Accelerating white clover breeding in Ireland

A TEAGASC project is developing approaches to accelerate delivery of improved white clover varieties that will contribute to the economic and environmental sustainability of grassland agriculture.

White clover in grassland agriculture

White clover can contribute to both the economic and environmental sustainability of pastoral-based production systems. Teagasc research demonstrates that incorporating white clover into grazing swards provides additional nitrogen (N) for herbage production (pastures receiving 150 kg N/ha had similar yields to perennial ryegrass-only pastures receiving 250 kg N/ha) and provides higher-quality forage leading to increased milk yields relative to grass-only swards. Furthermore, the incorporation of white clover into pastures has been identified as a greenhouse gas mitigation measure by Teagasc's Mitigation Abatement Cost Curve (MACC) analysis. Breeding new cultivars of white clover to support pastoral-based production systems is therefore a critically important goal for Ireland.

Breeding new varieties of white clover

Teagasc has been breeding white clover at Oak Park since the 1960s. Five new cultivars have been commercialised in the last 10 years. A typical white clover breeding programme uses genotypic recurrent selection to evaluate the genetic merit of candidates from progeny performance. This is facilitated through evaluating full-sib progeny in field plots in combination with perennial ryegrass over at least three years (following an establishment year) under mechanical cutting and sheep grazing. Evaluation in swards is crucial for improving traits like forage yield and compatibility with perennial ryegrass. Once the best full-sib families are identified, plants are selected within these families as parents to produce an improved synthetic population.

This process represents a single cycle of selection and takes at least seven years to complete from initial pair-crosses to producing a new

synthetic population. However, there are opportunities to accelerate genetic gain by using new technologies to reduce the length of the selection cycle and increase selection intensity.

Genomic selection in plant breeding

In recent years there has been an increased interest in the application of genomic selection in plant breeding, an approach to accelerate genetic gain. This has mainly been driven by a reduction in the cost of DNA sequencing and the availability of reference genomes for many plant species (the white clover genome sequence was published in 2019), making it now feasible to characterise genetic variation across large plant genomes. Genomic selection is a form of DNA-assisted selection that uses genome-wide DNA profiles to calculate genomic estimated breeding values (GEBVs). Selection of plants to use as parents for the next generation are subsequently based on GEBVs, thereby avoiding the need to evaluate the progeny of these plants in field trials. Crucially, calculating GEBVs and recombining selected plants can be completed within a single year, which in the case of white clover enables up to seven cycles of selection to be completed in the same time it takes to complete a single cycle with field evaluations. In white clover, genomic selection offers a huge opportunity to accelerate genetic gain for important traits such as yield and persistency.

Developing genomic selection models for forage yield

In order to develop genomic selection, we needed a reference population that had been: (i) genotyped with genome-wide molecular markers; and, (ii) accurately evaluated for the target traits under selection (**Figure 1**). At Teagasc Oak Park, we were able to



FIGURE 1: Developing genomic selection in white clover. In order to develop genomic selection, a reference population needs to be both genotyped with DNA markers and evaluated for target traits in multi-year field trials. These data are then used to develop statistical models that will enable us to predict a plant's breeding value when only presented with its DNA marker profile. Once prediction models are developed (steps shown in green) they can then be applied to white clover breeding material (steps shown in brown). This involves genotyping selection candidates with molecular markers by partially sequencing their genomes and using the prediction models developed above to calculate genomic estimated breeding values (GEBVs). Selections are then made using these GEBVs and plants are crossed to produce a new and improved population. Another cycle of genomic selection can then be completed using the new population as a starting point. New field evaluations are carried out to update and improve the statistical models.

exploit historical performance data that had been collected on more than 200 full-sib families over a three-year period from 2009-2011. White clover families had been transplanted into plots of perennial ryegrass (common variety across all plots) and total forage yield was measured at up to eight cuts each year.

Seed existed for each family and this was used to characterise DNA sequence variation across the genome of all families. This was achieved by sampling and sequencing a small region of the genome in each family, which enabled us to identify over 100,000 positions that showed variation among the white clover families. Associating genome variation with variation in field performance allowed us to build genomic prediction models for seasonal forage yield. These models can now be used to generate GEBVs for a new line when only provided with its DNA information. The advantage this offers is that DNA information can easily be obtained from three-week-old seedlings, meaning selections can be made without the need to develop full-sib families and carry out field evaluations. This reduces the length of a cycle of selection from seven years to a single year.

What are the next steps?

It is expected that we can more than double the rate of genetic gain for forage yield in white clover using the models developed at Oak Park. The next steps will involve taking these models and applying them to quantify genetic gain over multiple cycles of selection. This will enable us to validate prediction models while simultaneously developing new and improved synthetic populations.

Genomic selection in white clover will accelerate genetic gain for key traits such as forage yield. This will contribute to an increase in onfarm profitability through reduced inputs and increased productivity due to a better nutritional profile.

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