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SEEKING AN IDEAL BALANCE OF MOLECULAR AND PHENOTYPIC CHARACTERISTICS FOR DUS TESTING OF INBRED MAIZE LINES

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SEEKING AN IDEAL BALANCE OF MOLECULAR AND PHENOTYPIC CHARACTERISTICS FOR DUS TESTING OF INBRED MAIZE LINES.

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Introduction

The power of molecular markers to discriminate between varieties is well known and this, together with their independence from environmental influence, discriminative power, potential for greater cost-effectiveness form the basis of the rationale in favor of their adoption in testing for Distinctness, Uniformity and Stability (DUS). In addition, molecular marker data are increasingly becoming a regular component of plant breeding. However, existing (phenotypic) systems also give high levels of discrimination and can function even in the presence of environmental interaction. Existing morphologically based systems define current and historic variety boundaries and are thus still the primary focus towards which breeders aim to create and to describe a new variety (even where molecular data are available). The complexity of how to describe varieties *de novo* is evident and is reflected by the effort expended by the BMT in seeking ways forward.

Looking at the future use of molecular techniques in DUS testing there is a growing consensus that a combination of phenotypic and molecular markers might provide a path forward. If their combined usage is feasible for DUS the question then becomes; which phenotypic characteristics and which molecular markers to use?

Factors influencing this choice include:

- The effect on existing variety boundaries.
- The parallel requirement for uniformity.
- Relative discrimination powers of markers (& “redundancy”)
- Cost.

In an attempt to inform this debate we are in the process of dissecting a large database (consisting of full USA Plant Variety Protection (US PVP) phenotype descriptions for maize inbred lines, plus 400 SSR markers) to determine how the optimum combination of characteristics might be determined.

Materials and Methods

Phenotypic data for 687 maize inbred lines, collected over a period of 7 years, were supplied to NIAB. The data comprise characteristics collected under the US PVP system on a per plant and per plot basis. Within each year plot and plant data were collected from three sites, plant data were collected from 5 plants per site. All lines were represented in at least two of the seven years and a set of 22 lines was present in all years and have been used as controls.

The US PVP system makes use of continuous data and does not have descriptions according to the notes in the UPOV Test Guidelines (document TG/2/7). Continuous data

were converted to UPOV notes by dividing the observed range into an appropriate number of bins.

Not all the characteristics in the UPOV Test Guidelines are collected within the US PVP system and so sub-sets of characteristics were created to allow comparison of data.

The DUST software package was used for data analysis.

In order to examine the level of redundancy in the characteristic set an iterative dissection of the data was conducted in which characteristics were systematically removed from the analysis. At each iteration, the most discriminating characteristic was removed. Dissection continued until the percentage of variety pairs discriminated fell below 92%.

The effect on uniformity was determined by assessing the percentage of varieties exhibiting no uniformity problem in any characteristic. This was determined at each of the iterations described above.

Molecular data were provided on 275 inbred lines (a sub-set of those lines described above). Data from 424 SSR markers were supplied although, on average, 400 markers were reported for any one line (the minimum number of markers for any line was 165, maximum; 423). Data were presented as genotypes with two alleles reported; some lines had residual heterozygosity for some of the markers.

Molecular data were analyzed using PowerMarker, genetic distances between pairs of lines was calculated using the method of Nei (1983).

Summary of Results

Of the 275 inbred maize lines analyzed with SSR markers, 100% of all possible pairs of lines had a genetic distance >0, and thus might be said to be 'distinct' (depending on the definition of D that is adopted). This set of markers contains more than 50% redundancy; over half the markers can be disregarded before the level of discrimination begins to fall.

Within the larger set of 687 inbred lines 99% of all possible pairs could be discriminated (P=0.001) on a one year analysis using phenotypic characteristics. Redundancy of characteristics is lower than for the molecular markers (c. 15%; defined as above).

Within the phenotypic characteristics we found that the more precise the measurement (i.e. continuous data), the greater the discrimination power of the characteristic.

Considering uniformity we discovered that the risk of non-uniformity increased with increasing numbers of characteristics deployed, and that this relationship is largely explained as a probability function. In addition, increased precision of measurement led to increased risk of non-uniformity.

We briefly considered the uniformity of varieties with regard to the molecular data. If only those markers were selected which showed no variation within varieties/lines, then the level of discrimination fell to 66% of all possible pairs. The level of marker uniformity could probably be raised by further quality control in selection of the markers. However, it might be practically unfeasible and unnecessary to require complete uniformity for a large number of markers that are not under the direct selection by breeders.

Preliminary Conclusions

The optimum combined set of molecular markers and phenotypic characteristics will be constrained by:

- the ease of achieving uniformity for each characteristic;
- the need to preserve existing variety boundaries;
- the need to minimize the number of characteristics used (thus minimizing disuniformity risk)
- the cost of implementing and running any revised system.

Short term plan

- Seek advice from BMT on the questions which the BMT would like us to address in the ongoing research.

Long term plan

- Formally determine the current variety (distance) boundaries within the 687 lines.

- Use association genetics to identify sets of molecular markers and phenotypic characteristics giving similar information: this may allow replacement of phenotypic characteristics with groups of markers (Option 1)[*].

- Divide the population to allow the development and testing of model systems to proceed on independent populations.

- Quantify outcomes in terms of levels of discrimination; levels of dis-uniformity encountered and in terms of the level of agreement with existing variety boundaries and relative costs.

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[*] Option 1(a): Use of molecular characteristics which are directly linked to traditional characteristics (gene specific markers) – see documents TC/38/14-CAJ/45/5 andTC/38/14 Add.-CAJ/45/5 Add.)