (16/8h during each 24h cycle) in darkness & at 15/25°C (16/8h during each 24h cycle) with light during the high temperature period for 8h each day. Light was provided by a cool

white florescent source (1200 lux). First & final counts were taken at seven & 14 days, respectively.

Statistical analysis
In experiment one & three, percentage data were subjected to an arc sin square root percent transformation prior to ANOVA (Steel & Torrie, 1980). Transformed data from experiment one were analyzed using a split-plot design with cultivars as main plots & temperature as subplots (Minitab, 1996). Transformed data from experiment three were analyzed using split-plot design with cultivars as main plots & dormancy-breaking treatment as subplots. Levene's test (P=0.05) was used to test the homogeneity of variances (Minitab, 1996). For experiment two, percentage data were averaged over four replications & the means were tested to be significantly different from 98% germination (P=0.05) based on one-tailed t-tests.

Results

Level of dormancy
In 1998 & 1999, annual canarygrass cultivars developed deeper seed dormancy levels relative to the spring wheat control (Table 1). Annual canarygrass cultivars exhibited high levels of dormancy compared to the wheat control at germination temperatures of 20 & 25°C. Virtually no germination (0-1%) was detected in annual canarygrass cultivars after one week of incubation at 25°C, while Katepwa was 78% germinated in 1998 & 45% germinated in 1999. The highest percentage germination in annual canarygrass was observed at 15°C, averaging 95% (1998) & 77% (1999), with reduced germination above (20 & 25°C) or below (10°C) this optimum. In annual canarygrass cultivars, average percentage germinations were higher in 1998, ranging from 47 to 57%, than in 1999, ranging from 25-30%. Lower germination (%) was observed at 22°C in annual canarygrass cultivars following one, two, & three weeks of storage at 24°C in 1999 relative to 1998 (Figure 1). In annual canarygrass cultivars, germination (%) at 22°C was not significantly different from 98% (P=0.05), based on one-tailed t-tests, using two or more weeks of storage at 24°C in 1998 & four or more weeks of storage in 1999. In Katepwa wheat (1998 & 1999), germination (%) at 22°C was not significantly different from 98% (P=0.05) using zero to eight weeks of storage.

Dormancy breaking treatment & incubation temperature regime
In annual canarygrass (1998 & 1999), incubation at 15°C in darkness led to the highest germination levels followed by incubation at 15/25°C in darkness & then incubation at 15/25°C with 8h of light (Table 2). Germination in the Katepwa wheat control ranged from 97 to 100% across years, pre-treatments, & incubation regimes. Using an incubation regime of 15°C for one week, germination levels of 99% (1998) & 97% (1999) were observed after KNO_3 treatment in annual canarygrass. After a two-week incubation at 15°C, germination levels of 99% (1998) & 98% (1999) were observed after KNO_3 treatment. In 1999, significantly higher germination levels (97%) were observed using KNO_3 treatment relative to the other treatments, ranging from 66 to 89% germination. Using an incubation regime of 15/25°C (in darkness) for one week, significantly lower germination levels were observed in 1999 using each of the two currently recommended treatments for testing dormant seed of annual canarygrass (60% germination with KNO_3 & 72% germination with pre-chilling) relative to combining the two treatments (83% germination with a pre-chilling + KNO_3 treatment). An incubation regime of 15/25°C with light

(8h) inhibited germination relative to incubation at 15/25°C in darkness, particularly in 1999. Using an incubation regime of 15/25°C with light (8h), significantly higher germination levels were observed in 1999 using pre-chilling + KNO_3 (67%) relative to the other treatments (Table 2).

Discussion

The three annual canarygrass cultivars in this study exhibited significantly deeper seed dormancy than the standard red spring wheat cultivar control. At 20 & 25°C, the differences in dormancy between species were approximately three- & 15-fold, respectively. Similarly, averaged over years, the differential in germination between 10 & 20°C was nearly two-fold for annual canarygrass, but only 20% for spring wheat. Generally, low temperatures during grain-filling & seed maturation in grasses result in increased levels of seed dormancy in the mature seed compared to maturation at high temperatures (Simpson, 1990). Average monthly temperatures during grain-filling & seed maturation in 1998 ranged from 19°C (July) to 20°C (August) & in 1999 ranged from 16°C (July) to 17°C (August). Thus, seed development & maturation of seed lots used in this study occurred under cooler conditions in 1999 relative to 1998. The results from experiments one & two indicate that deeper dormancy levels in annual canarygrass were observed in 1999 relative to 1998. In experiment one, incubation at 10, 15, 20 & 25°C for one week led to higher percentage germination, averaged over temperatures & canarygrass cultivars, in 1998 (averaging 53%) relative to 1999 (averaging 27%). Deeper dormancy in grasses is expressed as a broadening of the range of temperatures, either side of some optimal value, in which germination is inhibited (Simpson, 1990). In experiment two, four weeks of storage at 24°C, prior to germination at 22°C for one week, were required to overcome dormancy (98% germination) in annual canarygrass in 1999 compared to two weeks of storage at 24°C in 1998. Averaged over annual canarygrass cultivars, incubation at 15°C for one week led to the highest percentage germination (95% in 1998 & 77% in 1999) followed by 10°C (79% in 1998 & 46% in 1999), 20°C (averaging 46% in 1998 & 8% 1999), & then by 25°C (1% in 1998 & 0% in 1999). The ability of mature seeds to germinate at low temperatures but not at all temperatures is known as thermal dormancy (Simpson, 1990). In addition, high temperatures (>23°C) can induce secondary dormancy, the re-introduction of dormancy after primary dormancy has terminated or almost terminated, in mature grains when they are soaked with water. Therefore, the inability of annual canarygrass cultivars to germinate at 25°C in both years of the study is likely a result of thermally-induced secondary dormancy.

The most effective method for breaking dormancy in freshly harvested seed of annual canarygrass cultivars was a KNO_3 treatment followed by incubation at 15°C for one week. Germination in Katepwa wheat ranged from 97 to 100% suggesting that the freshly harvested mature seed lacked dormancy. The recommended method for testing fresh or dormant samples of annual canarygrass (KNO_3 or pre-chilling treatment prior to incubation at 15/25°C, 16/8h during each 24h cycle, in darkness) led to unsatisfactory germination levels in annual canarygrass seed with deep dormancy after one week of incubation. Our results indicate that an incubation regime of 15/25°C with 8h of light inhibits germination in annual canarygrass cultivars relative to using an incubation regime of 15/25°C with 24 h of darkness. These results agree with previous studies in *Phalaris* because light has been shown to both inhibit & promote

germination in this genus (Nakamura, 1962; Landgraff & Juntilla, 1979). Dormancy in annual canarygrass was not completely overcome using an alternating temperature regime of 15/25°C suggesting that thermal sensitivity to germination at 25°C may be leading to unsatisfactory germination in seed with deep dormancy. Based on our results (Matus-Cadiz et al., In Press), we suggest using a KNO_3 pre-treatment followed by incubation at 15°C for two weeks, instead of the currently recommended method (Canadian Food Inspection Agency, 1997), to test germination levels of annual canarygrass seed samples. Seed should be air-dried at 24°C, under conditions of low relative humidity, for one-week prior to conducting germination tests. First & final counts should be taken at seven & 14 days, respectively.

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Table 1. Effect of incubation temperature on germination (%) of freshly harvested samples of three annual canarygrass cultivars & Katepwa wheat. Data are the average of four replications & based on counts taken at seven days. (-) Values between brackets are the means of untransformed data.

	Temperature				
Cultivar	10ºC	15ºC	20ºC	25ºC	Average
Keet (1998)	65.8 (83)	78.5 (96)	42.6 (46)	4.1 (1)	47.7 (55)
CDC Maria (1998)	58.9 (73)	74.6 (93)	36.9 (36)	2.0 (0)	43.1 (47)
CY 184 (1998)	63.9 (81)	77.5 (95)	48.9 (57)	5.8 (1)	49.0 (57)
Katepwa (1998)	90.0 (100)	87.1 (99)	82.4 (98)	61.9 (78)	80.4 (97)
LSD (0.05) between transformed means within a row = 13.0 or column = 12.1					LSD (0.05) = 5.0
Keet (1999)	47.3 (54)	66.8 (85)	16.7 (8)	2.0 (0)	33.2 (30)
CDC Maria (1999)	38.3 (38)	60.4 (75)	17.3 (9)	0 (0)	29.0 (25)
CY 184 (1999)	43.4 (47)	57.3 (71)	14.1 (6)	6.4 (1)	30.3 (25)
Katepwa (1999)	90.0 (100)	90.0 (100)	62.9 (79)	41.9 (45)	71.2 (90)
LSD (0.05) between transformed means within a row = 5.4 or column = 5.9					LSD (0.05) = 4.0

Table 2. Effect of dormancy-breaking treatments & incubation temperatures on germination (%) of freshly harvested samples of CDC Maria & Katepwa wheat. Data are the average of four replications & based on counts taken at seven days. (-)Values between brackets are the means of untransformed data.

Cultivar	Distilled water	GA_3	KNO_3	Pre-chilling	Pre-chilling & KNO_3	Average
	15ºC in darkness					
CDC Maria (1998)	78.7 (96)	80.3 (97)	85.1 (99)	80.0 (97)	71.8 (90)	79.2 (97)
Katepwa (1998)	90.0 (100)	88.0 (100)	86.0 (100)	88.0 (100)	90.0 (100)	88.4 (100)
LSD (0.05) between transformed means within a row = 7.5 or column = 9.1						LSD (0.05) = 2.1
CDC Maria (1999)	62.3 (78)	66.4 (84)	80.5 (97)	54.5 (66)	70.3 (89)	66.8 (85)
Katepwa (1999)	87.1 (100)	90.0 (100)	90.0 (100)	88.0 (100)	90.0 (100)	89.0 (100)
LSD (0.05) between transformed means within a row = 7.6 or column = 9.4						LSD (0.05) = 2.9
	15/25ºC 16/8h during each 24h cycle in darkness					
CDC Maria (1998)	75.4 (94)	74.7 (93)	72.1 (91)	77.8 (96)	72.9 (91)	74.6 (93)
Katepwa (1998)	86 (100)	90.0 (100)	90.0 (100)	90.0 (100)	90.0 (100)	89.2 (100)
LSD (0.05) between transformed means within a row = 6.8 or column = 8.2						LSD (0.05) = 2.2
CDC Maria (1999)	41.9 (45)	43.9 (48)	50.8 (60)	58.1 (72)	65.7 (83)	52.1 (62)
Katepwa (1999)	80.3 (97)	90.0 (100)	90.0 (100)	90.0 (100)	90.0 (100)	88.1 (100)
LSD (0.05) between transformed means within a row = 5.0 or column = 4.4						LSD (0.05) = 1.3
	15/25ºC 16/8h during each 24h cycle with 8h light					
CDC Maria (1998)	59.5 (74)	76.1 (94)	69.2 (87)	80.4 (97)	62.3 (78)	69.5 (88)
Katepwa (1998)	87.1 (100)	90.0 (100)	89.0 (100)	90.0 (100)	90.0 (100)	89.0 (100)
LSD (0.05) between transformed means within a row = 5.5 or column = 7.9						LSD (0.05) = 4.6
CDC Maria (1999)	17.8 (9)	37.0 (36)	28.9 (23)	37.7 (37)	54.9 (67)	35.2 (35)
Katepwa (1999)	80.4 (97)	90 (100)	90 (100)	85.9 (98)	90 (100)	87.3 (100)
LSD (0.05) between transformed means within a row = 7.0 or column = 10.4						LSD (0.05) = 6.4

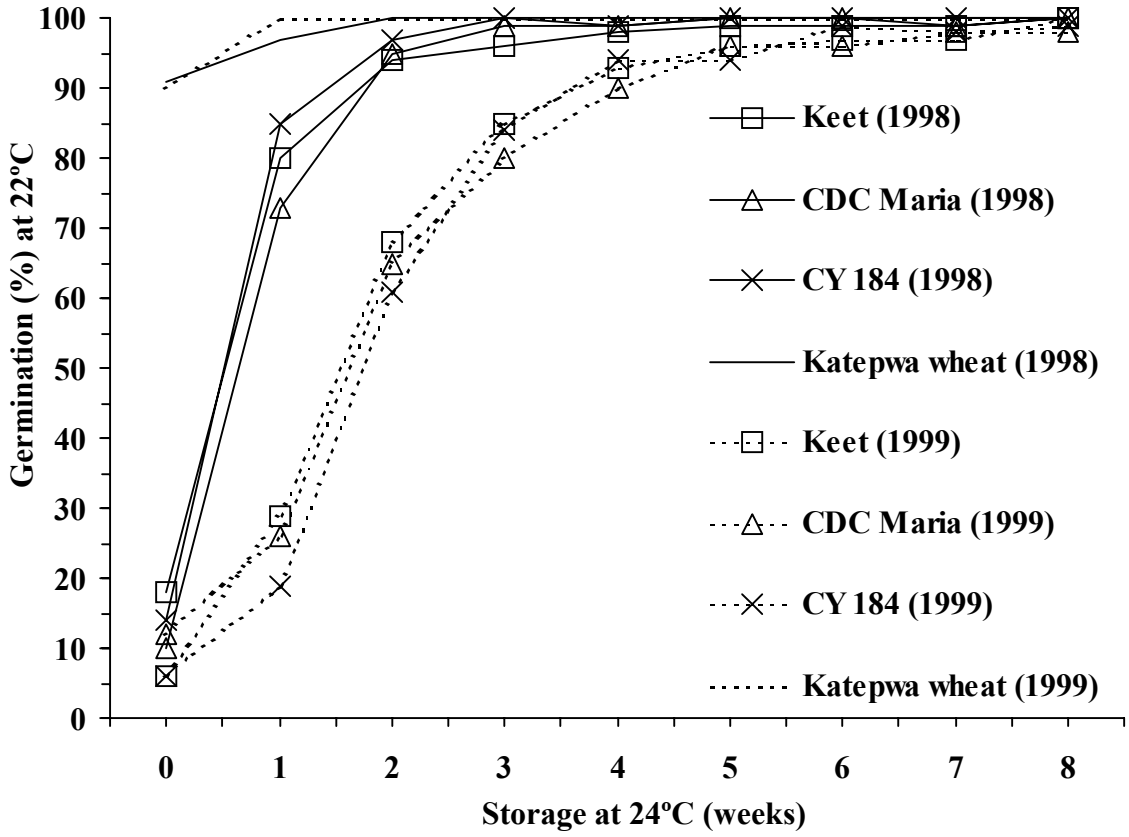

Figure 1. Effect of storage at 24ºC for zero to eight weeks, prior to germination at 22ºC for one week, on germination (%) of freshly harvested samples of three annual canarygrass cultivars & Katepwa wheat. Data are the average of four replications. Germination (%) was not significantly different from 98% (P=0.05), based on one-tailed t-tests, at 2-8 weeks in 1998 & 4-8 weeks in 1999 for annual canarygrass cultivars or at 0-8 weeks in 1998 & 1999 for Katepwa wheat.