Population improvement of seedling traits in beardless and Altai wildrye (Leymus spp.)
by Neal Robin Foster

A dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy In Crop and Soil Science
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Abstract:
Beardless wildrye [Leymus triticoides (Bulkl.) Pilg.] and Altai wildrye, [Leymus angustus (Trin.) Pilg.] are adapted to the western United States. Beardless wildrye is a native, rhizomatous, perennial grass adapted throughout the western United States. Altai wildrye is an introduced perennial grass adapted to the northern Great Plains. Plant characteristics of Altai wildrye include an active growth period in spring and summer, with a moderate regrowth rate, low moisture use, high drought and salinity tolerance, and low fertility requirements. Several factors severely limit the use of these species, including seedling vigor, poor stand establishment, erratic seed production, and seed dormancy in the case of beardless wildrye. By reducing these limitations beardless and Altai wildrye could be widely used for forage production, reclamation and phytoremediation of saline soils.

Studies were conducted to determine the extent of genetic and environmental variability of germination and seedling vigor traits in beardless and Altai wildrye. Wildrye populations were evaluated for seed weight, germination response to exogenous substances, speed of germination, field emergence and forage yield. Seedling vigor was evaluated by measuring seedling dry matter production.

Application of exogenous potassium nitrate significantly improved beardless wildrye germination. Following three cycles of recurrent genotypic selection, field emergence (146%) and forage yield (127%) were improved over Shoshone beardless wildrye. No significant differences occurred in seedling vigor. Exogenous gibberellic acid or indole acetic acid did not significantly improve germination of Altai wildrye populations. Following two cycles of recurrent genotypic selection, field emergence (37%) and forage yield (15%) were improved over Prairieland Altai wildrye.

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Neal Robin Foster

A dissertation in partial fulfillment
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Neal Robin Foster

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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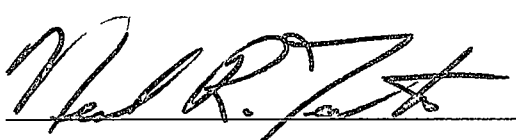
Signature 

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CHAPTER 1

INTRODUCTION

Beardless wildrye [*Leymus triticoides* (Bulkl.) Pilg.] and Altai wildrye [*Leymus angustus* (Trin.) Pilg.] are two grasses that have great potential benefit to regional producers due to adaptability and forage production (USDA, NRCS. 2001, USDA, ARS 2002).

Seedlings of both species develop slowly resulting in poor competition with weeds and other grasses. Once these wildrye grasses become established, they exhibit rapid vegetative spread by rhizomes (Alderson and Sharp 1994). Beardless wildrye has low and erratic seed production (Stroh 1968) and seed dormancy (Knapp and Wiesner 1978). By reducing these problems, beardless and Altai wildrye could be widely used for forage production, reclamation and phytoremediation of saline soils.

The AOSA defines seedling vigor "as those seed properties which determine the potential for rapid and uniform emergence, and development of normal seedlings under a wide range of field conditions" (AOSA 1983). Highly vigorous seedlings become established quickly, utilizing early season moisture and competing well with weeds. As a result of low seedling vigor and seed dormancy (in the case of beardless wildrye) neither species competes well against weeds or other crops during stand establishment (Knapp and Wiesner 1978; USDA-NRCS, 2001).

Factors affecting seed and seedling performance and vigor are seed size, conditioning and storage conditions such as time, temperature, and relative humidity.

Seed weight of grass seed is often correlated with seedling vigor (Rogler 1954; Kneebone and Cremer 1955). In crested wheatgrass [*Agropyron desertorum* (Fisch. Ex Link) Schult.], it was found that emergence from seed planted 3.81 cm deep increased as seed size increased (Rogler 1954). Seed size also impacts seed vigor of many native grass species. Increased vigor of buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], indiangrass [*Sorghastrum nutans* (L.) Nash], bluestems [*Andropogon* spp. L.], sideoats grama [*Bouteloua gracilis* (Kunth) Lag. Ex Griffiths] and switchgrass [*Panicum virgatum* L.] was accomplished simply by sizing seed, with the largest, heaviest seed having the most vigor (Kneebone and Cremer 1955). Seed conditioning will improve grass seed vigor by the removal of the smaller and lighter seed. Optimal storage conditions are necessary to maintain seed and seedling performance, while suboptimal conditions will allow the seed to rapidly deteriorate.

Environmental factors during seed production such as temperature, relative humidity, soil water and soil fertility have variable effects on dormancy levels among seed lots (Fairey and Hampton 1997; Juntilla 1977). Exogenous and endogenous seed dormancy is influenced by many stimulatory factors, including stratification, temperature, light and ionic solutions (Cohn et al. 1989; Roberts et al. 1987). Exogenous dormancy is reduced by water, gases or elimination of mechanical restriction (Adkins and Adkins 1994). Endogenous dormancy can be reduced by many compounds such as weak acid, alcohol, aldehyde, nitrile, and ketone (Adkins et al. 1985). The relative activity of many of these compounds in breaking dormancy is thought to be a function of the lipophilicity of the solvent to the cell membranes within the seed (Cohn et al. 1989).

Many grasses require exogenous potassium nitrate (KNO_3) when using standard AOSA germination procedures, including standard crested wheatgrass, creeping foxtail [*Alopecurus arundinaceus* Pior.], bluestem, grama, and bluegrass [*Poa* spp. L.] (AOSA 2000). Seedling vigor may also be affected by several endogenous hormones in the seed. These hormones include gibberellins (such as GA_3) and indole acetic acid (IAA). The oxygen (O_2) level of the tissue in and around the embryo may also affect vigor (Hsiao and Quick 1984).

Many compounds aid in the reduction of dormancy, and promotion of germination, and consequently seedling growth. Exogenous gibberellin (GA_3) was found to increase first internode length allowing for more emergence in deep seeded wheat (Chen et al. 2001). Scots pine [*Pinus sylvestris* L.] seedlings have insufficient indole acetic acid (IAA) for growth, requiring the addition of IAA for cell elongation (Ljung et al. 2001). Hydrogen peroxide (H_2O_2) has been shown to break dormancy in wild oats [*Avena fatua* L.]. This is thought to occur because as H_2O_2 deteriorates it yields O_2, increasing the respiration rate of the seed and inducing germination (Hsiao and Quick 1984). Ethanol (ETOH) is thought to induce germination by promoting respiration or providing a substrate of respiration (Adkins et al. 1985).

Potassium nitrate (KNO_3) has been used to help break dormancy in many types of seed, including buffalograss (Wenger 1941), poverty grass [*Danthonia spicata* L.] (Toole, 1939), bur buttercup [*Ranunculus testiculatus* Crantz)] (Young 1992) and *Acacia coriacea* DC. (Rehman, 1998). Many grasses require exogenous KNO_3 when using standard AOSA germination procedures, including standard crested wheatgrass, creeping foxtail, bluestem, grama, and bluegrass (AOSA 2000). Standard germination procedures

for beardless wildrye require KNO_3 (2×10^3 mgL^{-1}) as the moistening agent and an alternating temperature of 20/30°C (Chirco, 1986). The mode of action of KNO_3 in breaking dormancy is unknown.

Crabtree and Bazzaz (1993) evaluated the interaction of ammonia (NH_4) and nitrate (NO_3) for effects on germination and plant growth. It was found that an increased ratio of NH_4 to NO_3 improved birch [*Betula spp.* L.] species germination under high light conditions, while low light conditions shifted this ratio to favor NO_3. As the NO_3 ion became available in solution it may mimic a natural trigger which initiates germination (Crabtree and Bazzaz 1993).

Gibberellic acid (specifically GA_3) is present in dry seed at low levels and upon imbibition this level increases dramatically in maize, due to endogenous and *de novo* synthesis of gibberellin (White and Rivin 2000). Far red light induced dormancy of radish seed [*Raphanus sativus* cv. Eterna] was reduced by exogenous GA_3 (Schopfer et al. 2001). In deep-seeded (6 cm) wheat [*Triticum aestivum* L.], exogenous GA_3 increased first internode length allowing for increased depth of emergence (Chen et al. 2001). Germination of chickpea [*Cicer arientinum* L. cv. PBG-1] under salt stress was found to increase with 50 mgL^{-1} additional GA (Kaur et al. 1998). In a seed priming experiment, Carter (1997) exposed chile [*Capsicum annuum* L.] seed to a 4 ppm GA solution for 5d, then air dried for 2d, this treatment increased germination rate over the control. In a comparison of four inbred parent lines and their 12 F_1 hybrids in corn, [*Zea mays* L.], heterotic growth was found to be strongly correlated to GA concentrations (Rood et al. 1988).

At submicromolar amounts, IAA regulates plant growth and development. It is involved in apical dominance, shoot elongation, lateral root initiation and tropisms (Ljung et al. 2001). Dry seed corn contains submicromolar amounts of indole-3-acetic acid, and this small concentration meets the requirement of germination (Epstein et al. 1980). In Scots pine seedlings, endogenous IAA was not sufficient for growth (Ljung et al. 2001). Exogenous IAA was needed for cell elongation. Indole acetic acid is involved in the biosynthesis of gibberellins at the initiation of germination (Van-Huizen 1997). Indole acetic acid is transported from the endosperm to the embryo upon initiation of germination, it may be one of the limiting factors in rapid seedling development (Nowacki and Bandurski 1980).

Ethanol (ETOH) has been found to induce germination of lettuce [*Lactuca sativa* L.] (Pecket 1978), barley [*Horedum vulgare* L.] (Le Denuff 1983) and wild oat (Adkins et al. 1985). Ethanol is thought to induce germination by promoting respiration or providing O_2, a substrate of respiration (Adkins et al. 1985). Hydrogen peroxide (H_2O_2) has been shown to break dormancy in wild oats. This is thought to occur because as H_2O_2 deteriorates it yields O_2, increasing the respiration rate of the seed and inducing germination (Hsiao and Quick 1984).

Seed conditioning has a great impact on seed and seedling performance. In an earlier study by Gutormson and Wiesner (1987) on beardless wildrye it was found that scarification increased germination. During the conditioning process seed is often processed through a debearder. This removes awns and breaks apart multiple seed units while mildly scarifying the seed. This process will reduce seed dormancy while increasing germination (Coukos 1944). Optimal storage conditions will maintain seed

and seedling performance while suboptimal conditions will allow the seed to deteriorate at a faster rate.

Based on the potential utility of beardless and Altai wildrye for reclamation and forage, breeding projects were initiated to improve emergence and seedling vigor of these species. Seed of both species, breeding populations and varieties were produced in the same environment.

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CHAPTER 2

RESPONSE OF BEARDLESS WILDRYE [*LEYMUS TRITICOIDES* (BULKL.) PILG.] TO SELECTION FOR RAPID GERMINATION

Introduction

Beardless wildrye [*Leymus triticoides* (Bulkl.) Pilg.] is a native, rhizomatous, perennial grass commonly found throughout the western United States from Montana west to Washington and south to Texas. While drought tolerant, beardless wildrye thrives in standing water, and is adapted to wet saline-alkaline soils (USDA, NRCS. 2001). Beardless wildrye is highly palatable as forage, stabilizes soil and provides wildlife cover. Forage productivity of this species is good with high moisture and low to moderate salt levels. Beardless wildrye is an excellent species for saline seep reclamation, but due to seed germination problems it is often established from sprigs. Seed from this species exhibit dormancy, germinate slowly and have poor seedling vigor (USDA, NRCS. 2001). Seedlings develop slowly resulting in poor competition with weeds and other grasses. Once beardless wildrye becomes established, it exhibits rapid vegetative spread by rhizomes (Alderson and Sharp 1994).

There are several factors that severely limit the use of breadless wildrye including low and erratic seed production (Stroh 1968) seed dormancy (Knapp and Wiesner 1978) and poor seedling vigor (Gutormson and Wiesner 1987). By reducing these problems

beardless wildrye could be widely used for forage production, reclamation and phytoremediation of saline soils.

Many factors affect seed production such as temperature, relative humidity, soil water and soil fertility (Fairey and Hampton 1997; Juntilla 1977). Stimulatory factors, including stratification, temperature, light and ionic solutions (Cohn et al. 1989; Roberts et al. 1987) influence seed dormancy. Water, gases or elimination of mechanical restriction will reduce exogenous dormancy (Adkins and Adkins 1994). Weak acid, alcohol, aldehyde, nitrile, and ketone can reduce endogenous dormancy in many grasses (Adkins et al. 1985, Cohn et al. 1989).

Many compounds, such as gibberellic acid (GA_3) (Chen et al. 2001), indole acetic acid (IAA), (Ljung et al. 2001), hydrogen peroxide (H_2O_2), (Hsiao and Quick 1984), ethanol (ETOH) (Adkins et al. 1985) and potassium nitrate (KNO_3) (Wenger 1941; Toole, 1939; Young 1992; Adkins and Adkins 1994; Rehman, 1998; Chirco, 1986; Crabtree and Bazzaz 1993) aid in the reduction of dormancy, and promotion of germination, and consequently seedling growth.

Seed size, seed conditioning and storage conditions (time, temperature, and relative humidity) also affect seed and seedling performance (Rogler 1954; Kneebone and Cremer 1955; Gutormson and Wiesner 1987; Coukos 1944; Knapp and Wiesner 1978).

A major limitation to previous investigations of beardless wildrye seed dormancy or seedling vigor has been extreme variability among seed lots. Beardless wildrye is a cross-pollinated, heterogeneous, diploid (2n=28) species (USDA, ARS. 2002). While beardless wildrye seed production is limited, seed available on the market is almost

exclusively the cultivar Shoshone. The variability in seed production, seed quality, dormancy and seedling vigor of Shoshone beardless wildrye is apparently due to environmental (E) conditions, interactions of individual genotypes (G) with the environment of production (GxE), or possibly seed harvesting and processing methods.

Based on the potential utility of beardless wildrye for reclamation and forage, a small breeding project was initiated to improve speed of germination and seedling vigor of this species. Seed was produced after two cycles of recurrent genotypic selection for speed of germination. The objectives of this study were to: 1) evaluate beardless wildrye populations under current AOSA germination procedures to determine the extent of genetic and environmental variability and 2) determine if selection for speed of germination in the absence of KNO_3 effectively improved seeding vigor.

Materials and Methods

Populations and Evaluation

Seed of six beardless wildrye populations, produced in 1999 were evaluated. These populations included a certified commercial lot of Shoshone produced in Powell, WY and five breeding populations harvested from solid seeded blocks established in 1998 near Bozeman, MT (Table 1). The breeding populations all traced to Shoshone, and were: Cycle 0 (C_0) unselected Shoshone, and three generations (C_1, C_2, and C_3) developed by recurrent genotypic or phenotypic selection for speed of germination using standard AOSA laboratory conditions. Deionized H_2O was used as the germination medium rather than KNO_3 2×10^3 mgL^{-1} to prevent selection for faster germination due

to the effects of KNO_3. Each cycle of selection required two years to complete due to limited seed production in the year of transplanting. The C_1 generation consisted of 89

Table 1. Sources and descriptions of four breeding populations harvested in 1999 near Bozeman, MT and certified Shoshone beardless wildrye.

Population	Description
Shoshone	Certified Shoshone seed was produced 1999 near Powell, WY. Shoshone is a leafy, fine-stemmed and relatively high forage and seed producing cultivar for this species. Seed dormancy requires fall seeding. While Shoshone is primarily a forage crop it also performs well on saline-affected and saline-seep discharge areas (Alderson 1994).
C_0	Open-pollinated bulk seed of Shoshone was produced in 1996 in isolation, and then established as a solid-seeded increase block in 1997 and seed was harvested in 1999.
C_1	Synthetic of 89 half-sib families following one cycle of phenotypic selection for speed of germination in 1988 (Wiesner and Ditterline, unpublished data). The Syn 1 seed of C_1 was produced in isolation in 1996, and this seed was used to establish a solid-seeded increase block in 1997 and seed was harvested in 1999.
C_2FG	Synthetic bulk of seed from 23 half-sib families from C_1, selected genotypically for fast germination (FG, top 25% of mean from C_1 families). The Syn 1 seed was produced in isolation in 1996 and used to establish a solid-seeded increase block in 1997 and seed was harvested in 1999.
C_2LS	Synthetic bulk seed from 27 half-sib families from C_1, selected genotypically for both large seed and speed of germination (top 50% of C_1 families for both seed weight and speed of germination). The Syn 1 seed was produced in isolation in 1996, and this seed was used to establish a solid-seeded increase in 1997 and seed was harvested in 1999.
C_3 Elite	Synthetic bulk seed from 24 half-sib families from C_2 (C_2 recombined from C_2FG and C_2LS), selected for seed yield, seed weight and emergence. A space plant nursery was established in 1998 and seed was harvested in 1999.

half-sib families selected from Shoshone for rapid germination on blotters. The C_2 generation consisted of rapidly-germinating seedlings from 50 elite half-sib families. Twenty-three half-sib families were selected for speed of germination forming population C_2FG and 27 half-sib families selected for fast germination and high seed weight forming population C_2LS. The C_3 Elite population consisted of 24 families selected for seed yield, seed weight and emergence from the C_2 generation. Seed of the five breeding populations was hand harvested in August 1999, threshed with a rub board, screened and aspirated with a South Dakota seed blower (Hoffmanmfg.com). The South Dakota seed blower was adjusted to mimic commercial processing settings using a commercially cleaned seed lot.

Care was taken to handle each seed lot consistently to minimize differences due to processing. Prior to laboratory germination tests, seed weights of 100 seed samples (r=4) were recorded. Speed of germination was calculated using the method described in the Seed Vigor Testing Handbook (AOSA 1983).

Germination experiments were conducted in 10.5 x 10.5 cm plastic boxes (PioneerPlastics.com), containing two 10 x 10 cm steel blue blotters (AnchorPaper.com). An alternating germination temperature of 20/30°C was used with the low temperature cycling for 16 hours with no light and the high temperature cycling for 8 hours with light. Germination counts were made every other day from day 7 until day 35. Evaluation of seedlings was conducted according to AOSA Rules for Testing Seed (AOSA 2000a). Viability of ungerminated seeds was evaluated after the test period using 2,3,5-triphenyl tetrazolium chloride (TZ) staining techniques to determine dormancy described for grasses in the AOSA Tetrazolium Testing Handbook (AOSA 2000b). The viable ungerminated (dormant) seed was added to the 35 day germinated seed to compute the total viable seed count.

Seedling Vigor

Potential differences in seedling vigor after emergence, were evaluated by transferring 20 equally-sized beardless wildrye seedlings from each of the populations from blotters 11 days after planting into greenhouse conditions. Seedlings were transplanted into 3.8 x 21 cm cone-tainers (stuewe.com/products/rayleach.html) with 145g potting soil (equal parts of Bozeman Silt Loam Soil, washed concrete sand, Canadian sphagnum peat moss, plus AquaGro 2000 G (Aquatrols.com) wetting agent at

594g m^{-3} of soil mix. The mix was steam pasteurized at 80°C for 45 minutes. Growth conditions were daytime (16-hours light) temperature of 21°C, and nighttime (8-hours dark) temperature of 18°C. The seedlings were watered daily and fertilized as needed. Seedlings were clipped, and top growth was dried and weighed at 90 days after transplanting, allowed to regrow and clipped, dried and weighed again at 180 days after transplanting.

Field Establishment and Forage Yield

Forage yield trials were established in 1998 and 2000 near Bozeman, MT on dryland and irrigated conditions, respectively. Plots consisted of seven rows on 15 cm centers, with total plot area of 32 m^2 in a replicated (r=4) randomized complete block design. In the 1998 dryland trial 138 seeds m^{-2} of each population were planted. In the 2000 irrigated trial, 1000 seeds of each population were planted per plot to aid in stand counts of each population. Following the year of establishment, forage yields were evaluated one year in the 1998 dryland trial and two years 2000 irrigated trial.

The selected first and second cycle populations (C_0, C_1, C_2LS and C_2FG) were compared to Shoshone in the 1998 trial. This trial was harvested once in 1999. The irrigated trial included Shoshone, C_1, C_2 (recombined C_2LS and C_2FG) and C_3 Elite. Field stand counts were taken biweekly in July of 2000 and forage yields were obtained twice in 2001 and twice in 2002 in the irrigated trial.

Germination Substrate Evaluation

Two experiments were conducted to determine if selection had changed the response of germination to hormones or growth promoters. In the first experiment, germination tests were conducted using KNO_3, GA_3, 2,4-dichlorophenoxyacetic acid (2,4-D) (as a source of IAA), ETOH, and H_2O_2 respectively, on a commercial lot of Shoshone beardless wildrye. Levels of GA_3 tested were 200, 400 and 800 mgL^{-1}, KNO_3 at 5×10^3, 10×10^3, and 20×10^3 mgL^{-1}, ETOH, H_2O_2 and 2,4-D (IAA) at 0.05, 0.1 and 0.2 mgL^{-1}.

In the second experiment, beardless wildrye breeding populations were evaluated in standard laboratory germination experiments at the MSU State Seed Testing Laboratory in 2000 and 2001. Fifteen factorial treatment combinations (five seed lots x three KNO_3 levels) were evaluated in a completely randomized design with eight replications. The three KNO_3 levels consisted of deionized water (0), 2×10^3 mgL^{-1} KNO_3 (the AOSA standard) and 5×10^3 mgL^{-1} KNO_3. Each replication consisted of 25 seeds of each treatment in a germination box.

Statistical Analyses

Data for all laboratory experiments were analyzed as a completely randomized design using SAS (SAS 2000). Field experiments were analyzed as a randomized complete block design in templates developed with Microsoft Excel (Microsoft.com).

Results and Discussion

Population Responses to Selection

From 1988 through 1999, three cycles of recurrent selection for rapid germination and other traits were conducted on populations derived from Shoshone beardless wildrye. During the development of these breeding populations, a number of seed and seedling vigor characteristics were measured. Seed weights of the C_1 half sib families of beardless wildrye ranged from 1.5 to over 4.1 mg seed $^{-1}$ (Figure 1). The majority of families (73 of 89) had seed weights between 2.4 and 3.5 mg seed^{-1}.

After 35d, the C_1 mean germination was 68% (Figure 2). The majority of families had germination ranging from 51 to 85% (66 of 89 progeny). The mean speed of germination index for the 89 C_1 families was 2.0, with the majority between 1.8 and 2.5 (48 of 89 progeny) (Figure 3). Seed size and germination rate appeared to be normally distributed in the C_1 generation. These indicate that a significant level of variability exists to allow further improvement by selection. The AOSA standard test for germination specifies a 35-day test (Chirco 1986). Figure 2 suggests that most C_1 families tended to have fairly high germination rates, however it is likely that a 35-day test may overestimate potential field emergence.

Germination percentage and rate were significantly ($P<0.05$) and positively associated to seed weight (Figures 4 and 5). Up to approximately 3.3 mg seed^{-1}, these relationships were positive but at seed weights higher than 3.3 mg seed^{-1} the relationship diminished (Figure 5). A similar trend occurred with speed of germination (Figure 4).

While these relationships were fairly weak (R<0.30), it appeared that further concurrent selection for seed size and germination rate would be possible in the C_1 generation.

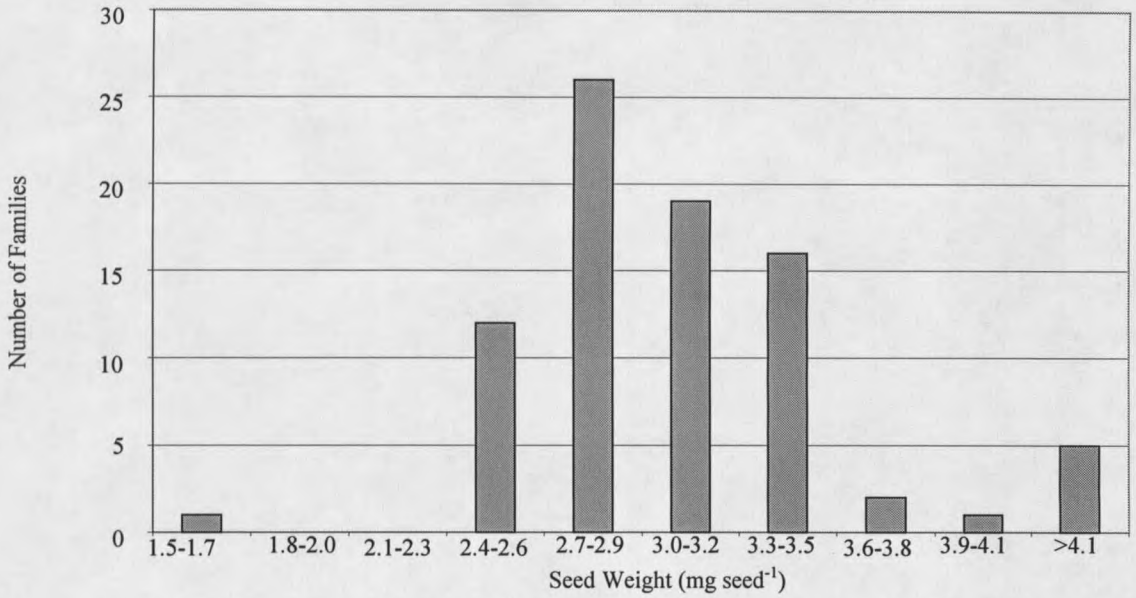

Figure 1. Seed weights of 89 C_1 half sib families of beardless wildrye produced in 1989 (Mean was 3.1 mg seed^{-1}).

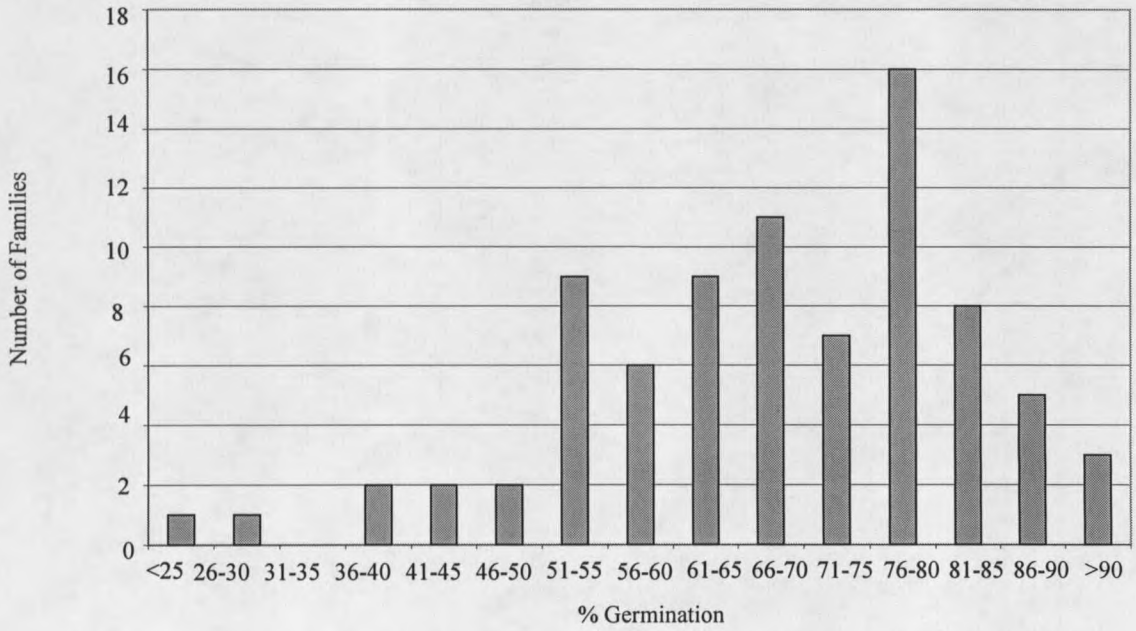

Figure 2. Germination after 35d of 89 C_1 half sib families of beardless wildrye produced in 1989. (Mean was 68%).