PLANT BREEDING PROGRAMME FOR 1935 RELATIVE TO GRASSES AND CLOVERS.

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The purpose of this paper is to outline the general breeding technique adopted at the Plant Research Station in regard to the improvement of grasses and clovers to pedigree standard. The following species are under study:- perennial ryegrass, Italian ryegrass, cocksfoot, Agrostis canina, white clover, red clover, and Lotus major.

The first stage in the procedure is large-scale testing of samples obtained from representative lines of seed from diverse hobitzts both within New Zealand 2nd overseas. These lines are tested in broadcast plots which are subjected to various methods of management so that data are obtained on such characteristics as establishment, yield, type, seasonal growth, persistency, and disease resistance. Four thousand to six thousand plants of each species from the most promising lines are put out for study is single plants, and these are observed over a period of one to two years. At the end of that time a selection is made, approximately one hundred of the best plants being chosen for further study.. These are now split up and planted out to forn a tiller-row approximately ten feet in length. Single plants from tillers are also set out so that a close study of each selected plant as a tiller row and tiller single plant my be carried a stage further. Observations are continued and examinations recorded at regular periods, points being allotted for such characteristics as production, recovery after cutting, growth-form, persistency, freedom or otherwise from winter-burn and rust. In this way, after three to four years, the agronomic qualities of the single plants may be gauged.

From this point in the programme there are two methods of attack by which further improvements nay be effected -

1. A modified form of mass selection. Those plants of outstanding agronomic merit are tested by diallel crossing and selfing to ascertain whether their progeny contain any serious defects. If, after this test, the parents prove to be sufficiently desirable they are isolated together to form a selection.

2. A method involving further refinement in the plant material. This involves much more intensive breeding work and aims at the elimination of any undesirable characteristics so that a pedigree line is obtained which contains the least possible proportion of inferior typos; in effect, by this method the less desirable characters which may be present in the plant-material are sifted out.

Although the first method is an important phase in the improvement of a cross-fertilised species such as perennial ryegrass in so fnr as it produces some rapid improvement, it is regarded as only a stage in the final production of pedigree seeds. It, however, enables a selection to be obtained in the minimum of time which is probably superior to the best commercial lines and thus satisfies a demand until such time as a more highly improved product can be obtained.

The next stage, then, after producing a selection from grouping "original" desirable plants is to effect further improvement by the adoption of methods which provide more precise information on the genetic constitution of the plant-material, so that inferior characters may be eliminated. The plants secured as a result of selfing and diallel crossing of "original" plants are carefully studied, and from these a fairly accurate opinion may be formed of the breeding behaviour of the parent plants. It is not likely, however, in cross-fertilised species as those with which we are dealing, that "original" plants will be sufficiently homozygous to warrant their acceptance as the basis of a highly improved strain. It is usually essential to take the best plants from the most promising L_1 and F_1 families, and by some method of inbreeding, such as selfing or sib crossing, to eliminate undesirable characters and to increase the homogeneity of the family. When a number of sufficiently homogeneous unrelated plants or families have been obtained, and before they are used as a nucleus for a selection, they are crossed in all directions to ascertain which combinations produce the most vigorous families. After this test the approved plants are massed together under controlled isolation conditions to produce the nucleus for the final selection, which is tested on a broadcast sward and single-plant basis before acceptance into the pedigree class.

We have given an outline above of the general technique in the breeding programme, and it may now be of some interest to discuss briefly the results likely to be obtained by such methods and their applicability to the species being considered.

The two breeding methods employed are (1) crossing unrelated plants, and (2) various types of inbreeding.

(1) Crossing unrelated plants.

As a method of progeny-testing of unrelated plants, crossing has its limitations in that recessive factors present in the heterozygous state in one of the parents may still be concealed in the F_1 generation. However, this is not always the case, as cross-fertilised plants such as those we are considering may be heterozygous for many similar factors, so that by crossing such plants we do often obtain some information as to their factorial constitution. Dominance of certain characters often becomes apparent and valuable evidence may be obtained on the value of the combination of the factors from these two plants.

(2). Inbreeding.

For studying the mode of inheritance of characters some method of inbreeding is of outstanding value. It also offers a ready means of eliminating undesirable factors and obtaining a greater degree of homozygosity for desirable factors than obtained in the original parent plant. On the whole, however, inbreeding results in a reduction in vigour which is simply a manifestation of the segregation of dominant vigour factors and increase in homozygosity of these factors and their recessives. By appropriate combinations of inbred progenies vigour can be regained.

The methods of inbreeding adopted with the species being worked with at the Station depend on the degree of self-fertility of the plant-material. As regards white clover and red clover very little work has been carried out at the Station on the degree of self-fertility, but Williams at Aberystwyth has shown that on the whole these species are highly self-sterile. Selfing therefore cannot be used to any great extent with these clovers so that the method of inbreeding is to make sib crosses with F_1 plants.

In the case of the ryegrasses, however, self-fertility is on the whole much higher than with the clovers, so that selfing may be used to some extent. The seed-setting figures obtained by selfing 71 unrelated "original" ryegrass plants are shown in table 1.

Table 1.

Self-fertility of 71 "original" plants, (approximate figures).

Seeds per 100 spikelets.	% of plants.
0	7
0.1 to 1.0	14
1.1 " 3.0	18
3.1 " 5.0	13
5.1 " 10.0	14
10.1 " 20.0	10
20.1 " 50.0	11
50.1 " 100.0	6
Over 100.0	7

These figures are in accord with those obtained by Jenkin at Aberystwyth. It will be seen that 79% of the plants set over 1 seed per 100 spikelets. Although 1 seed per 100 spikelets may seem a low figure it is comparatively easy to obtain an L_1 generation of twenty to thirty plants from a single plant with such self-fertility figure.

Whether selfing methods can be used with L_1 material depends upon the genetic constitution of the parent plant. When L_1 plants are selfed, a wide range in self-fertility of the L_1 plants may be obtained, as will be seen in the following results of selfing L_1 plants from three unrelated original plants, A10, A18, and B35.

Plant A10 was self-fertile to the extent of 80.1 seeds per 100 spikelets. Of its twelve L_1 plants self-pollinated, six produced no seeds. The figures for the other six plants were 0.3, 1.0, 1.8, 2.2, 8.5, and 71.1 seeds per 100 spikelets.

Another plant, A18, produced on self-pollination 23.0 seeds per 100 spikelets. Ten of its thirteen L_1 plants selfed produced no seeds, while the remaining three set 0.6, 5.3, and 30.5 seeds per 100 spikelets.

The self-fertility figure of the remaining plant, B35, was 42.0 seeds per 100 spikelets. Eleven L_1 plants of B35 were selfed end three produced no seeds, while the figures for the other eight were 0.4, 0.8, 3.2, 4.0, 4.4, 14.0, 20.4, and 49.6 seeds per 100 spikelets.

In all three cases there is a range in the L_1 plants from complete self-sterility to self-fertility as high as or higher than that of the parent. It appears that self-fertility has a genetic basis and segregation for self-fertility or self-sterility factors has taken place as a result of selfing. The genetic constitution of the respective parent plants will therefore decide the degree of self-fertility of the L_1 plants and the use that can bo made of further selfing in this generation. In practice any ryegrass plant used for crossing is always at the same time selfed.

In view of the general reduction in vigour after selfing it would probably not be advisable to select highly self-fertile lines for the purpose of building up a composite strain, as the amount of self-fertilisation that would probably take place in the field might be sufficiently great to affect, the vigour of the strain as a whole. It is possible, however, that some highly self-fertile strains may undergo very little reduction in vigour on selfing, and in perennial and Italian ryegrass, as in timothy end cooksfoot, it might be possible to produce a self-fertile strain which is quite as vigorous as the best commercial lines but much more homogeneous.

The general procedure to be followed in our breeding programme as outlined above may be varied ii? accordance with results which are obtained from time to time. Iv this connection reference will be made to a modification in regard to testing for the presence of factors producing a type of chlorophyll deficiency in perennial ryegrass which we may term "tiller albinism". This type of chlorophyll deficiency, which has not been encountered before in our material, occurs in the progeny of one of our most promising parent plants, B35. Up to the first tiller stage the seedling is usually normal, but its first tiller and often subsequent tillers are devoid of chlorophyll. As the chlorophyll-deficient leaf develops it becomes somewhat green and appears variegated. As the older leaves die off the plant becomes normally green.

The intensity of the development of the albinism varies and sometimes makes tht identification of "tiller albino" plants rather difficult.

From a practical point of view it is debatable whether this character is sufficiently harmful to warrant elimination, but we are of the opinion that it is at least desirable to know which plants are heterozygous for the factor concerned.

In table 2 are shown the results from selfing B35 and also seven L_1 plants of B35.

Table 2.

Plant No.	Tiller do. green.	Albinism. No. albino'.	Ratio. green.	:	albino.
B35'	414	119	3.5	:	1
B35/1	424	65	6.5	:	1
/24	35				
/31 31	24	8 0	35.0 3.0	:	01
/34	24				
/53 54	14	11 3 0	14.c 15.8 8.0	:::	1
/54	174				
/58	128	23	5.6	:	1

These figures appear to indicate a mono-factorial inheritance of tiller albinism with "green" dominant to chlorophyll deficiency. The number of green plants is in most cases too high, particularly in the progeny of B35/54 where the numbers indicate 8 15 : 1 rather than a 3 : 1 ratio. However, it is probable that the excess of green plants is due to the difficulty of identifying some of tht. chlorophyll-deficient plant s.

In view of th; large amount of promising plant material consisting of approximately 2,700 plants many of which may contain the recessive factor for "tiller albinism", some simple large-scale method of testing the most valuable of these plants for the presence of this factor is desirable. A large number of the plants may be selected later for further progeny-testing, and if some indication can be obtained as to the presence of the factor for "tiller albinism" while the plants are under test in the single-plant plot much time will be saved. It is thought that the following method may furnish this information.

When the seedlings were put out as single plants into the plot one row of equal numbars of chlorophyll-deficient seedlings and tillers of the parent plant B35 (which is heterozygous for tiller albinism) was planted to two rows of "green" plants so that each row of the latter was adjacent to a row of plants with the recessive "tiller albino" factor. . All of the plants in the block will be permitted to flower. There should be about equal proportions of pollen carrying the dominant factor for "green" and that with the recessive factor producing "tiller albinism", so that the chances of any plant not being fertilised by some pollen carrying the recessive factor are quite remote . The heterozygous plants fertilised by pollen carrying the "tiller albinism" factor will produce some chlorophyll-deficient seedlings while those homozygous for "green" will produce only green progeny. A seed sample will be taken from each plant which it is desired to test and sown separately only for testing purposes. In this way the plants homozygous for "green" may be determined at the same time as the plants are in the plot for observation of other characters.

When testing of breeding behaviour on a large scale is to be carried out the plant breeder has to use methods which give the desired result and enable him to utilise his time to the fullest advantage.

It should sometimes be quite possible to employ some such simple method of large-scale testing as that outlined above, especially in regard to the detection of easily distinguishable recessive characters after some knowledge of their mode of inheritance has been acquired from preliminary genetical work. But the importance of preliminary fundamental work can hardly be over-estimated, for without it as a foundation the plant-breeder is not likely to attain the desired objective in the minimum of time. Such knowledge is essential to enable him at a later stage to employ large-scale methods on material from which he is likely to obtain the greatest reward, and to save him from working along lines which may ultimately prove not to lead in the desired direction.
