UPOV

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IDENTIFYING LEVELS OF DIVERSITY AND DEVELOPING MARKERS TO ASSIST IN MANAGING THE DUS REFERENCE COLLECTION OF FIELD BEANS (VICIA FABA)

Document prepared by an expert from the United Kingdom

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The annex to this document contains a copy of a presentation on “Identifying levels of diversity and developing markers to assist in managing the DUS reference collection of field beans (Vicia faba)”, prepared by an expert from the United Kingdom, to be made at the twentieth session of the BMT

[Annex follows]

Identifying levels of diversity and developing markers to assist in managing the DUS reference collection of field beans (*Vicia faba*)

UPOV BMT 20 (September 2021)

Background

- Field beans (*Vicia faba*) are an important crop in the United Kingdom.
  - Small but active breeding community
- TG/8/7 – 23 characteristics
- Effective Grouping characters
  - Wing: melanin spot
  - Plant: growth type
  - Seed: black pigmentation of hilum
- …but limited if the candidates cover the selection
  - Eg in a year where both “absent” and “present” black pigmentation of hilum exist in the candidate varieties then the full reference collection is included in the growing trial.

Marker selection

- Two main approaches have been employed to generate informative SNP markers in *V. faba*, suitable for conducting genetic investigations:

  1. High-throughput next generation sequencing to genotype and fingerprint.
     - Kaur *et al*. 2014, https://doi.org/10.1016/j.plantsci.2013.11.014
     - DArT-Seq uses restriction digestion to reduce genomic complexity that generates thousands of SNP and presence/absence markers
     - Killian *et al*. 2012, https://doi.org/10.1007/978-1-61779-870-2_5

Marker selection (continued)

2. Gene-linked markers by sequencing RNA from target genotypes
   - Webb *et al*. 2016, https://doi.org/10.1111/pbi.12371
   - www.viciatoolbox.org
   - Subset of 200 Kompetitive Allele Specific PCR (KASP) markers were selected using the following criteria:
     - Robustness to amplify in different genetic backgrounds
     - Location on the *V. faba* consensus linkage map
     - Polymorphic Information Content (PIC)
     - Ease of scoring using KASP platform

Materials and methods

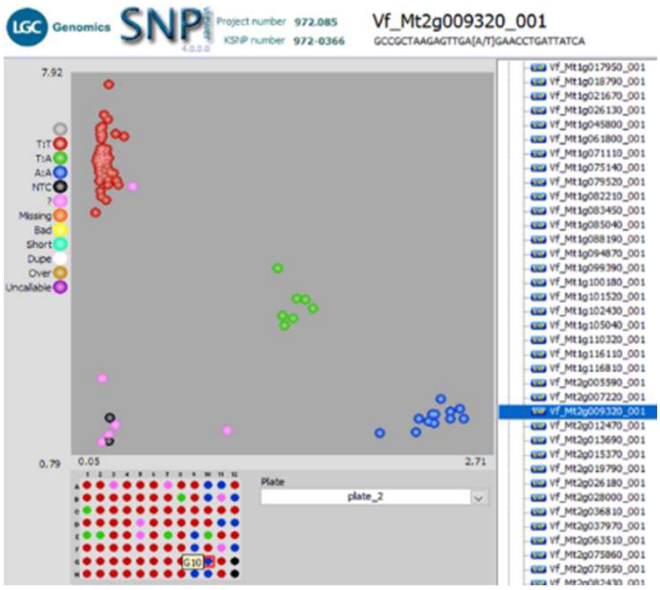
- 88 varieties were selected to provide the greatest range of material, including those that had failed DUS testing
- Twelve individual seeds from the 88 varieties were grown to second true leaf stage.
- DNA extracted from individuals using CTAB method.
- DNA from ten individuals were mixed in equimolar concentrations to obtain a pooled sample.
- Ten individuals from 30 varieties to provide templates for genotyping single plants.

Results

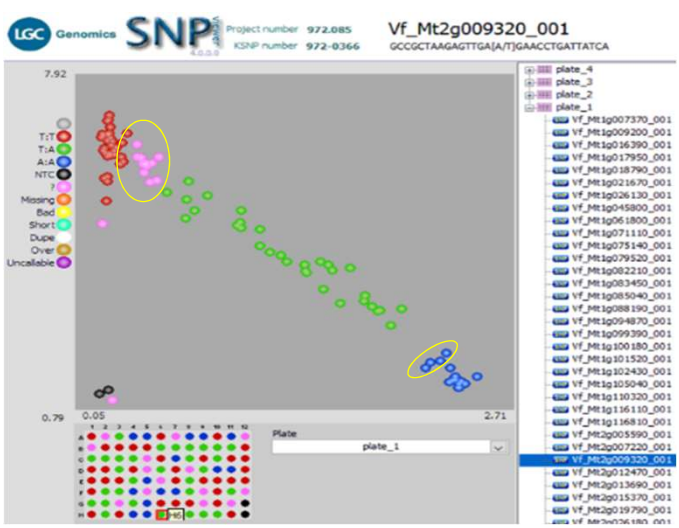
- DArT-Seq data is under analysis, so results are unavailable at present.
- Of the 200 KASP markers:
  - 190 returned genotyping data.
- 18 of those markers were excluded due to
  - inconsistent or low amplification
  - Ambiguous clustering
  - Monomorphic for all pooled calls

Therefore 172 KASP markers were analysed

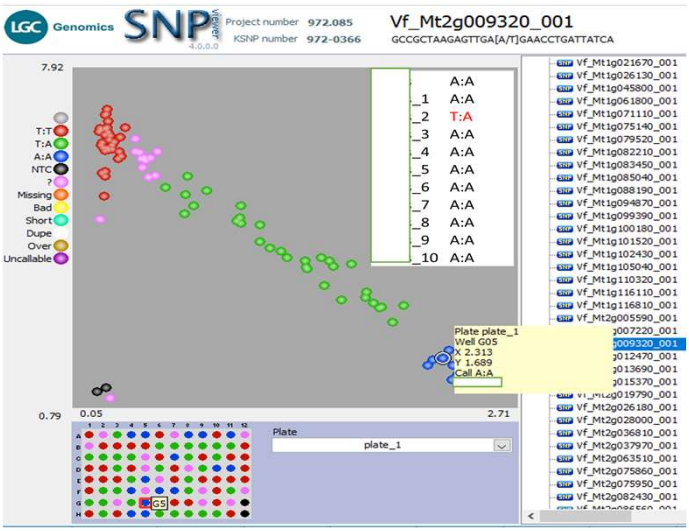
Clustering pattern for samples of individuals



Clustering pattern for pooled samples – heterozygous calls scatter more broadly between homozygous clusters. Some calls at the edges potentially being miscalled or labelled no-calls (yellow ellipses)



Clustering pattern showing genotype of pooled samples of one variety which included incorrect calls



- *V. faba* are partially allogamous and varieties are generally bred using multiple parents
- The level of heterozygosity ranged from 8.1% to 79.5%.
- Heterogeneity is of little concern when dealing with individuals, but in pooled samples heterozygous calls cannot be used effectively for genotyping
  - cannot be interpreted quantitatively
- Despite the high level of heterogeneity however, all varieties could be distinguished using the panel of 172 SNP markers

Genetic diversity

- The Euclidean distance between pairs of varieties ranged between 4.78 (71 polymorphisms) and 15.61 (96 polymorphisms)
- The smallest Manhattan distance (22.86, 22 polymorphisms) was between the same two varieties. The largest was 145.54 representing 90 polymorphic markers.
- Most polymorphisms represent a difference of a single allele.
- Manhattan distances were used to construct a balanced minimum evolution phylogenetic tree
  - Purportedly related varieties and varieties developed by specific breeders grouped together
  - Two distinct clusters were observed

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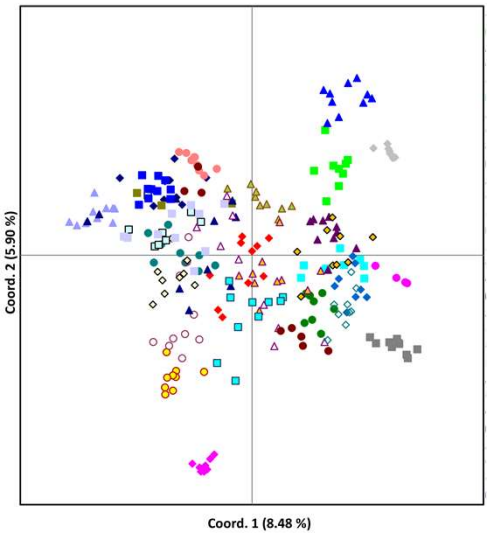
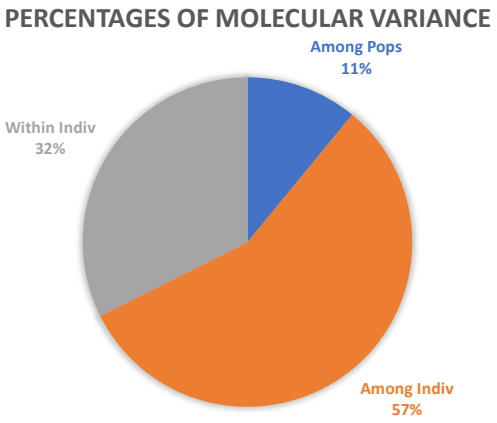
Principal Coordinates (PCoA) codominant allelic distance

- Based on Wright's fixation indices (*Fst*) of each pooled accession to assess for differentiation in the population caused by genetic structure.
- Lowest pairwise *Fst* value (0.03) between △ and ●
- Highest pairwise *Fst* value (0.797) between ◆ and ◆



AMOVA

Fst values were used to implement an Analysis of Molecular Variance (AMOVA) to assess levels of genetic variation within and amongst populations and individuals



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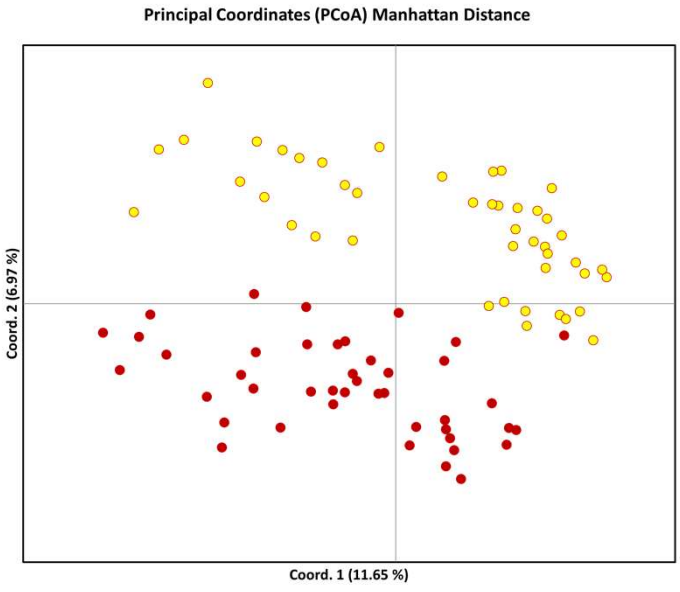
PCoA plot for reference panel based on Manhattan Distances using Fisher's exact test to test the population assignment.

	Spring	Winter
Yellow	44	1
Red	17	26



Summary of outcomes

- Public set of KASP-markers were screened using a subset of varieties from the field bean DUS variety collection.
- Some markers were rejected because of:
  - low quality calls requiring a considerable amount of manual interpretation
  - low information content
- 172 markers successfully discriminated all varieties in the panel – some with only a few polymorphisms different.
- Bulk samples derived from multiple field bean individuals can be used to represent a variety but may mask diversity within a variety and require markers with low proportion of heterozygous calls.
- DArT-Seq information will provide a larger data set to mine
- Development of standardised marker set for DUS testing will require genotyping of individuals of all reference varieties for identification of suitable bulk markers
- Further consideration is required to determine:
  - Appropriate implementation into routine DUS testing
  - Suitable quality thresholds for the marker data
  - Effect on Combined-Over-Years analysis for Distinctness (COYD) and Uniformity (COYU)

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