Research & Development

DLF
SEEDS & SCIENCE

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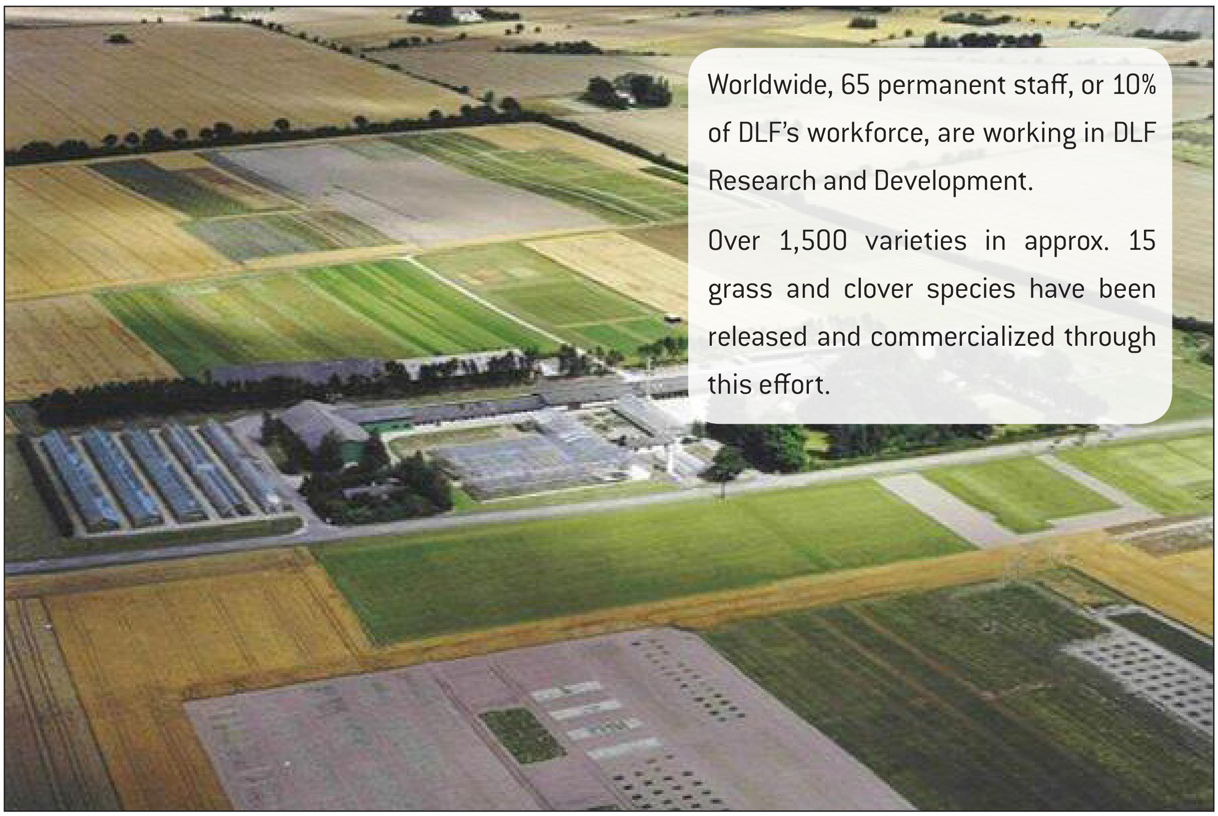
Research & Development

Background and History of R&D in DLF

The DLF Group is headquartered in Denmark, where it started with selection work in grasses and clovers over 100 years ago. Today, DLF is the world's largest producer and marketer of cool season forage and turf grasses. We have been able to reach that position by offering a portfolio of top performing varieties combined with efficient seed production and distribution. Over the course of the past 100 years, the variety improvement methods have dramatically improved and resources DLF has allocated to this effort increased substantially. The original simple selection methods were complemented with pair crossings, creation of tetraploids, molecular marker-assisted breeding and inclusion of novel endophytes. Genome Wide Selection is the latest tool in our variety improvement program.



The DLF Research and Development center in Store Heddinge, Denmark.

Global Research Network

Variety adaptation to local conditions is paramount and our Research Stations are strategically located around the globe to develop the varieties that meet that requirement. All our Research Stations are fully integrated in our global research network, complemented with independent third party locations to extend the environments in which our newest developments are trialed. It allows for screening for certain diseases in a broad array of breeding lines in locations where the disease pressure is high and predictable. The data from all trial locations is fed back to our breeders, who analyze this data to select the varieties with exceptional performance and the broadest adaptation.

The data generated through this intensive trial program is also utilized in the product development and marketing phases of the resulting varieties. Besides yield, disease resistance and quality data, it provides data on suitability for a certain use, adaptation to climatic and soil conditions, etc. These are all traits that are important to the end users of our varieties.

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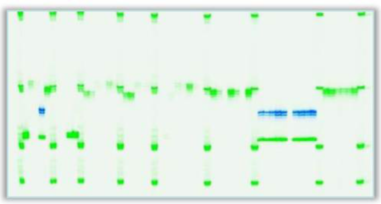
The latest breeding techniques

TILLING

TILLING stands for: ***Targeting Induced Local Lesions in Genomes.*** It is a non-GMO DNA marker technology which allows scientists and breeders to pinpoint valuable variations in the genome that are associated with beneficial traits. Such variations may be derived from nature itself or induced by various seed treatments. Efficient utilization of TILLING in plant breeding requires extensive knowledge about how and which genes control different traits. For digestibility, as an example, several genes involved in the hardening of the cell wall have been identified during the past ten years. These genes control the production of the compound *lignin*, which significantly reduces digestibility. By the use of TILLING, DLF has identified lines in which a particular DNA variation has led to a significant reduction in lignin content. These lines are already advancing to become the next generation of varieties.



Genome Wide Selection (GWS)

Today, breeders select material for a new variety based on different trial data that has been collected during the growth season. Such data may cover dry matter yield, seed yield, heading date and disease resistance. Typically, only the top 5-10% are selected for further breeding while the rest is discarded. Same selection criteria are applied in the second year until the improvements that will qualify the variety for National listing have been obtained. This system is now facing a radical new technological development which may lift the trait values to unseen heights.

The technology, called “Genome Wide Selection” (GWS), bases itself on the genetic potential which is hidden in the plant genome. In 2011, DLF was the first to start implementing genomic selection in grass breeding in collaboration with the Institute of Genetics and Biotechnology at Aarhus University in Denmark. This technology of genomic selection has already prompted major progress in cattle breeding. It provides a new and revolutionary tool for securing the high-quality sustainable grass varieties of the future.

Genome and GWS

The genome in all organisms consists of long DNA chains (chromosomes), which harbor genes that control different traits. The building blocks in DNA consist of four different ribonucleic acids, which are designated by A, T, G, and C. Ryegrass has seven chromosomes and contain approximately 2.7 billion bases and 40,000 genes.

It is the differences in the DNA codes that account for the observable differences between two genetically different individuals. Some traits are controlled by a single gene, but most are controlled by multiple genes.

In order to apply GWS in breeding, it is not necessary to know all DNA differences between the individual plants, but preferably there should be at least 100,000 or more.

GWS consists of selecting breeding material on the basis of the plants’ DNA code instead of their trial data. In order to do so, the first task is to determine which of the DNA variations contribute positively or negatively to various crop traits. This is done by aligning DNA codes from many plants with their trial data from the field. For more than a decade, DLF has recorded both trial data and DNA code from thousands of breeding families which are well suited for such an alignment. Once an association is established, the genetic potential for any trait can be calculated for each plant and those with the highest breeding value can be selected. This adds completely new perspectives and will alleviate some of the limitations associated with the current breeding system.

One of the current limitations lies in the ability to select for more traits simultaneously. Today the development of a new variety with superior yield typically requires 500 field plots. If the new variety is also going to be superior in disease resistance, the breeding process requires 500 x 500 (= 25,000) field plots. This is an impossible task, which in practice means that you can select for one trait (i.e. yield) and then only hope to find variation for other traits in your selected material. GWS can overcome these limitations because it can dissect which part of the genome controls different traits. Thereby it is possible to select plants with the highest breeding values for both traits. In the long term, GWS may also reduce the number of expensive field trials because selections are made on calculated breeding values derived from genomic data. Value and precision will increase as data from more and more breeding lines is applied to the underlying calculation model.

The DLF Breeding Station in Moerstraten, Holland.

DLF now continuously collects data on valuable traits for developing genomic selection in forage grass. Besides traditional traits such as dry-matter yield, seed yield and disease resistance, this also includes fiber and sugar content, nitrogen-use efficiency (NUE) and salt tolerance. All data is associated to a detailed genetic profile made for each of the many numbers of breeding lines. Using comprehensive computations, the genetic potential is predicted for each trait in every new breeding line. This data enables us to select future varieties with several considerably improved traits and may shorten the variety development phase with several years. The implementation of GWS in breeding will result in varieties with dry matter yields well above current top varieties in combination with other improved traits, such as disease resistance, increased forage quality and traits for tolerance to agronomic stresses.

Improving digestibility and energy content of forage grass will increase milk production or weight gain per ton of forage. An additional benefit of increased digestibility is the reduction of methane emissions. Improvement in nitrogen-use efficiency enables production of the same volume of dry matter using less input of inorganic nitrogen. Such quality traits are not visible in dry matter yield data, the most commonly used parameter to determine varietal improvement. However, they affect the growers' bottom line just as much.

Trial and evaluation network

DLF plants over 140,000 trial plots each year to evaluate the performance of new crosses and selections made. Besides the breeding stations within the DLF global research network, a large number of sites in the market areas, close to our customers, are used to test and evaluate these new selections. Trials can be focused, for instance, on traits specific for certain environmental stress conditions, always in combination with forage yield or turf quality. In seed production areas, we conduct seed yield trials to ensure economic seed production in combination with the improvements in forage or turf quality.

Forage quality has a high correlation with the genotype. Selection for improved quality traits has high priority on the breeding stations in the DLF network. DLF has installed online NIRS readers on its plot harvesters, allowing for efficient and detailed data collection. From each selection at each cut and each trial replication, a broad range of quality data is collected in real time, including dry matter and content of fiber, water soluble carbohydrates and protein. It provides direct quality data used for further variety development and to communicate the important resulting quality parameters RFV, RFQ, milk/ton and milk/acre with our customers.

Turf quality requirements are highly dependent on the intended use: sports field, golf course, home lawn, parks or roadsides, each use has its demands of varieties. Wear tolerance and performance under different cutting heights are critical for sports fields and the varieties developed for this market are also used for parks and home lawns that benefit from our rigorous selection process. We select varieties that offer excellent performance under reduced input of fertilizers, pesticides and water, as well as under salt stress. These are important factors for commercial applications and home owners alike, who have increasing awareness of the environmental impact of these factors and the associated regulations.

High digestibility produces more milk

Digestibility is one of the most important goals for the quality of coarse forage, and both the content and digestibility of cell walls (NDF) are of great importance. Grass scores top marks on both counts. High cell wall content is particularly important for a cow's health and production capacity. For each percent the digestibility of the NDF fraction increases, the daily milk yield rises by ½ lb per cow. That is why we focus on breeding varieties with the highest possible digestibility.

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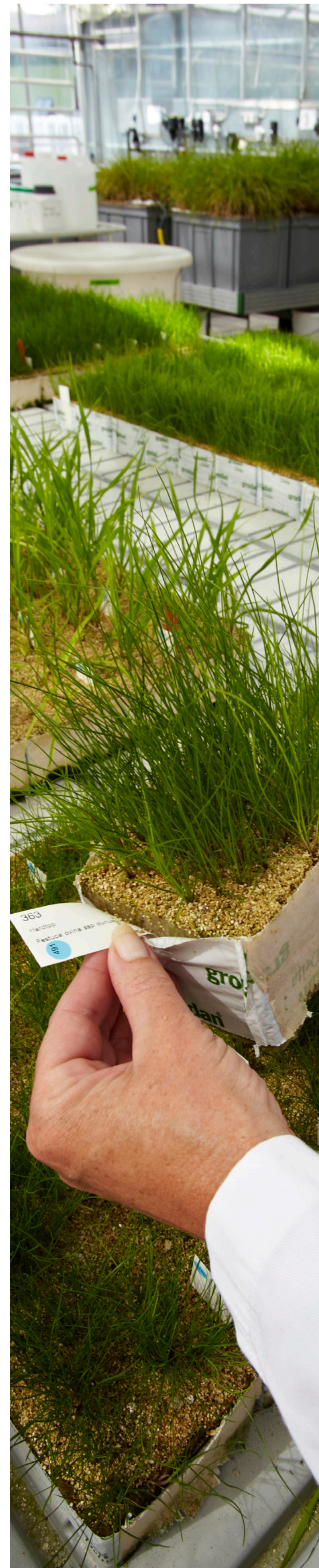


Endophytes

Endophytes were initially discovered in the more persistent varieties and only later the beneficial symbiotic relationship between endophytic fungi and the host plant was understood and put to use. In turf grass, inclusion of endophytes improves insect tolerance, stress tolerance and persistency. In forage grass, the initial focus was on eliminating any endophyte from the plants to prevent the animal health problems it potentially causes. For over a decade DLF has been involved in endophyte research. Through collections of wild endophytes and cooperation with universities, selection has been made for endophytes that provide the positive symbiosis with the host plants while not producing any of the ergo-alkaloids that are harmful for livestock. These are known as novel endophytes. Various genotypes of the target host species, mostly ryegrasses and tall fescue, are then inoculated to select a stable host genotype-endophyte combination. Today, DLF can identify and select specific endophytes with the aid of molecular marker technology, vastly improving the accuracy and time line for development. Successfully inoculated host genotypes are multiplied and tested for good propagation through seed and stability in seed storage. They are field tested to confirm the absence of ergo-alkaloids through lab analyses and animal feeding studies. Improved performance of the grass stand is expressed by increased insect and stress tolerance, resulting in better persistency.

Targeted research utilizing the wide variation within endophyte species has resulted in the release of several novel endophyte - host grass combinations, each providing the host plant the protection we seek and improve its persistency, for forage as well as turf varieties:

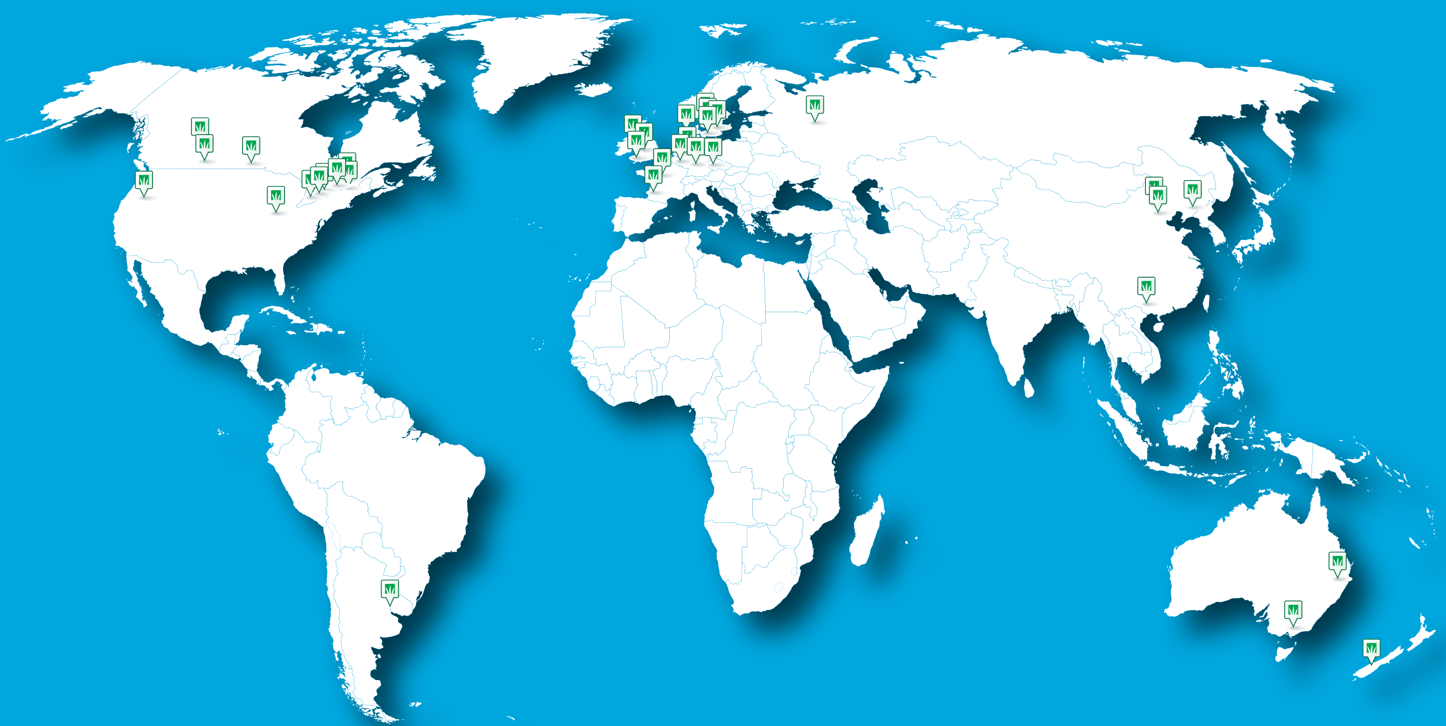
- **AR1® endophyte:** A ryegrass novel endophyte, in cooperation AgResearch in New Zealand
- **HAPPE® endophyte:** A ryegrass and tall fescue novel endophyte, with a specific and unique activity in the root system of the plant, potentially providing protection against soil borne insects.
- **Gold® endophyte:** A tall fescue novel endophyte, in cooperation with the University of Arkansas
- **Protek™ endophyte:** A tall fescue novel endophyte, proving to be very stable in combination with the host genotypes.

Research and Development Results

Here are some examples of DLF's contributions to larger and better forage production:

- Approximately ten new forage varieties of grass and clover from our breeding program are accepted on National variety lists every year
- Perennial ryegrass varieties with a high content of digestible cell walls, NDF (Neutral Detergent Fibre), increasing milk/ton
- Special hardy varieties to meet the requirements of very dry, cold, wet or alkali conditions
- Development of the world's largest breeding program in Festulolium
- Top NTEP rated turf varieties, year after year

Global Research Network



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