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# DEVELOPMENT AND CHARACTERIZATION OF ENDOPHYTE-FREE TALL FESCUE VARIETY, GREENMASTER3ho

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## ABSTRACT

A new tall fescue variety (*Festuca arundinacea* Schreb.), named Greenmaster 3ho, was developed by the National Institute of Animal Science, Rural Development Administration in Republic of Korea from 2010 to 2014. For synthetic seed production of this new variety, five superior clones, 09XFa02, 09XFa03, 09XFa11, 09XFa13, and 09XFa14 were selected and polycrossed. The agronomic growth characteristics and forage production capability of the seeds were studied at Cheonan from 2010, and regional trials were conducted in Cheonan, Hoengseong, Jeju, and Jinju from 2012 to 2014. Greenmaster3ho showed enhanced disease resistance, persistence, and regrowth ability as compared to Fawn. The dry matter yield of Greenmaster3ho was 29% higher (15,119 kg/ha) than that of Fawn. However, the nutritive value of both varieties was similar. This study developed a new tall fescue variety with excellent environmental adaptability, aiming to make a contribution to the vitalization of the Korean grassland industry.

**Keywords:** Tall fescue, Variety, Greenmaster 3ho, Grassland, Breeding.

## INTRODUCTION

Korea's grassland area was 417,000 ha in the 1970s, and intensive grassland area formation was initiated, but it stands at 35,763ha as of 2014, and the area is decreasing gradually. Grass cultivated in Korea has been mostly introduced from overseas countries, and the area of grassland is continuously on the decline because of frost damage during winter, high temperatures during summer, high humidity and impediments to growth, the development of grass resulting from concentrations of precipitation during the rainy season when grassland is formed, and moisture insufficiency in soil due to draughts from autumn to early summer of the next year.

Therefore, for the development of Korea's grassland industry, the development of new grass varieties appropriate for the domestic climate environment is very important. In particular, its importance has become greater in relation to research on activation of the recent eco-livestock breeding in mountains.

Regarding the development of a new variety of tall fescue 'Greenmaster'. was developed for the first time in 2008 (Choi *et al*., 2010), and tall fescue 'Purumi'. (Lee *et al*., 2012) was developed in 2010. Registration of such breeds for breed protection has been completed. In addition, in order to develop a new breed of endophyte-free tall fescue, diverse domestic and overseas genetic resources were collected and their agricultural characteristics were analyzed; through synthesis of new systems, research on the development of new breeds of endophyte-free tall fescue is actively taking place. In the cultivation of tall fescue, its adaptability to the Korean climate is excellent but its use has limitations due to the problem of infection by seed endophytes. Adaptability to the environment of the plant body of seeds infected with the tall fescue endophyte may be strengthened, but it has been reported that they produce alkaloid toxic materials, and cattle eating tall fescue infected with endophytes, changes body temperature, occur reproductive disorders and subsequently reduced daily milk yield (Bouton, 2002; Walls and Jacobson, 1970; Hohenboken, 1991; McMurphy, 1990).

Accordingly, this study developed Greenmaster3ho, a new breed of tall fescue whose adaptability to the climate environment has been improved and which is not infected with endophytes. Greenmaster3ho exhibited excellent adaptability in Korean climatic condition.

## MATERIALS AND METHODS

**Selection of genetic resources and production of cross-combination:** From 2008, diverse endophyte-free tall fescue genetic resources were collected in and out of Korea, and their characteristics were evaluated in the greenhouse.Nutrition systems of the tall fescue with excellent agricultural traits were formed; five good genetic resources (09XFa02, 09XFa03, 09XFa11, 09XFa13, and 09XFa14) with excellent growth traits and similar heading stages were selected and their cross combinations were produced in 2010 (Fig. 1).

**Seed synthesis:** The nutrition systems of the produced cross combinations were proliferated with asexual breeding and in 2010 polycross packaging was formed for seed synthesis of new systems with a polycross design using five-system triangle polycross technique. Seeds were produced by cultivating rye nearby in order to block pollen scattering.

**Investigation of unique characteristics:** The investigation of unique characteristics of the newly synthesized growing system was conducted in the 2010 trial packaging. The investigation was done according to the Korea Seed & Variety Service's tall fescue characteristics investigation method.

**Tests on regional adaptability:** The regional adaptability test was conducted in Cheonan, Hoengseong, Jeju, and Jinju, Korea, from 2012 to 2014. The tested breed was compared to Fawn, its contrast variety, in order to test its regional adaptability and growth characteristics. The seed amount was applied 30 kg/ha while seeds were putted in 20 cm drill. As for the amount of fertilizer and its application method, the composed fertilizer, N-$P_2O_5$-$K_2O$ = 80-200-70 kg/ha, was applied during sowing; the management fertilizer was N-$P_2O_5$-$K_2O$ = 210-150-180 kg/10a. Nitrogen was applied differently at 30%, 30%, 20%, and 20% during early spring, after the first round of harvest, after the second round of harvest, and after the third round of harvest, respectively, and phosphate and potassium fertilizers were equally applied at 50% during early spring and after the third round of harvest. The morphological characteristics of the breeds were investigated in Suwon and the quantity was investigated by harvesting all treatment groups from the four tested regions.

**Analysis of feed values:** Analysis of feed values of the synthesized system was performed in the same way as that used by Park *et al*. (2012). The content of crude protein (CP) was analyzed using a auto sampler system (Kjeltec™ 2400, FOSS Analytical, Denmark) according to the method recommended by the Association of Official Agricultural Chemists (1990), and the content of neutral detergent fiber and acid detergent fiber was analyzed with an Ankom fiber analyzer (ANKOM technology, 2005a; 2005b) according to the method of Goering and Van Soest (1970). The in vitro dry matter digestibility (IVDMD) was analyzed using the method devised by Tilley and Terry (1963) and modified by Moore (1970).

**Investigation of the endophyte infection of the seeds:** Whether the mature seeds were infected with endophytes was investigated according to the protocol of the Phytoscreen Seed Endophyte Detection Kit (Agrinostics, Ltd. Co., Watkinsville, GA, USA).

## RESULTS AND DISCUSSION

**Unique characteristics of the Greenmaster3ho, a new breed of tall fescue:** Table 1 shows the major growth characteristics of the tall fescue breed Greenmaster3ho. Greenmaster3ho is an allohexaploid variety and its leaf color is green, the length of its branches during the earing season is a medium length of 18.1 cm, the width of its branches is narrow at 5.4 mm, the length of its ears is 23.2 cm, and the length of the longest stem is long at 115cm. Greenmaster3ho is a medium-maturing variety whose earing period is around May 14, which is late by three days compared to Fawn, its contrast variety.The leaf length during the earing season was about 76 cm.

**Dry matter yield of tall fescue variety Greenmaster3ho:** Regional adaptability was conducted for three years in four areas of Cheonan, Hoengseong, Jeju and Jinju to investigate tall fescue dry matter yield; the results are shown in Table 2. The average dry matter yield of the four regions was 14,386kg/ha for Greenmaster3ho, which was about 30% greater than Fawn variety, and the former's productivity was better than its contrast variety Fawn's in three areas (except for Cheonan).

In particular, in Hoengseong, Jeju, and Jinju, the dry matter yields of Greenmaster3ho exhibited higher that was approximately 20%. Possibly, such difference were due to growth characteristics under natural environment stresses such as drought damage, high temperatures, and dryness are directly related to dry matter productivity (Fig. 2). Previously, disease resistance, high forage quality, super re-growth ability, and persistence of Greenmasters cultivars (Gm1, Gm2) were evaluated by NIAS (2014) compared to Fawn. In this study, we introduced and release Greenmaster3ho tall fescue cultivar that is better compared to Fawn as commercial cultivar.

However, Greenmaster3ho is better in adaptability to Korea's climate, environment, and perpetuity than its contrast variety Fawn, and is judged to be suitable for grassland area formation utilized for several years after it is composed once.

**Feeding value of Greenmaster3ho, a new tall fescue variety:** The feeding value of the tall fescue new variety Greenmaster3ho was better than Fawn, with crude protein (CP) at 15.5%, IVDMD at 70.4%, total digestible nutrients (TDN) at 63.4%, neutral detergent fiber (NDF) at 60.8%, and acid detergent fiber (ADF) at 33.4% (Table 3).

**Analysis of seed endophytes:** Whether the tall fescue mature seed was infected with endophytes was analyzed using the Phytoscreen Seed Endophyte Detection Kit.The resultsare shown in Fig. 3. The endophyte-free system collected in and out of the nation was used as breeding

parents to produce the synthesis system, and Greenmaster3howas not infected with endophytes.

This test was performed by the Grassland & Forages Division, National Institute of Animal Science, Rural Development Administration, Korea, from 2008 to 2014 in order to develop a new endophyte-free tall fescue variety. A new tall fescue variety,Greenmaster3ho, is an allohexaploid variety and its leaf color is green, its earing season is about May 16, which is later by 3 days than Fawn, its contrast variety, and the length of its leaf is 76cm. The dry matter yield of Greenmaster3ho in the four areas where the regional adaptability test was performed was 14,386kg/ha, and its productivity was better than Fawn's by about 30%. The feeding value of the tall fescue new variety Greenmaster3ho was better than Fawn's, with CP at 15.5%, IVDMD at 70.4%, TDN at 63.4%, NDF at 60.8%, and ADF at 33.4.

In particular, Greenmaster3ho is not infected with endophytes and is better in adaptability to Korea's climate, environment, and perpetuity than Fawn, and is judged to be suitable for grassland area formation utilized for several years after it is composed once. It can be cultivated across the nation.

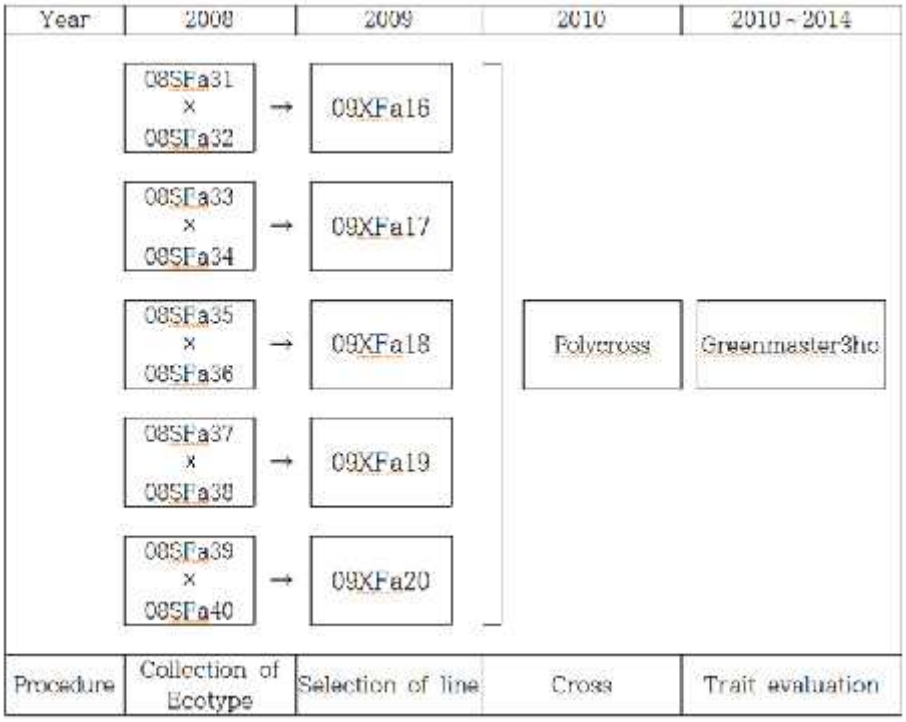


**Fig. 1. Pedigree diagram of a new tall fescue variety, 'Greenmaster3ho'.**


**Fig. 2. Comparison of tall fescue growth and development at Cheonan in 2012.** (A) Evaluation of the regional adaptability of tall fescue. (B) Contrast variety Fawn (more blights in summer). (C) New variety Greenmaster3ho (fewer blights in summer).

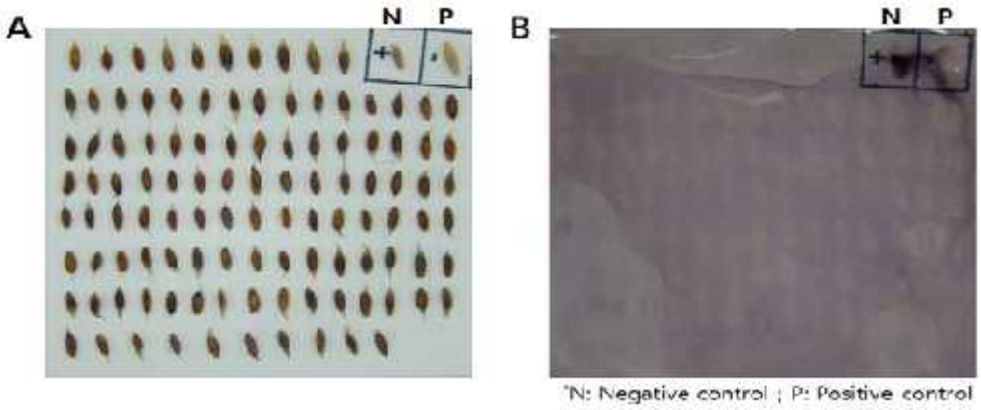


**Fig. 3. Detection of endophytes in tall fescue with five synthetic elite tall fescue lines harvested at seed maturity.**

**Table 1. Agronomic and botanical characteristics of Greenmaster3ho in cheonan, 2012.**

| Characteristics | Fawn | Greenmaster3ho |
|---|---|---|
| Ploidy of chromosome | Hexaploid | Hexaploid |
| Leaf color | Green | Green |
| Leaf width | Medium | Medium |
| Leaf quality | Medium | Medium |
| Plant type(Heading stage) | Half-erect | Half-erect |
| Heading time | May 11 | May 16 |
| Heading stage | | |
| - Flag leaf length | 18.4cm | 18.1cm |
| - Flag leaf width | 5.8mm | 5.4mm |
| - Plant height | 84.2cm | 83.3cm |
| Length of longest stem | 116cm | 115cm |
| Length of upper internode | 37cm | 35cm |
| Inflorescenc length | 23.7cm | 23.2m |

**Table 2. Dry matter yield of tall fescue varieties cultivated in Cheonan, Hoengseong, Jeju, and Jinju from 2012 to 2014.**

| Years Trial region | Fawn(compared) | | | | Greenmaster3ho | | | |
|---|---|---|---|---|---|---|---|---|
| | '12 | '13 | '14 | Average | '12 | '13 | '14 | Average |
| Cheonan | 14,535 | 15,190 | 17,696 | 15,807 | 13,385 | 15,319 | 15,427 | 14,710 |
| Hoengseong | 3,125 | 13,577 | 5,584 | 7,429 | 5,767 | 15,539 | 10,350 | 10,552 |
| Jeju | 11,990 | 17,060 | 14,570 | 14,540 | 15,100 | 20,210 | 18,160 | 17,823 |
| Jinju | 3,539 | 10,809 | 12,461 | 8,936 | 8,317 | 17,709 | 17,344 | 14,457 |
| Average | 8,297 | 14,159 | 12,578 | 11,678 | 10,642 | 17,194 | 15,320 | 14,386 |

**Table 3. Crude protein (CP), in vitro dry matter digestibility (IVDMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrient (TDN) of tall fescue varieties cultivated in Cheonan from 2012 to 2014.**

| Varieties | CP (%) | IVDMD (%) | NDF (%) | ADF (%) | TDN (%) |
|---|---|---|---|---|---|
| Fawn | 13.2 | 67.5 | 61.9 | 34.8 | 62.2 |
| Greenmaster3ho | 15.5 | 70.4 | 60.8 | 33.4 | 63.4 |

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