LABORATORY METHODS FOR THE DETERMINATION OF PLANT TYPE.

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Following developments in the segregation of superior types of pasture plants in many countries attention is being focused on the associated problem of the establishment of simple and reliable methods of type or strain diagnosis in the laboratory; the primary object in the search for suoh methods being a very considerable reduction in the time required for type identifioation in the field or in. plot trials.

Many possibilities have been investigated, mainly by Seed Testing institutions, in the hope of establishing the existence of seed or seedling characteristics which would prove to be oonsistent determinators of plant type.

Specific morphological differences are exhibited by the seeds of most genera, and many of these differences are obvious to the untrained eye, but in. some instances they become apparent only by critical examination or by special technique.

On the other hand morphological and physiological differences within species are not usually associated with structural differences in the, seed, or with form or growth behaviour of the seedling..

Certainly; the relatively small size of the seeds of many wild forms of @asses and clovers is a significant characteristic., but 'a's size may be influenced by various other factors- it cannot be employed with any degree of certainty as a type determinator except possibly with pure lines representing extremes.

The absence of morphological differences in seed or in seedlings has resulted in a concentration on the physiological and bio-chemical fields and it has been shown that with the application of certain physical and chemical treatmants, certain definite differential responses can be obtained. It is an interesting fact however that the degree of success obtained in the,, application of the various methods is by no means the same Indifferent countries and would appear to be dependent either on the homogeneity of type in the seed ofcommerce or to the type standards adopted for the common pasture plants.

The scope of this paper is not sufficiently wide to permit of more than a passing reference to the use of phenol and chloral hydrate in specific and varietal differences in seeds of the Brassica family - confined almost entirely to Europe and Russia, of the measurement by--the speed of germination, of the absorption by seed of standard cane sugar solutions - termed "suction force", of the differentiation between seed samples representative of early and late flowering red clovers by the respective photoperiodic reactions

in the seedling stages. Therefore discussion herein will be confined to the two methods which have proved to be of practical value in this country, namely the use of screened Ultra Violet light. in the determination of peienniality in ryegrass, and of the picrlc acid test in type determination of white clover.

In 1929 Gentner recorded the fact that most seedlings of Italian ryegrass and some seedlings of Perennial, when germinated in contact with filter paper, produced a substance which was fluoresce& in Ultra Violet light. His observations attracted the attention of several workers, who immediately either sought a means of exploiting the phenomenon as a diagnostic characteristio, or endeavoured to show the genetical relationship of the factors responsible for fluorescence. In 1930 the work in New Zealand of segregating superior types of perennial had been attended with success, and the possibilities and the value particularly in the commercialisation of superior perennial types, of a simple laboratory method of type diagnosis were very obvious. The possibilities of the U.V. light examination were then investigated and it was shown that a relationship existed between the content of fluorescent reactors presentin. line of seed and its agronomic7 value.

It was apparent that a high measure of corelation existed between the proportion. of fluorescent seedlings and the Agrostologist's type classification, and furthermore, that a constant assooiatiqn of low content with a high degree of persistence and that conversely a high content of reactors was associated with non-psrsistenoe, characterised by poor types of false perennial or Italian.

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The application of the test, therefore,. appeared to be quite Simple, the content of phsture reactors was expressed as a percentage ,of the total number of seedlings and the position of the line of seed fixed accordingly in the perennial scale, which position indioated directly agronomic value.

Later work showed however that the method could not be used to distinguish between regional strains of perennial representative of the Hawke's Bay OF Poverty Bay types and of those saved from old pasture in the Manawatu, Canterbury, and Southland districts. In brief this means that although lines from Hawke's Bay and Canterbury may both shoa an identical proportion of positive reacting seedlings, they represent potentially different types - morphologically and in some respects agronomically. However, although this difference exists, it does not appear to be sufficiently wide to warrant condemnation as perennial types, of those lines of low U.V. test grow-n outside of the Bay districts. Therefore although the U.V. test has failed to provide a means of distinguishing the regional strains within th6 true perennial group, there is more than ample scope for its useful employment. For some years it has been used as a basis of classification as perennial of ryegrass seed samples submitted by the Trade, which service has proved to be of a very real value to seed merchants.

The fact that the content of positive reactors is expressed 'as a percentage is responsible for misinterpretation of the results when obtained by inexperienced persons, who invariably apply them quantitavely - that is, in terms of so much perennial and so much non perennial. Obviously such an interpretation

is misleading, as the percentage may be regarded as nothing more than an index figure, by which the position of seed may be indicated on the perennial scale, and generally is therefore qualitative in its application.

There is however, one.exception to this 'generalisatiod, when the test is allowed a partial qualitative interpretation, and that is with seed which has been produced from regional strains. The proportion of positive reactors is then used as ad indication of the degree of contamination by hybrids or Italian.

The present position in the application of this test is that three distinct services are provided by the Department of Agriculture, one, the testing of samples of Certified seed of the Hawke's Bay type previously approved on plot trial and field inspection, and which is in effect an estimation of the degree of contamination of the are8 producing the seed; two, the testing of officially drawn samples of seed provisionally sealed for the purposes of a decision as to qualification or rejection of the line. from Certified Commercial perennial; and 'three, the testing of privately drawn samples with a system of classification designed to indicate the possible chances of qualification as Commercial Certified. For the two last named services the test results are interpreted qualitatively, qualification. being determined by a fixed maximum allowable percentage of positive reactors.

It is convenient-here to review various hypotheses concerning th6 nature of the fluorescent material and inherited factors responsible for its expression.

Dealing first with the nature of the fluorescent material,. Gentner suggested that an exudation from the seedling rootlets reacted on the paper substratum and effected the hydrolysis of some constituent, of the paper to a dextrin compound which is fluorescent in Ultra Violet light. A chemical study of this reaction would prove of value and of interest. Although the expression of the characteristic is made possible by physical means: it is evident that it is fundamentally bia-chemical, and as most botanical investigators are aware, bio-chemical differentiation is not unusual in morphologically different species and varieties, *and* that characteristics so classified may readily be transmitted to hybrid progeny.

Both Corkill and Lineham and Mercer showed by genetical studies that fluorescence is a simple MENDELian character dominant to non fluorescence. Lineham and Mercer showed further that no genetical linkage existed between fluorescence and the presence of the awn of the flowering glume, nor between fluorescence and the revolute vernation characteristic of Lolium multiflorum. Trumble and Phipps have also shown that there is no linkage between fluorescence and the annual habit.. It is definitely established that Lolium perenne and Lolium multiflorum are fertile and that hybridization, occurs readily between the two species. From the progeny of such hybrids we may in the light of the findings just stated expect to observe some plants in which the morphologic81 characters of Italian ryegrass have become segregated, together with the character of non fluorescence; and other plants showing the morphologic81 characters of perennial ryegrass associated with fluorescence. Such plants are not infrequent and it is reasonable to believe that they take their origin from interspecific hybrids.

No acceptable suggestion has so -far been put forward to render intelligible that where ryegrass is,. preserved for many years without reseeding, @ants bearing the fluorescent character are almost entirely eliminated. A suggestion which it is desired to offer is founded on the presumption that persistency is based on several or many independent genetical factors. Among a hybrid population few plants would occur in which all these factors had by chance become reunited and at the same time associated with the factors for fluorescence. Hence in an old pasture 'the natural elimination of the plants not bearing a full complement of the factors for persistence would at the same time greatly reduce the proportion of plants bearing the factor for fluorescence.

It has been demonstrated that there is no linkage between non fluorescence and any of the morphological characters which distinguish the species Lolium perenne. Neverthelass the possibility of the existence of an association between non fluorescence and some physiological character which under appropriate conditions is of material importance in effecting the selective survival of the plant, cannot altogether be 'disregarded. This association might be of the nature of genetical linkage or possibly physiological dependence for as yet nothing is known of the physiological significance of the chemical substance causing fluorescence. .

In order to indicate the manner in which an'obscure physiological or histological character may determine the incidence of natural selection, reference will be made firstly to the-susceptibility of Lolium perenne to the fungus causing the loss of viability of the seed, and the relative immunity of Lolium multiflorum to this disease; secondly to the differences in susceptibility of different strains of ryegrass to the attacks of the rust fungus, *and* thirdly, the differences in palatability which marks the various strains and result in, selective- grazing- by stoak.

'No evidence to' show that these characters -are associated with fluorescence or non fluorescence can be presented, but it must be emphasised that we cannot ,-dismiss the possibility of the instance of some obscure character which is linked with fluorescence and which is the deciding factor under some conditions in determining the survival or non survival of the plant.,

The established relationship between, the content of fluorescent reactors and agronomic value may now be considered in the light of the hypotheses that have been presented. The suggestion offered by the senior writer in nis first paper on this subject in 1931, that the major cause of degeneration in perennial ryegrass is attributable to hybridization with. *Lolium* multiflorum has 'received support, from later work and is now generally accepted.

The degree of degeneration of a strain of perennial ryegrass is proportionate -to the extent to which hybridization with Italian ryegrass has occurred, Furthermore the percentage of fluorescent reactors is dependent on the same factor. Thus the percentage of fluorescent reactors is a measure of tho agronomic value of the strain despite the fact that fluorescence, owing to the absence of genetical linkage, may in individual plants of a hybrid strain be associated with various

combinations of the characteristics of the two species.

The U.V. test is therefore to be regarded as a gauge of the proportion of inheritance of Italian "blood" - if the term is permissable - in the hybrid strain as a whole.

This hypothesis is advanced in respect of the major group wherein agronomic degeneration is due in greater part to hybridization; there- may exist a minor group wherein agronomic degeneration of perennial would be due to the effects of unfavourable environmental influences, and in consequence the content of fluorescent reactors would nst be relative to agronomic value. The fact that lines of seed produced in New 'Zealand are almost wholly representative of the major hybrid group and rarely of the degenerative group justifies the application of the U.V. test in this country. Elsewhere, particularly in the Northern Hemisphere, much of the seed of commerce appears to be representative of the minor degenerate group.

The second method of plant type diagnosis referred to herein; the picric acid test for white clover, is based upon the fact that certain plants of Trifolium repens are cyanopheric and that a relationship exists between strain and the H.C.N. content of the line as a whole. In 1912 Mirande recorded the presence of a cyanopheric glucoside in white clover and in 1913 Armstrong and Horton reported that plants of Wild White clover contained cyanide and that cultivated' Dutch white was free from it. Later Pethybridge carried out tests concurrently with germination tests, using the picric acid method of determination, with a view to ascertaining whether the test could be used to distinguish between samples of wild white and Dutch white clovers. As, ho&ever, he found that in later plantings a small proportion of the plants from -high cyanopheric testing samples were acyanopheric, and the reverse for low testing . . samples, he concluded that the test was not infallible and: could not be used. His conclusions also suggested that because of the nonconformity of the reaction of individual plants the material used by him was not pure.

In 1933 Mr. B.W. Doak, of the Plant Research Station, Palmerston North, published an account of a chemical method for the determination of type in white clover, which method was based on the hydrocyanic acid content of clover herbage, measured by a distillation method evolved by that investigator.,, As a result of this work Doak concluded that (a) there is a correlation between H.C.N. content and type of white clover.

(b) that the most highly producing and persistent lines are invariably associated with high H.C.N. content; while the poorer short-lived types are low in this respect.

(c) that single plant studies supportthe findings of&her workers, that all the plants of the same line do not contain the same amounts of H.C.N.

The difference between the herbage distillation and the picric acid method-s b-s that in the last named, the test is made with sight day seedlings, and that the H.C.N. content is estimated colorimetrically whereas in the distillation method this content is

expressed as a percentage of H.C.N. in green herbage.

The technique involved in the picrfc acid test is a relatively simple one. Seed is germinated on filter pads in full light and on the eighth day when the exposed cotyledons are a dark green,' fifty seedlings are taken at random and inserted into .a three inch glass test tube and firmly packed at the base of the tube,. Two or three drops of toluene or chloroform are then added which have the effect of anaesthising the seedlings in which condition the enzymes which liberate hydrocyanic acid from the cyanogenetic glucosides become active. A small strip of picrate paper (filter paper immersed in, a solution of picric acid and sodium carbonate and stored only partially dry) is then inserted, and when the tube is stoppered the paper is held and lies along the length of the tube.

Four tubes from each germinated sample are prepared in this manner and then incubated for -48 hours at 30° C .

Upon the completion of this period, the amount of H.C.N. gas liberated is shown by the changes in colour of the originally lemon coloured paper and the tubes are then classified -into groups according to the degree of the colour change from dark orange to the unchanged lemon.. As the greater proportion of the samples were supplied by the Agrostologist we have been able to corelate colour classification with the plot classifications reported by that officer,

Our results conform within reasonable limits to those obtained by the herbage-distillation method and our conclusions arb in accord with those-of Doak, that for New Zealand white clover there: is a corelation between 'cyanopheri-c oontent and plant type. Approximately one thousand tests have been made representing certification trial samples'; trade samples, and a considerable number of check tests for the purposes of the development and refinement of .a consistent reliable technique.

Tests for 460 odd samples representing lots submitted for certification trials, and for which the Agrostologist's final plot classification is available, 'have been completed and summarised.

Covering all plant type classification recognised by the Agrostologist'these results show an agreement of 85%, and on a pass-reject basis for the purposes of certification, an agreement of 94% which must be regarded as being reasonably satisfactory.

The relationship of the cyanide content and combinations of the standard plant types 1, 2, and 3, represented in some samples; is being studied, and the result-s of this work, together with data relative to the genetical status of the factors responsible for the reaction will be presented later in a published paper.