Genetic analysis of production characters in *Lolium*

1. Triple test cross analysis of spaced plant performance

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**Summary.** The genetical control of heading date and dry matter production in an 'F2' population of *Lolium perenne* are presented from analyses of triple-test-crosses and basic generations. The data is derived from spaced plant trials at sites in the United Kingdom (UK) and Italy in different years. Despite the wide initial cross between UK bred material and an Italian accession, there was no significant evidence for epistasis, while additive and dominance variation were generally present with partial to complete dominance for all traits. Linear regression onto the environmental means accounted for all the GxE variation for dry matter production in the establishment and aftermath cuts, but not for heading date or the hay cut. The b values measuring responsiveness to the environment were clearly heritable and showed partial dominance. Predictions of the likely performance of recombinant inbred lines and second cycle hybrids were sufficiently promising to support further investigation of these approaches to breeding in this crop.

**Key words:** *Lolium* – F1 hybrids – Recombinant inbred lines – Triple test crosses – Genotype by environment interaction

**Introduction**

The synthetic variety has been the system of variety construction adopted for the majority of grasses, of which *Loliums* are the predominant species in temperate agriculture. Traditional breeding methods have involved phenotypic selection at the spaced plant level, polycross progeny, testing under simulated sward conditions, followed by the identification of superior clones for the establishment of a synthetic variety. In spite of the considerable effort that has gone into breeding along these lines, only a limited improvement in annual dry matter production has been achieved. In comparison with many other crops which have attained an increase of about 1% per annum in harvestable yield, ryegrass improvements, as measured by cutting trials, are only of the order of 0.2% for diploid cultivars (Hayward, unpublished). Greater success has been achieved in other objectives such as seasonal production and herbage quality.

Several reasons have been put forward to account for this limited improvement in annual yield, such as: spaced plant versus sward relationships (Lazenby and Rogers 1964), the low heritability of production characters and the intensity of selection (van Bogaert 1977; Hayward 1983). To overcome some of these problems alternative selection systems have been proposed such as the use of physiological selection criteria (Wilson 1976) or selection under competitive conditions (van Dijk and Winkelhorst 1978; Hayward and Vivero 1985).

To date such selection procedures have led to limited success at an experimental stage only. They are based on the premise that extensive genetic variation is present in the source material for all characters of interest and that those characters are directly correlated with sward performance. These novel approaches are still, however, based upon exploitation of the selected individuals through a synthetic variety.

The synthetic system of variety construction utilizes additive (and to a lesser extent dominance) and epistatic variation (Breese 1972) and is an ideal method for cross-pollinated species such as *Lolium*. It has several advantages in terms of population improvement as a means of recombining variation prior to further
cycles of selection (Wright 1974; Gallais 1977). This characteristic can, however, create problems in that it provides an avenue for the release of variation during generations of seed multiplication, with the further opportunity for selection and change in varietal performance to take place. Some recent studies in *Lolium perenne* have shown, for example, that even where selection has been intensively applied and a positive response achieved, there is still sufficient residual variation present for rapid loss of the desired trait to occur (Hayward and Abdullah 1985).

A further problem of the synthetic is that it is dependent on the identification of a number of superior individuals which will combine well together. It has been suggested that too few a number may lead to inbreeding depression, while too many will lower the overall performance (van Bogaert 1984).

In order to overcome these difficulties in ryegrass breeding and to more fully exploit the considerable variation which is known to exist within the species (Breese and Tyler 1986), it has been proposed that alternative systems of variety construction be adopted, namely F1 hybrids or recombinant inbred lines (Hayward 1985).

Techniques for the production of F1 hybrids in ryegrass have been hampered by a lack of effective pollination control mechanisms, although several attempts have been made using male sterility (Kobabe 1983) and the semihybrid system (Foster 1971). Earlier studies of the genetic organization of some natural populations of *Lolium perenne* showed an absence of the appropriate forms of gene action desirable for the production of F1 hybrids (Hayward and Breese 1968; Breese and Hayward 1972). More recently however, hybridization of geographically isolated and genetically very distinct populations has shown that considerable heterosis may be encountered (Hayward and McAdam 1983; Humphreys 1984; Wilkins 1985). The present series of investigations are aimed at gaining an understanding of the genetic basis of such heterosis and determining if it is a type which may only be utilized in hybrids or, as seems more likely from studies of this phenomenon, that it may eventually be captured in superior inbred lines (Jinks 1981).

The ability to produce recombinant inbred lines in ryegrass as an alternative system of variety synthesis requires the breakdown of the genetically controlled incompatibility system and the establishment of self fertility. It has long been known that inbred lines in ryegrass can be obtained by enforced selfing (Jenkin 1931; Utz and Oettler 1977) or by the possible transfer of self-fertility genes from the closely related self-compatible members of the genus (Nitzsche 1983). The production of such inbred lines offers the opportunity for overcoming some of the difficulties pertaining to the synthetic variety. The present series of experiments are aimed at assessing the optimum strategy for variety synthesis in ryegrass, as well as assessing some of the other difficulties of selection such as performance under spaced plant versus sward conditions, adaptation across seasons and sites and indirect selection criteria.

### Materials and methods

#### Material

The material described in this paper derives from three different sources of *Lolium perenne*, the synthetic variety $24$ and wild collections from the Po Valley in Northern Italy and from the Monmouthshire Moors in Wales. The $24$ and Po Valley material are relatively short-lived, early flowering, hay types while the third is a longer-lived pasture type (Hayward and Breese 1968).

Three plants were chosen at random from both the $24$ and Po Valley material and three sets of inter-population crosses set up reciprocally to produce derived populations D/E, F/G, H/I (Cornish 1979). A seventh population (P) was produced from the Monmouthshire Moors material.

Population F was chosen for detailed biometrical analysis, and the two parents P1 (Po Valley) and P2 ($24$) together with one of their F1's (F 4) were used to produce a set of standard “basic generations” – i.e. parents, F1, F2 and backcrosses – and also to provide the testers (pollen parents) for a “triple-test-cross” (TTC) mating design (Kearsey and Jinks 1968). The seed parents of the TTC were obtained not from selfing the F1 (F4) but by intercrossing with another plant from the same population. They thus constituted a pseudo F2. Although neither parents were inbred, it was assumed that given their different origins and phenotype, there would be much more genetic variation between them than within them.

Some 35 'F2' were each split into 4 cloned replicates, one each for crossing to the three testers and the fourth to check on selfing rates. Selfs and crosses were produced independently in 1981, 1982 and 1983. In 1981 and 1982 the seed parents involved in crosses were all hand emasculated at Birmingham. It was not possible to obtain sufficient seed of all the necessary “basic generations” in 1981 or 1982, so only TTC seed was used. Moreover, only those families for which there were 100 or more seeds were grown. Since tests of selfing rate among the seed parents proved very low (< 0.5%), subsequent seed was produced in pollen proof isolation chambers without emasculation at the Welsh Plant Breeding Station (WPBS). This facilitated the production of larger quantities of seed both from crosses and from selfs, so that TTC and "basic generation" seed was available for the 1984 trials.

Seven separate trials are reported, three of which derived from seed produced in 1981 and 1982 and do not include the "basic generations" while the other four contain all material. Four control varieties ($24$, Premo, Mantilla and Cropper) were also raised in every trial. Six of these trials were located in the UK – Birmingham 1982, 1983 & 1984 (B82; B83; B84); Aberystwyth 1982 & 1984 (A82; A84); Edinburgh 1984 (E84) – while the seventh was in Perugia, Italy in 1984 (P84).

In all trials every family was represented by five plants with complete individual randomisation in a single block from the date of sowing. Seed for all trials was sown in early spring in glasshouses, and transplanted to the field at 60 cm spacings in early summer in each year.