

## Marker Assisted Selection in Forage Breeding: A Broadened Vision

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Forage breeding programs do not yet widely use molecular markers. Reasons include meager breeding program resources and mass selection's ease and cost competitive nature (despite its extreme inefficiency). Most traditional forage breeding methods are based on  $\Delta G = kh\sigma_A$  (i.e., selection gain [ $\Delta G$ ], selection intensity [ $k$ ],  $\sqrt{}$  narrow sense heritability [ $h$ ], and  $\sqrt{}$  additive genetic variance [ $\sigma_A$ ]). Breeders seek to increase  $h$  by minimizing non-additive genetic variance effects through replicated selection unit testing. However, such methodologies require selection unit phenotyping. With the advent of molecular markers, correlated selection response strategies were proposed (i.e.,  $\Delta G = kr\sigma_A$ ,  $r$  replaced  $h$ ), allowing selection in non-target environments, such as greenhouses, without phenotyping. Worries about minimizing non-additive genetic variance effects in  $h$  were replaced by marker-trait linkage decay worries in  $r$ -based methodologies. Very dense molecular marker genome coverage coupled with residual linkage disequilibrium-based correlated selection response strategies (e.g. whole genome selection) laid linkage worries to rest. Cost remains the only hurdle to implementing "correlated" selection methodologies (i.e.,  $\Delta G = kr\sigma_A$ ); which costs are currently prohibitive in most forage breeding programs. For cost-sensitive forage breeders with minimal molecular genetic infrastructure, what alternate marker assisted selection strategies exist? The simplest is selecting on molecular marker-determined population "structures" (i.e.  $\Delta G = kh\sigma_A$ ). Pre-molecular marker breeders selected on population structures such as maternal halfsib families. Using a few molecular markers (e.g. 15 to 20), parentage-based population structures (i.e. pollen-parent or both seed parents) can be defined for isolated polycrosses with all potential parents known. Parentage testing requires no functional genomic or marker-trait linkage information, can be accomplished with any molecular marker type (in species with any ploidy configuration), and is more efficient at higher ploidy levels (i.e., more information per marker). The drawback of parentage testing marker-assisted breeding is that family structure at most only defines 50% of  $\sigma_A^2$  and selection unit phenotyping is required each selection cycle. Theoretically, selection on non-parentage-based population structures could be considered; although *a priori* defined  $\sigma_A^2$  for selection would be difficult to estimate (parent-offspring regressions could be used to estimate  $\sigma_A^2$  post selection). Linkage-based marker-assisted selection strategies become attractive with progeny parentage fully known, particularly if parentage testing markers have known trait-marker linkage. Selecting under maximum linkage equilibrium conditions should be considered if only a few markers are genotyped since marker-trait proximity is less critical than with residual linkage equilibrium marker-trait correlations. Linkage-based strategies are better suited to small polycrosses, while parentage testing is better suited to larger polycrosses due to achievable selection intensities. It should be noted that maximum linkage disequilibrium and parentage-based selection can be orthogonal. As high genome density genotyping costs decline, whole genome selection would eclipse other marker-assisted selection strategies. Compared to mass selection and simple phenotyping, even simple genotyping remains costly; a key consideration for breeding programs is where in the breeding processes markers should be implemented. For example, combined selection strategies could be considered that select old sward survivors (i.e., mass selection for persistence) followed by marker-assisted culling based on family origin or marker-trait linkage for persistence or other traits. Despite genotyping costs declining, plant sample collection and organization for DNA extraction will always be required; logistical optimization of this task is central to successful marker-assisted selection implementation. Currently available marker-assisted selection strategies supplicate for immediate implementation in medium to large forage breeding programs.