

**AN ASSESSMENT OF GRASS REGENERATION NURSERIES AT THE WESTERN  
REGIONAL PLANT INTRODUCTION STATION, 1994-1997**

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**Abstract**

The Western Regional Plant Introduction Station (WRPIS), Pullman, WA, USA, maintains over 17,000 accessions of forage and turf grasses that are mostly wind cross-pollinated and highly heterogenic. Regeneration procedures have been refined over the past nine years to include improved isolation distance and increased plant populations for regeneration. The grass regeneration nurseries planted from 1994 through 1997 were evaluated using data recorded in the Germplasm Resources Information Network (GRIN) and it was found that approximately 78% of the regenerations were successful. Reasons for failures were contributed to inadequate plant number, presence of disease, seed shattering, and unsuitable growing environment. Several solutions have been identified and implemented.

**Keywords:** Regeneration, grass, diversity

## **Introduction**

The WRPIS, located in Pullman, Washington, is part of the United States National Plant Germplasm System (NPGS), and manages almost 69,000 germplasm accessions representing more than 2,600 plant taxa. The mission of the unit is to acquire, regenerate, evaluate, document, and distribute plant germplasm for research purposes.

A major collection at the WRPIS is the temperate forage and turf grasses. Ten genera represent 73% of the total collection (Table 1). Most of these grass accessions are wind cross-pollinated and highly heterogenic. To maintain the genetic identity of accessions they must be isolated from accessions of the same species (Johnson et al, 1996), and the number of plants grown should be adequate to prevent genetic drift (Johnson, 1998). Past regeneration procedures at the WRPIS did not adequately address isolation distances or regeneration population size. To remediate these problems, original seed has been planted, when possible, for regeneration since 1992, and the regeneration population number of plants has been increased from 30 to 60.

Because the WRPIS grass germplasm regeneration program has undergone major modifications it is vital that an assessment of regeneration quality and efficiency be performed. Accessions were considered to be successfully regenerated if at least 8,000 seeds were produced and the germination rate was 75% or higher. The objective of this study was to determine the regeneration success of the field grass nurseries planted from 1994-1997 using GRIN data fields.

## **Material and Methods**

The WRPIS utilizes two distinct environments for grass regeneration nurseries. A dryland site at Pullman, Washington (approximately 800 m in elevation) and an irrigated site at Central Ferry, Washington (approximately 200 m in elevation). The Central Ferry location's growing season

is approximately 30 days longer than Pullman's. Accessions were assigned to either environment by evaluating their suitability according to passport data and taxonomy.

Sixty cardboard bands (50 mm x 50 mm x 80 mm height) containing a steam sterilized soil mix consisting of one-third each sand, peat moss, and Spofford soil were placed into metal flats (50 cm x 30 cm x 60 mm deep). Seeds were treated with captan<sup>1</sup> fungicide and planted into flats. Original seed was used when quantities allowed, otherwise, seed of the generation closest to the original seed source was used. After germination plants were thinned to two per band. These plants were initially established and maintained in a greenhouse for approximately one month and then placed in a lath house about ten days prior to field transplanting.

The field plot area was cultivated, fertilizer was incorporated based on soil tests, and fields were rolled in preparation for planting. The exterior bands were removed as the plants were placed into double furrows approximately 0.25 m wide, 0.30 m apart, and 0.10 m deep formed by two V-blades attached to a lawn tractor, and seedling roots were covered with soil. Each accession was isolated by at least 25 m from other accessions of the same species.

The regeneration nurseries remained in the field for two complete seasons. Typically, first year accessions were mowed before seed formation. However, if an accession was an annual, had few plants, or might suffer substantial winter-kill, seeds were allowed to form and were harvested.

Accessions were harvested by hand-rubbing seed into plastic pans, or by hand cutting. Seeds were placed into labeled paper bags and dried at about 25°C. After drying for at least a month, seeds were threshed, cleaned, and stored in paper envelopes at 4°C and a dew point of -10°C.

Germination tests were performed on newly regenerated seed if quantities were adequate for distribution (>8,000 seeds). Seed quantity, first and second year plant number, and germination rate data were recorded in GRIN. These data were used in assessing the success of the grass nurseries

planted in 1994-1997.

## **Results and Discussion**

In the 1994-1997 nurseries, 2,500 accessions were grown and 1,949 (78%) were successfully regenerated (Table 2). Germination data was not available for all accessions that produced 8,000 or more seeds. However, of the 2,500 accessions grown, 1,175 were tested and the mean germination rate was 91%. This indicates that most of the seed produced in the 1994-1997 nurseries was high quality.

In 1997, 485 of the 665 accessions planted produced adequate seed. Of the unsuccessfully regenerated accessions, 60% produced seven or fewer plants. Other factors such as plant diseases, harvesting time and technique, and suitability of the growing environment may also have contributed to the low seed production. These problems are discussed below.

**Inadequate number of plants produced.** Low plant populations are a major concern as they create a genetic bottleneck (Sackville Hamilton and Chorlton, 1997). Inadequate number of plants established was a major factor in unsuccessful regenerations. Because germination rate information for most original seed lots was not available, seed with low (or no) viability was planted for some accessions, which resulted in limited plant numbers. Low plant population also occurred when only a few seeds had been provided by the donor or when previous unsuccessful regeneration attempts had depleted the seed supply.

To lessen the impact of this problem a back-up of another seed inventory lot, if available, is now planted. These seeds are treated with captan fungicide and placed in a germination box of damp vermiculite. If the original seed does not produce 30 plants or more, the back-up seedlings are transplanted into the flat. This procedure was started on a small scale

in 1999 and will be expanded. Also, germination testing of newly acquired original seed is being completed when we receive at least 3,000 seeds.

**Plant diseases.** In some years, stem rust (*Puccinia graminis* Persoon) and ergot (*Claviceps purpurea* Tul.) have severely limited seed production in a number of species. As a result, we routinely apply Tilt<sup>®</sup> (propiconazole) fungicide for disease control. The 1999 applications at Central Ferry appear to have been adequate to control these diseases, however, more testing is needed to verify these results in different years. A drip irrigation system will replace the overhead sprinkling system in the 2000 grass nursery at Central Ferry, which should also aid in lowering the occurrence of fungal diseases.

**Timely harvest/harvest technique.** Since all plants within an accession rarely mature at the same time, we now carefully monitor plant development and harvest individual plants within accessions at or near physiological maturity. This practice greatly improves the probability of acquiring an adequate quantity of high quality seed from each plant.

**Suitability of the growing environment.** Suitability of the growing environment is a factor influencing seed production of exotic accessions. Before planting, the passport data for each accession was reviewed to determine if the accession was best adapted to the Central Ferry or Pullman location. Sometimes key data was missing or incomplete. Problems with stand establishment, lack of pollination or poor winter survival were also encountered. These difficulties can be minimized when complete passport data is furnished by those collecting and donating accessions.

Germplasm regeneration at the WRPIS is a compromise of factors to maintain the diversity of accessions with available resources. We will continue to evaluate the success of our program and implement improvements. Problems resulting in poor seed quality and quantity are

being resolved, and our goal is to increase successful regenerations to 90% or higher by 2005.

### References

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<sup>1</sup> Mention of trademark or proprietary product does not constitute a guarantee or warranty by the USDA and does not imply its approval over other suitable products.

**Table 1** - The ten major Western Regional Plant Introduction Station grass genera.

Genus	Number of Accessions
<i>Festuca</i>	2,137
<i>Elymus</i>	1,954
<i>Dactylis</i>	1,536
<i>Eragrostis</i>	1,298
<i>Lolium</i>	1,271
<i>Bromus</i>	1,127
<i>Poa</i>	909
<i>Elytrigia</i>	863
<i>Phalaris</i>	761
<i>Agropyron</i>	744
TOTAL	12,600

**Table 2** - Summary statistics of the Western Regional Plant Introduction Station grass regeneration nurseries, 1994-1997.

Year nursery was planted	Number of accessions planted	Number of successfully regenerated accessions†	Number of unsuccessfully regenerated accessions	Percentage of successfully regenerated accessions
1994	656	520	136	79
1995	623	542	81	87
1996	556	402	154	72
<u>1997</u>	<u>665</u>	<u>485</u>	<u>180</u>	<u>73</u>
Total	2,500	1,949	551	78

†Greater than 8,000 seeds produced with germination 75% or greater.