TRUE-TO-TYPE VERSION 2 - HIGH RESOLUTION GENOTYPING PLATFORM FOR PARENTAL IDENTIFICATION

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850

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SureSawit™ True-to-Type genotyping platform (Version 1) consisting of 24 genome-wide single nucleotide polymorphism (SNP) markers that are stably inherited, was introduced in 2018. The Version 1 genotyping platform proved useful in discriminating individual palms, even in full sib families and allowed assignment of palms to a family when the expected parents are known. In such a guided-analysis, Version 1 was used to validate the legitimacy of controlled crosses across a broad range of genetic backgrounds. The genotyping platform also proved useful for quality control in tissue culture. However, in cases where the parentage information is not available, reduced accuracy was observed in assigning the individuals to a family. To accurately assign each and every individual palm to their biological parents found in seed garden is important especially, in breeding programmes.

Following the feedback received on Version 1 especially its importance and limitations, MPOB has now developed an advanced platform namely, TRUE-TO-TYPE VERSION 2 that is able to assign a seedling or plantlet to the actual palms used as parental lines even when the expected parentage information is not available. The genotyping resolution has been maximised with a very powerful set of 110 SNP markers, 4X more in relative to Version 1. This means that other than for the parental identification, the latest version has all the features originally designed (for Version 1) but with better resolution. The current genotyping platform (TRUE-TO-TYPE VERSION 2) will also aid in assigning legitimate palms identified in a family to its true parental-pair in the seed garden with the condition that all potential parental-pairs are also genotyped. This will ensure that all seedlings of a breeding programme in the nursery can be assigned to their respective families thus, improving overall efficiency of the process. The new genotyping platform as such, allows for better quality control in breeding and tissue culture procedures and accelerates development of new varieties. More importantly, the new platform allows breeders to establish a clear and unambiguous DNA fingerprint database of their seed garden. This is especially useful for seed producers to avoid infringement of their respective company brand name for sale of inferior seeds and seedlings.

NOVELTY OF TECHNOLOGY

TRUE-TO-TYPE VERSION 2 offers

i. a very high-resolution genotyping platform for accurate parental identification for the *Elaeis guineensis* materials;
ii. an improved genotyping resolution in detection of illegitimate palms and tracking of genetic lineage in breeding, germplasm materials and clonally propagated lines; and
iii. to facilitate establishment of a comprehensive DNA fingerprint database of seed gardens to ensure protection against any infringements or disputes under Plant Variety Act.

Development and verification of the TRUE-TO-TYPE VERSION 2 SNP panel

i. Identifying optimal number of SNP markers required for parental identification

From a pool of 2280 carefully selected SNPs showing Mendelian inheritance, 17 panels with increasing number of SNPs: 24 (of Version 1), 50, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 180, 200, 500 and 1000 SNPs were tested in a blinded study of 20 *dura* x *pisifera* (DxP) crosses consisting of 1108 progeny (45-60 progeny/cross) and a pool of 38 candidate parental palms. Analysis was carried out using Cervus 3.0 (Marshall *et al.* 1998) for the assignment of individual palms to their respective parental-pair in each cross (*Figure 1*). The result showed that the 110 SNP panel (Version 2) was the optimal size for high accuracy (99.4 %) parental prediction. Increasing the number of SNP markers beyond 110 provided minimal additional benefit for parental identification.

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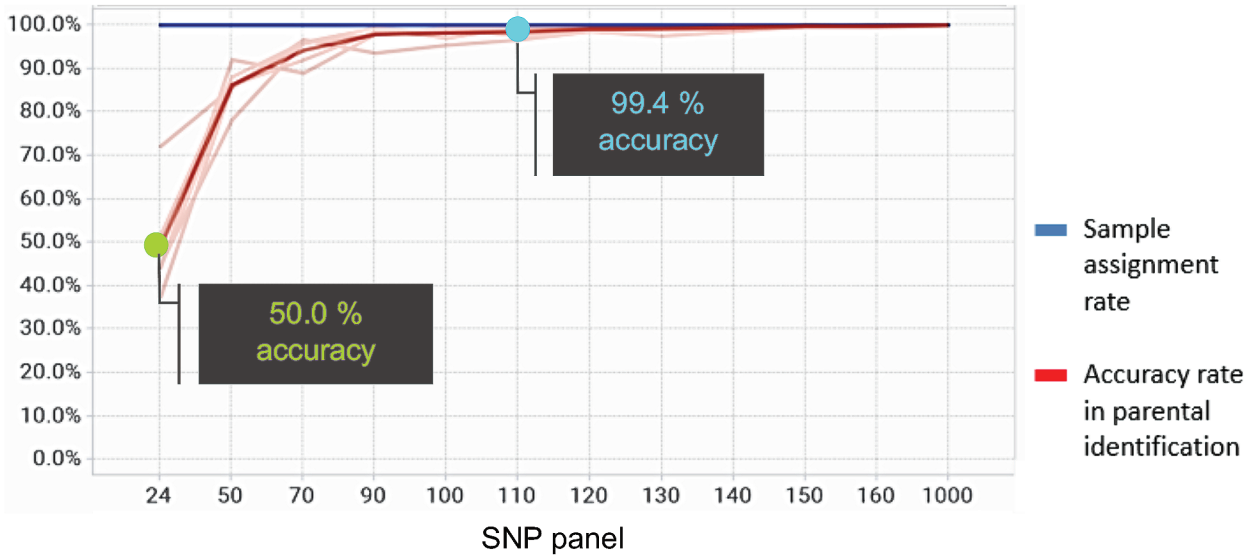

Figure 1. Determining the optimal SNP panel size for TRUE-TO-TYPE VERSION 2 by the blinded analyses. A total of 1108 palms from 20 crosses and 38 parental candidates were genotyped and an attempt was made to assign each genotyped palm to their true parents. The panel with 110 SNPs (indicated by the fluorescent blue dot) was selected as the optimal SNP number for high confident (>95.0 %) parental prediction while the 24-SNP panel of Version 1 (indicated by the fluorescent green dot) only encountered ~50.0 % accuracy.

The selection criterion for 110 SNPs are as follows:

a. distribution across 16 pseudo-chromosomes;
b. have Mendelian segregation; and
c. have high minor allele frequencies across multiple genetic backgrounds.

ii. The TRUE-TO-TYPE VERSION 2 validation

a. Genotyping success rate

Genotyping assay design was attempted for the 110 selected SNPs where, primers could be designed for 109 SNPs. Genotyping was performed for 2951 palms from various genetic backgrounds and 108 SNPs (99.1%) yielded high performance genotype calls as summarised below:

Sample pass rate (%)	: 99.6
Overall genotype calling rate (%)	: 99.0
Average SNP calling rate per sample (%)	: 99.0
Genotype reproducibility across replicates (%)	: 100.0

b. Detection of illegitimacy

Marker data for 177 progeny palms from eight bi-parental crosses (*Table 1*) were subjected to legitimacy check. The result showed that, one to eight progeny palms were identified as contaminant in the first six crosses while both parental palms were not the true parents for the last two crosses (No. 7 and 8). This demonstrates that the TRUE-TO-TYPE VERSION 2 is capable for screening illegitimate palms at both offspring and parent levels, across a wide range of genetic backgrounds.

c. Parental identification efficiency

Marker data for 220 progeny palms from 10 confirmed full-sib crosses (*Figure 2*) were subjected to parental prediction in the blinded triad analysis. The result showed 100.0 % accuracy in all the prediction made across the full-sib progeny palms with different genetic backgrounds, demonstrating that the TRUE-TO-TYPE VERSION 2 is highly reliable for parental identification even when, only a small number of the progeny palms could be genotyped.

BENEFITS TO THE INDUSTRY

The availability of the TRUE-TO-TYPE VERSION 2 genetic test will:

i. allow for high precision DNA fingerprinting and identification of contaminants in breeding populations and controlled crosses;
ii. assist breeders in establishing a comprehensive DNA fingerprint database for all materials in their seed garden to protect their breeding lines especially those used for commercial seed production;
iii. enable breeders to identify the true parental-pairs of all their breeding materials;
iv. improve the management and quality control of the propagated materials in breeding and commercial nurseries as well as in tissue culture laboratory;
v. accelerate the development of new oil palm varieties; and

TABLE 1. DETECTION OF ILLEGITIMATE PALMS IN VARIOUS BI-PARENTAL CROSSES INVOLVING ADVANCED BREEDING LINES (ABL) AND GERMPLASM MATERIALS (GERM)

No	Material	Parental fruit form	Total progeny	Illegitimate progeny
1	ABLxABL	DxD	23	1
2	ABLxABL	DxD	23	1
3	GermxABL	DxP	23	1
4	GermxABL	DxP	22	2
5	GermxABL	DxP	23	5
6	GermxGerm	DxD	17	8
				Illegitimate parent
7	GermxABL	DxT	23	2
8	GermxGerm	DxD	23	2

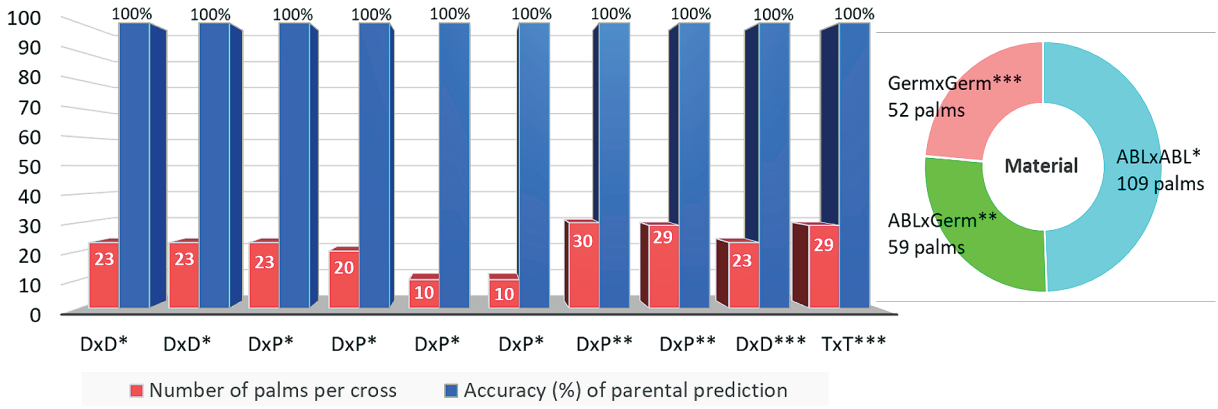

Figure 2. Highly accurate prediction for parental identification by the TRUE-TO-TYPE VERSION 2. The result showed 100.0 % accuracy in all the prediction made across different genetic backgrounds of advanced breeding lines (ABL) and germplasm (Germ).

vi. serve as an important platform for enforcement to track lineage in cases of dispute.

ECONOMIC ANALYSES

The SNP panel has good economic potential especially for an entity already having basic molecular biology facilities. The additional capital cost required is estimated at RM287 700.00. The economic perspectives are feasible with a Net Present Value (NPV) of RM784 611.00 and Internal Rate of Return (IRR) estimated at 68.6%. The expected payback period is three years.

REFERENCES

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