Theor Appl Genet (1985) 70:48–51

Selection and stability of synthetic varieties of *Lolium perenne*

1. The selected character and its expression over generations of multiplication

M. D. Hayward and I. B. Abdullah *

The University College of Wales, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth SY23 3EB, Dyfed, Wales

Received August 20, 1984; Accepted November 26, 1984
Communicated by A. Kahler

Summary. The performance of three experimental cultivars of *Lolium perenne* selected for yield or water soluble carbohydrate content was monitored over four generations of seed multiplication under relaxed selection. In each variety the selected trait regressed towards that of the base population from which the selection line derived. This could be accounted for by residual genetic variation within the lines for the selected trait, and in some instances, by association of this variation with the fitness character, seed numbers produced. These results emphasize the need for practical breeding programmes to consider the nature of the gene action controlling the selected trait, if additive, directional selection should be effective in increasing the expression of the character. Where ambidirectional dominance and epistasis are important, consideration should be given to means of achieving reassortment of the controlling genes prior to selection.

Key words: *Lolium perenne* – Selection – Multiplication – Stability

Introduction

The benefits of a plant breeding programme are only realized when sufficient seed is commercially available to satisfy farming requirements. In order to achieve this it is essential that seed supplies be built up rapidly after release by the breeder, but at the same time it is important to maintain the integrity of the varietal characteristics.

In outbreeding species such as the forage grasses, it is well known that 'shift' may take place during the seed production sequence leading to the loss of superior varietal performance; for instance, studies by Davies (1954); Cooper (1959) and Kelley and Boyd (1966) on perennial ryegrass (*Lolium perenne*) have shown progressively earlier heading with generations of seed multiplication. Such changes in flowering time are usually accounted for by selection taking place for earlier heading and concomitantly with it, seed production, as a result of climatic and management factors. Strains may also shift, however, simply through an increase in the frequency of certain genotypes following recombination and segregation during multiplication (Knowles and Christie 1972). This could account for the significant changes in morphology and growth habit following seven generations of multiplication of the hybrid ryegrass variety 'Manawa' (*L. perenne* × *L. multiflorum* (Rumball 1970)).

These observations so far have been made on a range of characters displayed by varieties which have not undergone strong directional selection for a specific trait. The present series of investigations examines the performance of varieties which have been intensively selected over a number of generations for an individual character. This initial experiment assesses the expression of the selected trait in three experimental varieties of *Lolium perenne* over four generations of multiplication.

Materials and methods

Three experimental cultivars of *Lolium perenne* were examined, all deriving from the variety 'S.23' by selection for individual characters. Cultivar 'Ba 8674' had been selected for two generations for yield as measured under spaced plant conditions, and a 28% improvement had been achieved (see Hayward 1983 for further details) whereas cultivars 'Ba 8903' and 'Ba 8904' were selections for high and low water soluble carbohydrate content, respectively. These latter two varieties had undergone three generations of selection. The selection intensity was 2.5%, and a cyclical mating scheme with 12

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* Present address: MARDI, P.O. Box 202, Serdang, Selangor, Malaysia

plants were used to reduce inbreeding depression (Breese and Thomas personal communication). Five plants formed the parents of 'Ba 8674' and 12 different plants were used for each of 'Ba 8903' and 'Ba 8904'.

Three generations of multiplication Syn I, Syn III and Syn IV were available for 'Ba 8674' and four generations, Syn I–IV, were available for the remaining two varieties. The plant material assessed derived from the seed stocks, as multiplied, for 'Ba 8674'. In order to provide more detailed information on the genetic variability present within each generation of the carbohydrate selections, a North Carolina Model II (NCM II) (Comstock and Robinson 1952) mating design was imposed. From each generation 40 plants were sampled at random and allocated to 10 sets of 4; within each set one plant was crossed with each of three other plants using the automatic cross pollination technique of Jenkin (1931). Reciprocal crosses were included and seed harvested from each was pooled, thus providing three families per set. All crosses for each generation of 'Ba 8904' produced sufficient seed for sowing purposes in all 120 families, whereas a total of only 96 families was available for 'Ba 8903' over the four generations.

Seeds of the various generations of the three varieties were sown in a glasshouse and seedlings then transplanted to the field in a spaced plant (60 × 60 cm) experiment. Sixty plants of each generation of 'Ba 8674' were planted out as part of a more comprehensive experiment for the assessment of synthetic varieties of ryegrass (see Abdullah 1980). The layout consisted of six replicates, with individual randomization of the ten plants of each generation within a replicate. Twenty plants per family were established for the high and low carbohydrate selections; again in an individually randomized layout of two replicates of ten, including all generations and families of the two lines.

Characters measured

Four cuts were taken to assess productivity of 'Ba 8674', two during the establishment year, and two during the first harvest year: the first, a conservation cut at three weeks post inflorescence emergence followed by the second, an aftermath cut, two months later. Yield was determined as dry matter production per plant for the establishment year cuts and fresh material for the subsequent cuts. These four cuts were comparable to those on which the initial selection programme had been based (Hayward 1983).

Carbohydrate content was determined on plants from all generations of 'Ba 8903' and 'Ba 8904'. Approximately 5 g of fresh material was sampled in September of the establishment year from each plant of one replicate only and dried overnight in an oven at 80 °C. Samples of plant material within a family were pooled because of the large numbers involved. Water soluble carbohydrate (WSC) content was determined by the automated procedure of Thomas (1977).

Results

'Ba 8674' – the high yield selection

The mean productivity of the various generations at each of the cuts is shown in Table 1 together with the corresponding analysis of variance. Averaged over cuts the generations show a regular decline in yield, Syn III was 90% of Syn I, whereas Syn IV was only 77% of Syn I, which was comparable to the base population from which the selection line derived (see Hayward 1983). Differences between cuts and the interaction term generations x cuts were both significant. The largest differences between the Syn I and subsequent generations occurred at the hay and aftermath cuts, corresponding to the growth period which had the greatest influence on the selection criteria (see Hayward 1983).

Table 1. (**a**) Mean yield per plant – dry matter (g) for first and second cut and fresh wt (g) for hay and aftermath cut, for the three generations of 'Ba 8674' together with (**b**) the analysis of variance

(**a**)

Synthetic generation	First cut	Second cut	Hay cut	Aftermath cut	Generation mean
I	117	92	608	229	262
III	103	86	544	198	233
IV	101	74	454	178	202

(**b**)

Item	d.f.	$MS \times 10^{-3}$	P
Generations	2	216	0.001
Cuts	3	7,821	0.001
Cuts × generations	6	64	0.001
Error	702	14	

Table 2. Analyses of variance for total water-soluble carbohydrate content in high and low selection lines

Item	High – 'Ba 8903'		Low – 'Ba 8904'	
	d.f.	M.S.	d.f.	M.S.
Between generations	3	27.6*	3	34.5**
Within generations	89	10.9	116	7.1

* $P = 0.10–0.05$, ** $P = 0.01$

'Ba 8903' and 'Ba 8904' – the carbohydrate selections

The data for the WSC content of the four synthetic generations of both the high and low selected lines is presented in graphical form in Fig. 1. The analysis of variance (Table 2) clearly shows that the generations within each line differ significantly from each other. The WSC content declined regularly from 25.9% in the Syn I of 'Ba 8903' to 23.5% for the Syn IV, whereas the low line increased from 19.1% to 21.6% over the four generations of multiplication and returned to the level of S.23, i.e. 22.7% (see Walters and Evans 1976). The phenotypic variances of the generations are homogenous when compared by a Bartlett's test: χ^2 for 'Ba 8903' = 1.59, 'Ba 8904' = 0.53. Partitioning of the

Table 3. Mean squares of the analyses of the NCM II mating design applied to each generation within the high and low carbohydrate lines

Generation	High – Ba 8903				Low – Ba 8904			
	I	II	III	IV	I	II	III	IV
Between sets	13.0	10.6	13.8	7.9	12.7**	14.7*	4.4	9.9
Between families within sets	10.4	8.3	14.4	10.4	2.2	6.6	4.4	7.9
d.f. sets/families	7/12	9/12	9/17	7/12	9/20	9/20	9/20	9/20

* $P = 0.10$–0.05, ** $P = 0.01$

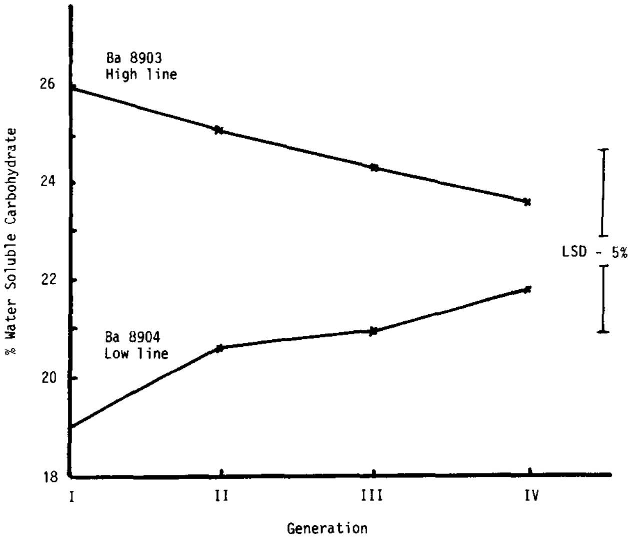

Fig. 1. Changes in the water soluble carbohydrate content of two selected lines of ryegrass over four generations of multiplication. The vertical bars represent the LSD at $P = 0.05$

within generation term of Table 2 into between and within sets within each generation showed that only in the first and second generations of 'Ba 8904' were there significant differences between sets (Table 3). This would suggest the presence of additive genetic variation for this character in these generations, but the absence of replication does not allow precise estimates of the various genetic components to be made.

Discussion

This analysis of the performance of various generations of the three experimental varieties has shown a common pattern, in that mean performance of the selected character returned towards that of the base population from which they derived. Thus, for 'Ba 8674' a 28% increase in spaced plant yield achieved by selection regularly declined over generations of multiplication; whereas for the high and low carbohydrate lines WSC content in the former ('Ba 8903') decreased and the latter ('Ba 8904') increased such that the differences between the two lines diminished considerably.

Although it appears that selection for specific characters can be effectively achieved, herbage breeders may find it difficult to maintain full expression of the selected character during multiplication under relaxed selection. Such an observation has been seen regularly in outbreeding organisms subject to strong directional selection e.g. *Drosophila* (Mather and Harrison 1947). Two inter-related factors concerning the genetic architecture of the character may account for this phenomenon. First, the nature of the gene action responsible for the character and second, the association of this genetic control with the fitness of the population.

If the character is under polygenic control by genes showing ambi-directional dominance or epistasis, selection will favour genotypes which have the potential for maintaining the full range of variability of the original population. Under relaxed selection equilibrium of genotype frequencies will be rapidly re-established and with it the regression of the phenotypic expression to that of the base variety. The rate of change will depend upon the linkage relationships of the genes concerned, the number of basic plants of the variety, their breeding compatibilities, and the population size during the few generations of multiplication under consideration. Such changes will be influenced by another factor, that of association of the selected genes with those controlling fitness characters like seed production. Genetic variation for the selected character has been observed for Syn I and II of 'Ba 8904'. The analyses, as conducted, did not allow tests for the presence of dominance or epistatic effects to be carried out neither did they eliminte the possible occurrence of these effects within the other lines and generations. Further studies have shown that this variation within generations is correlated with variation in seed numbers produced for the first and second generations of the high line ($r = -0.51$ for Syn I and $r = -0.43$ for Syn II, $P = 0.01$ for both coefficients) (Hayward et al., in preparation).

These results emphasize the need for practical breeding programmes to consider the nature of the gene action controlling the characters of interest together with the influence of the selection procedures adopted on possible correlated effects on fitness characters. Where only simple additive genetic control is operative directional selection should be effective, leading to stability of any derived synthetic variety, provided that residual variability is minimal. If however dominance and epistasis are important, consideration should be given to means of achieving genetic reassortment prior to the identification of the basic plants of the synthetic variety. This may be possible by inbreeding (Karp and Jones 1983) or the introduction of B chromosomes (Cameron and Rees 1967), both of which influence the site of recombination, or by the application of physical factors which can affect the number and location of chiasmata (Rees 1961). The current state of knowledge of these processes however is such that the desired precision of control is not yet attainable (see Riley et al. 1981).

Acknowledgements. We wish to thank Dr. E. L. Breese, Welsh Plant Breeding Station for his continuing interest in this work. One of us, I.A.B., acknowledges receipt of a Malaysian Agriculture Research and Development Institute, scholarship.

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