

Title: Methods for Obtaining High-Yielding Oil Palm Plants**Technical Field**

This application relates to methods for obtaining high-yielding plants, and more particularly to methods for obtaining oil palm plants that are high-yielding with respect to producing palm oil.

Background Art

5 The African oil palm *Elaeis guineensis* Jacq. is an important oil-food crop. Oil palm plants are monoecious, i.e. single plants produce both male and female flowers, and are characterized by alternating series of male and female inflorescences. The male inflorescence is made up of numerous spikelets, and can bear well over 100,000 flowers. Oil palm is naturally cross-pollinated by insects and wind. The female inflorescence is a
10 spadix which contains several thousands of flowers borne on thorny spikelets. A bunch carries 500 to 4,000 fruits. The oil palm fruit is a sessile drupe that is spherical to ovoid or elongated in shape and is composed of an exocarp, a mesocarp containing palm oil, and an endocarp surrounding a kernel.

 Oil palm is important both because of its high yield and because of the high
15 quality of its oil. Regarding yield, oil palm is the highest yielding oil-food crop, with a recent average yield of 3.67 tonnes per hectare per year and with best progenies known to produce about 10 tonnes per hectare per year. Oil palm is also the most efficient plant known for harnessing the energy of sunlight for producing oil. Regarding quality, oil palm is cultivated for both palm oil, which is produced in the mesocarp, and palm kernel
20 oil, which is produced in the kernel. Palm oil in particular is a balanced oil, having almost equal proportions of saturated fatty acids ($\approx 55\%$ including 45% of palmitic acid) and unsaturated fatty acids ($\approx 45\%$), and it includes beta carotene. The palm kernel oil is more saturated than the mesocarp oil. Both are low in free fatty acids. The current combined output of palm oil and palm kernel oil is about 50 million tonnes per year, and
25 demand is expected to increase substantially in the future with increasing global population and per capita consumption of oils and fats.

Although oil palm is the highest yielding oil-food crop, current oil palm crops produce well below their theoretical maximum. Moreover, conventional methods for identifying potential high-yielding palms for use in crosses to generate progeny with higher yields require cultivation of palms and measurement of production of oil thereby over the course of many years, which is both time and labor intensive. In addition, conventional breeding techniques for propagation of oil palm for oil production are also time and labor intensive, particularly because the most productive, and thus commercially relevant, palms exhibit a hybrid phenotype which makes propagation thereof by direct hybrid crosses impractical. Accordingly, a need exists to improve oil palm yields through improved methods for obtaining and identifying high-yielding palms.

Transgenic approaches offer potential solutions to the general problem of the need to increase plant yields. For example, transgenic modification of crops such as soy and corn by the introduction of pest resistance genes derived from other organisms is now well known as a means for increasing crop yields. Moreover, methods for increasing plant yields by increasing or generating in the plant activities of particular proteins have also been disclosed, for example by Schön et al., WO 2010/046221. However, transgenic modification of crops raises potential concerns regarding unintended detrimental effects on individuals and ecosystems.

Metabolomics, which encompasses the study of the metabolite complement of a cell, tissue, or organism, also offers potential solutions to the general problem of increasing plant yields. For example, metabolic profiling, corresponding to non-biased screening of biological samples for changes of metabolite levels relative to control samples, provides a means to determine whether and to what extent the presence and levels of particular metabolites may differ between biological samples subjected to particular treatments or at particular points in development or time, as discussed for example in J. Kopka, *Gas Chromatography Mass Spectrometry*, in 57 PLANT METABOLOMICS, 3, 3-4 (K. Saito, R.A. Dixon & L. Willmitzer eds., 2006), and Weckworth, W., *Metabolomics in Systems Biology*, 54 Annual Reviews Plant Biology 669-689 (2003). This approach can be applied for crop improvement by targeted breeding, as discussed for example with respect to breeding tomato for improved stress resistance and nutrition, by Schauer, N. et al., *Metabolic Profiling of Leaves and Fruit of*

Wild Species Tomato: A Survey of the *Solanum lycopersicum* Complex, 56 Journal of Experimental Botany 297-307 (2005). This approach can also be applied toward improving understanding of plant lipid metabolism, as discussed for example with respect to targeted profiling of fatty acids and related metabolites of the model plant *Arabidopsis thaliana* and oilseed rape *Brassica napus* in T.R. Larson & J.A. Graham, *Targeted Profiling of Fatty Acids and Related Metabolites*, in 57 PLANT METABOLOMICS, 211, 211-228 (K. Saito, R.A. Dixon & L. Willmitzer eds., 2006). However, given that plant metabolomes are extraordinarily complex and diverse, encompassing about 200,000 metabolites across the plant kingdom and about 15,000 metabolites per plant species, and that regulation of fatty acid metabolism during seed development is not yet well understood, whether and how metabolomics can be used to improve oil palm yields through improved methods for obtaining and identifying high-yielding oil palm plants remains to be determined.

15 **Disclosure of Invention**

A method is provided for obtaining a high-yielding oil palm plant. The method comprises determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof. The first metabolite is selected from the group consisting of malic acid and pyruvic acid. The second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid. The method also comprises determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The method also comprises selecting progeny of

the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

Also provided is a method for predicting oil yield of a test oil palm plant. The method comprises determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof. The first metabolite is selected from the group consisting of malic acid and pyruvic acid. The second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid. The method also comprises determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The method also comprises predicting the oil yield of the test oil palm plant based on the difference.

Also provided is a kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant. The kit comprises a first reagent for quantitative detection of a first metabolite. The first metabolite is selected from the group consisting of malic acid and pyruvic acid. The kit also comprises a second reagent for quantitative detection of a second metabolite. The second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid. The kit also comprises an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof. The kit also comprises instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the

mesocarp tissue of the fruit of the reference oil palm plant. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant.

Also provided is another method for obtaining a high-yielding oil palm plant. The method comprises determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof. The first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate. The second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate. The method also comprises determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The method also comprises selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

Also provided is another method for predicting oil yield of a test oil palm plant. The method comprises determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof. The first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate. The second metabolite is selected from the group consisting of 3-

phosphoglyceric acid and ribulose-5-phosphate. The method also comprises determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The method also comprises predicting the oil yield of the test oil palm plant based on the difference.

Also provided is another kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant. The kit comprises a first reagent for quantitative detection of a first metabolite. The first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate. The kit also comprises a second reagent for quantitative detection of a second metabolite. The second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate. The kit also comprises an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof. The kit also comprises instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant. The difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at

least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant.

Brief Description of Drawings

FIG. 1 is a graph of results of principal component analysis with respect to metabolites of mesocarp tissue of fruits of five high-yielding oil palm plants and five low-yielding oil palm plants at week 16 post-pollination.

FIG. 2 is a graph of ion chromatograms of spermine as detected in samples prepared from mesocarp tissue of fruits of high-yielding oil palm plants and low-yielding oil palm plants at week 16 post-pollination.

FIG. 3 is a graph of results of Orthogonal Partial Least Square-Discrimination Analysis, i.e. supervised analysis, with respect to metabolites of mesocarp tissue of fruits of five high-yielding oil palm plants and five low-yielding oil palm plants at week 16 post-pollination.

FIG. 4 provides a heat map indicating relative levels of various metabolites of the citric acid cycle metabolic pathway, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 5 is a graph of relative concentration versus time (weeks 12 to 22) post-pollination for various metabolites of the citric acid cycle metabolic pathway, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants.

FIG. 6A provides a heat map indicating relative levels of various metabolites of amino acid metabolic pathways, as determined for mesocarp tissue of fruits of high-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 6B provides a heat map indicating relative levels of various metabolites of amino acid metabolic pathways, as determined for mesocarp tissue of fruits of low-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 7 is a graph of relative concentration versus time (weeks 12 to 22) post-pollination for various metabolites of amino acid metabolic pathways, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants.

FIG. 8 provides a heat map indicating relative levels of various metabolites of purine and pyrimidine metabolic pathways, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 9 is a graph of relative concentration versus time (weeks 12 to 22) post-pollination for various metabolites of purine and pyrimidine metabolic pathways, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants.

FIG. 10 provides a heat map indicating relative levels of various metabolites of oxidative phosphorylation metabolic pathways, i.e. electron transport chain and ATP synthesis, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 11 is a graph of relative concentration versus time (weeks 12 to 22) post-pollination for various metabolites of oxidative phosphorylation metabolic pathways, i.e. electron transport chain and ATP synthesis, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants.

FIG. 12 provides a heat map indicating relative levels of various metabolites of glycolytic pathways, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants, at weeks 12, 14, 16, 18, 20, and 22 post-pollination (increasing darkness of shading indicates increasing relative level of metabolite).

FIG. 13 is a graph of relative concentration versus time (weeks 12 to 22) post-pollination for various metabolites of glycolytic pathways, as determined for mesocarp tissue of fruits of high- versus low-yielding oil palm plants.

Best Mode for Carrying Out the Invention

The application is drawn to methods for obtaining high-yielding oil palm plants, methods for predicting oil yield of test oil palm plants, and kits for obtaining high-

yielding oil palm plants. As disclosed herein, the levels of a first metabolite and a second metabolite in mesocarp tissue of a fruit of an oil palm plant can be used for obtaining a high-yielding oil palm plant and for predicting oil yield of a test oil palm plant. First and second metabolites useful in this regard include (i) malic acid and pyruvic acid, and (ii) 2-oxoglutaric acid, isocitric acid, and citramalic acid, respectively. Additional first and second metabolites useful in this regard include (i) fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and (ii) 3-phosphoglyceric acid and ribulose-5-phosphate, respectively.

Accordingly, the application provides methods for obtaining high-yielding oil palm plants comprising determining the levels of one of each of the above-noted first and second metabolites in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof, determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plants.

Moreover, the application provides methods for predicting oil yield of test oil palm plants comprising determining the levels of one of each of the above-noted first and second metabolites in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof, determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first

metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second
5 metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and predicting the oil yield of the test oil palm plant based on the difference.

In addition, the application provides kits for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant comprising a first reagent for quantitative detection of one of the above-noted first metabolites, a second reagent for
10 quantitative detection of one of the above-noted second metabolites, an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof; and instructions indicating use of the first and second reagents for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also
15 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil
20 palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant.

Without intending that the invention be bound by any theory by which it achieves its advantageous result, it is believed that using the levels of one each of the above-noted
25 first and second metabolites in mesocarp tissue of a fruit of an oil palm plant for obtaining a high-yielding oil palm plant and for predicting oil yield of a test oil palm plant provides greater than expected results than, for example, using the level of only one of either the first metabolite or the second metabolite, by increasing specificity of the processes of obtaining the high-yielding oil palm plant and predicting oil yield of the test
30 oil palm plant.

Definitions

The term "parental oil palm plant," as used herein, means an oil palm plant from which progeny have been generated, are generated, or will be generated during the course of carrying out methods for obtaining a high-yielding oil palm plant as disclosed herein or using kits for obtaining a high-yielding oil palm plant as disclosed herein.

The term "test oil palm plant," as used herein, means an oil palm plant which has been subjected, is subjected, or will be subjected to a step of determining the level of a metabolite in mesocarp tissue of a fruit thereof during the course of carrying out methods for predicting oil yield of the plant as disclosed herein.

The term "reference oil palm plant," as used herein, means an oil palm plant used as a basis for comparison in determining oil palm yield traits. The reference oil palm plant can be, for example, an oil palm plant that produces high, average, or low amounts of palm oil, depending on the context of the particular application. For example, the reference oil palm plant can be an oil palm plant that produces 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 tonnes of palm oil per hectare per year.

The terms "high-yielding," "low-yielding," and "oil yield," as used herein with respect to the methods and kits disclosed herein, refer to yields of palm oil in mesocarp tissue of fruits of oil palm plants.

The singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Method for Obtaining a High-Yielding Oil Palm Plant

As noted above, a method is provided for obtaining a high-yielding oil palm plant. The method comprises: (i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof, (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15%

- higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and (iii)
- 5 selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

Metabolites

- In accordance with this method, the first metabolite is selected from the group
- 10 consisting of malic acid and pyruvic acid, and the second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid. These metabolites are associated with the citric acid cycle, which plays a central role in metabolism by providing biosynthetic precursors and sources of energy for many metabolic pathways. Strikingly, although fatty acid biosynthesis and triacylglycerol
- 15 biosynthesis require biosynthetic precursors and sources of energy, it would not have been apparent whether levels of each of the first metabolites, i.e. malic acid and pyruvic acid, and of each of the second metabolites, i.e. 2-oxoglutaric acid, isocitric acid, and citramalic acid, in mesocarp tissue of a fruit of a parental oil palm plant exhibiting increased production of fatty acids and triacylglycerols would have been higher, the
- 20 same, or lower, than levels in mesocarp tissue of a fruit of a reference oil palm plant, e.g. whether higher levels of the first and second metabolites might drive production of fatty acids and triacylglycerols, or whether the levels may have no effect on, and be unaffected by, production of fatty acids and triacylglycerol, or whether the levels may be reduced because of increased production of fatty acids and triacylglycerols, or some combination
- 25 thereof.

Determining levels of the first and second metabolites

- The level of the above-noted first metabolite and the level of the above-noted second metabolite in mesocarp tissue of a fruit of a parental oil palm plant may be
- 30 determined by any of numerous techniques that are known in the art, as described for example throughout 57 PLANT METABOLOMICS (K. Saito, R.A. Dixon & L. Willmitzer

eds., 2006) and references cited therein. For example, the levels of the first and second metabolites can be determined in a preparation of metabolites from mesocarp tissue, e.g. a crude preparation, a minimally purified preparation, or a highly purified preparation of mesocarp metabolites. The preparation may be obtained from an extract of mesocarp tissue, e.g. a lipid phase extract based on lyophilization followed by isopropanol extraction and recovery of the lipid phase in chloroform, or an aqueous phase extract based on mechanical homogenization followed by recovery of the aqueous phase in water. The preparation may include total metabolites or a subset of total metabolites, e.g. metabolites soluble in polar solvents, metabolites soluble in nonpolar solvents, metabolites amenable to gas chromatographic separation, metabolites amenable to liquid chromatographic separation, or metabolites amenable to capillary electrophoretic separation, among others.

The mesocarp tissue of the fruit of the parental oil palm plant may be obtained and tested at a particular developmental stage of the fruit from which it is derived, e.g. 11 to 21 weeks after pollination thereof, i.e. after the pollination event from which the fruit originated. For example, the mesocarp tissue may be obtained and tested 11-19 weeks, 11-17 weeks, 11-15 weeks, 11-13 weeks, 13-21 weeks, 13-19 weeks, 13-17 weeks, 13-15 weeks, 15-21 weeks, 15-19 weeks, 15-17 weeks, 17-21 weeks, 17-19 weeks, 19-21 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or 21 weeks after pollination thereof.

In some embodiments the step of determining the levels of the first and second metabolites in mesocarp tissue of a fruit of a parental oil palm plant may be carried out by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay.

In some embodiments the step of determining the levels of the first and second metabolite in mesocarp tissue of a fruit of a parental oil palm plant may be carried out by determining (i) the mean level of the first metabolite in mesocarp tissue of at least one fruit of at least one parental oil palm plant, and (ii) the mean level of the second metabolite in mesocarp tissue of the at least one fruit of the at least one parental oil palm

plant. The mean levels of the first and second metabolites may be determined, for example, by determining the levels of the first metabolite in mesocarp tissue of each of a plurality of fruits of at least one parental oil palm plant, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. Alternatively, the mean level of the first metabolite may be determined by determining the levels of the first metabolite in mesocarp tissue of at least one fruit from each of a plurality of parental oil palm plants, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. As another alternative, the mean levels of the first and second metabolites may be determined by determining the levels of the first metabolite in mesocarp tissue of a plurality of fruits from each of a plurality of parental oil palm plants, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. As a further alternative, the mean levels of the first and second metabolites may be determined by combining mesocarp tissue from at least one fruit of at least one parental oil palm plant and then determining the levels of the first and second metabolites in the combined mesocarp tissue to obtain the mean levels of the first and second metabolites. As a still further alternative, the mean levels of the first and second metabolites may be determined by various combinations of the above-noted approaches.

In some embodiments, levels are determined for more than one of each of the first and second metabolites. For example, in some embodiments levels are determined for more than one of the first metabolites, i.e. for both malic acid and pyruvic acid, as well as for at least one of the second metabolites, i.e. 2-oxoglutaric acid, isocitric acid, and/or citramalic acid. Also for example, in some embodiments levels are determined for at least one of the first metabolites, i.e. for at least one of malic acid and pyruvic acid, as well as for more than one of the second metabolites, i.e. for two or more of 2-oxoglutaric acid, isocitric acid, and/or citramalic acid.

In any case, as one of ordinary skill will appreciate, determining the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is necessarily transformative of the mesocarp tissue. The levels cannot be determined, for example, merely based on appearance of the mesocarp tissue. Rather, determination of the levels of the first and second metabolites in the mesocarp tissue requires physical disruption and/or chemical treatment of the mesocarp tissue.

As one of ordinary skill also will appreciate, the levels of the first and second metabolites can be expressed, for example, in absolute quantitative terms, e.g. mass of metabolite per mass of mesocarp tissue, or in relative terms, e.g. intensity of signal of metabolite relative to intensity of signal of standard, or units of area under the curve ("AUC").

Determining difference between levels of metabolite

The step of determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant may be carried out by determining the levels of the first and second metabolites in mesocarp tissue of a fruit of a reference oil palm plant, for example by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay, as described above, and then checking for a difference between those levels and the levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant. Like for the mesocarp tissue of the fruit of the parental oil palm plant, the mesocarp tissue of the fruit of the reference oil palm plant may be obtained and tested at a particular developmental stage of the fruit from which it is derived, e.g. 11 to 21 weeks after pollination thereof, or 11-19 weeks, 11-17 weeks, 11-15 weeks, 11-13 weeks, 13-21 weeks, 13-19 weeks, 13-17 weeks, 13-15 weeks, 15-21 weeks, 15-19 weeks, 15-17 weeks, 17-21 weeks, 17-19 weeks, 19-21 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17

weeks, 18 weeks, 19 weeks, 20 weeks, or 21 weeks after pollination thereof. Like for the levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant, the levels of the first and second metabolites in the mesocarp tissue of the fruit of the reference oil palm plant may be determined as mean levels of the first and
5 second metabolites, respectively.

The levels of the first and second metabolites in mesocarp tissue of a fruit of a reference oil palm plant may be determined before, during, or after determination of the levels of the first and second metabolites in mesocarp tissue of the fruit of the parental oil palm plant.

10 In some embodiments the step of determining whether there is a difference is considered to reveal a biologically and/or statistically significant difference based, for example, on the difference being that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, i.e. the
15 ratio of the level of the metabolite in the mesocarp tissue of the fruit of the parental oil palm plant to the level of the metabolite in the mesocarp tissue of the fruit of the reference oil palm plant is 1.15:1 or higher, and wherein the p value for the comparison based on a T-test is less than 0.1, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the
20 level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, i.e. the ratio of the level of the metabolite in the mesocarp tissue of the fruit of the parental oil palm plant to the level of the metabolite in the mesocarp tissue of the fruit of the reference oil palm plant is 0.95:1 or lower, and wherein the p value for the comparison based on a T-test is also less than 0.1.

25 For example, in some embodiments such a comparison is considered to reveal a biologically and/or statistically significant difference based on the difference being that the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is, for example, at least 25% higher, at least 50% higher, or at least 100% higher, than the level of the metabolite in the mesocarp tissue of the fruit of the reference oil
30 palm plant, wherein the p value for the comparison based on a T-test is, for example, less than 0.05, less than 0.025, or less than 0.01, and wherein the level of the second

metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is, for example, at least 10% lower, at least 25% lower, or at least 50% lower, than the level of the metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, wherein the p value for the comparison based on a T-test is, for example, less than 0.05, less than
5 0.025, or less than 0.01.

In some embodiments the difference relates to a mean level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and/or the reference oil palm plant, and/or a mean level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and/or the reference oil palm plant. For example, in
10 some embodiments the difference is that the levels of the first and second metabolites in the mesocarp tissue of a fruit of the parental oil palm plants are higher than the mean levels of the first and second metabolites, respectively, in the mesocarp tissue of at least one fruit of at least one reference oil palm plant. Alternatively, in some embodiments the difference is that the mean levels of the first and second metabolites in the mesocarp
15 tissue of at least one fruit of at least one parental oil palm plant is higher than the levels of the first and second metabolites, respectively, in the mesocarp tissue of a fruit of the reference oil palm plant. As another alternative, in some embodiments the difference is that the mean levels of the first and second metabolites in the mesocarp tissue of at least one fruit of at least one parental oil palm plant is higher than the mean levels of the first
20 and second metabolites in the mesocarp tissue of at least one fruit of at least one reference oil palm plant. As a further alternative, in some embodiments the difference is that the mean levels of the first and second metabolites in the mesocarp tissue of a plurality of the fruits of a plurality of the parental oil palm plants is higher than the mean levels of the first and second metabolites, respectively, in the mesocarp tissue of a
25 plurality of the fruits of a plurality of the reference oil palm plants. As a still further alternative, the difference may relate to various combinations of the above-noted differences.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil
30 palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the malic

acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to 17 weeks after pollination thereof, and the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the parental
5 oil palm plant 11 to 15 weeks after pollination thereof is 50% to 69% lower than the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil
10 palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to 17 weeks after pollination thereof,
15 and the level of the isocitric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 13% to 35% lower than the level of the isocitric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and
20 the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp
25 tissue of the fruit of the reference oil palm plant 15 to 17 weeks after pollination thereof, and the level of the citramalic acid in the mesocarp tissue of the fruit of the parental oil palm plant 17 to 19 weeks after pollination thereof is 30% to 45% lower than the level of the citramalic acid in the mesocarp tissue of the fruit of the reference oil palm plant 17 to 19 weeks after pollination thereof.

30 In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil

palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the

5 mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof, and the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 50% to 69% lower than the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof.

10 In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after
15 pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof, and the level of the isocitric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 13% to 35% lower than the level of the isocitric acid in the mesocarp tissue of the fruit of the reference oil
20 palm plant 11 to 19 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after
25 pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof, and the level of the citramalic acid in the mesocarp tissue of the fruit of the parental oil palm plant 17 to 19 weeks after pollination thereof is 30% to 45%
30 lower than the level of the citramalic acid in the mesocarp tissue of the fruit of the reference oil palm plant 17 to 19 weeks after pollination thereof.

Selecting progeny

The step of selecting progeny of the parental oil palm plant based on the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant to obtain the high-yielding oil palm plant may be carried out, for example, by choosing a parental oil palm plant for propagation based on the difference and crossing the plant with another oil palm plant, e.g. another oil palm plant also exhibiting the same or a similar difference with respect to one of the above-noted first metabolites, i.e. malic acid and pyruvic acid, and one of the above-noted second metabolites, i.e. 2-oxoglutaric acid, isocitric acid, and citramalic acid, by conventional breeding techniques to obtain progeny corresponding to the high-yielding oil palm plant.

As is well known in the art, fruit type is a monogenic trait in oil palm that is important with respect to breeding and commercial production of palm oil. Specifically, oil palms with either of two distinct fruit types are generally used in breeding and seed production through crossing in order to generate palms for commercial production of oil (also termed "commercial planting materials" or "agricultural production plants"). The first fruit type is *dura* (genotype: sh+ sh+), which is characterized by a thick shell corresponding to 28 to 35% of the fruit by weight, with no ring of black fibres around the kernel of the fruit. For *dura* fruits, the mesocarp to fruit ratio varies from 50 to 60%, with extractable oil content in proportion to bunch weight of 18 to 24%. The second fruit type is *pisifera* (genotype: sh- sh-), which is characterized by the absence of a shell, the vestiges of which are represented by a ring of fibres around a small kernel. Accordingly, for *pisifera* fruits, the mesocarp to fruit ratio is 90 to 100%. The mesocarp oil to bunch ratio is comparable to the *dura* at 16 to 28%. *Pisiferas* are however usually female sterile as the majority of bunches abort at an early stage of development.

Crossing *dura* and *pisifera* gives rise to palms with a third fruit type, the *tenera* (genotype: sh+ sh-). *Tenera* fruits have thin shells of 8 to 10 % of the fruit by weight, corresponding to a thickness of 0.5 to 4 mm, around which is a characteristic ring of black fibres. For *tenera* fruits, the ratio of mesocarp to fruit is comparatively high, in the

range of 60 to 80%. Commercial *tenera* palms generally produce more fruit bunches than *duras*, although mean bunch weight is lower. The extractable oil to bunch ratio is in the range of 20 to 30%, the highest of the three fruit types, and thus *tenera* are typically used as commercial planting materials.

5 *Dura* palm breeding populations used in Southeast Asia include Serdang Avenue, Ulu Remis (which incorporated some Serdang Avenue material), Johor Labis, and Elmina estate, including Deli Dumpy, all of which are derived from Deli *dura*. *Pisifera* breeding populations used for seed production are generally grouped as Yangambi, AVROS, Binga and URT. Other *dura* and *pisifera* populations are used in Africa and
10 South America.

Accordingly, in some embodiments the parental oil palm plant is a *dura* palm selected from the group consisting of Deli *dura*, Serdang Avenue *dura*, Ulu Remis *dura*, Johor Labis *dura*, Elmina estate *dura*, and Deli Dumpy *dura*. Alternatively, in some
15 embodiments the parental oil palm plant is a *pisifera* palm selected from the group consisting of Yangambi *pisifera*, AVROS *pisifera*, Binga *pisifera*, and URT *pisifera*.

Oil palm breeding is primarily aimed at selecting for improved parental *dura* and *pisifera* breeding stock palms for production of superior *tenera* commercial planting materials. Such materials are largely in the form of seeds although the use of tissue culture for propagation of clones continues to be developed. Generally, parental *dura*
20 breeding populations are generated by crossing among selected *dura* palms. Based on the monogenic inheritance of fruit type, 100% of the resulting palms will be *duras*. After several years of yield recording and confirmation of bunch and fruit characteristics, *duras* are selected for breeding based on phenotype. In contrast, *pisifera* palms are normally female sterile and thus breeding populations thereof must be generated by crossing
25 among selected *teneras* or by crossing selected *teneras* with selected *pisiferas*. The *tenera* x *tenera* cross will generate 25% *duras*, 50% *teneras* and 25% *pisiferas*. The *tenera* x *pisifera* cross will generate 50% *teneras* and 50% *pisiferas*. The yield potential of *pisiferas* is then determined indirectly by progeny testing with the elite *duras*, i.e. by crossing *duras* and *pisiferas* to generate *teneras*, and then determining yield phenotypes
30 of the fruits of the *teneras* over time. From this, *pisiferas* with good general combining ability are selected based on the performance of their *tenera* progenies. Intercrossing

among selected parents is also carried out with progenies being carried forward to the next breeding cycle. This allows introduction of new genes into the breeding programme to increase genetic variability. Using this general scheme, priority selection objectives include high oil yield per unit area in terms of high fresh fruit bunch yield and high oil to
5 bunch ratio (thin shell, thick mesocarp), high early yield (precocity), and good oil qualities, among other traits.

Accordingly, in some embodiments, the parental oil palm plant is a *dura* breeding stock plant, the progeny comprises an oil palm plant selected from the group consisting of a *dura* breeding stock plant and a *tenera* agricultural production plant, and the high-
10 yielding oil palm plant is selected from the group consisting of a *dura* breeding stock plant and a *tenera* agricultural production plant. For example, in some embodiments the method is carried out with the purpose of generating improved *dura* breeding stock, in which case the parental *dura* breeding stock plant is crossed with another *dura* breeding stock plant to obtain a high yielding oil palm plant directly among the progeny, which
15 will also be *dura* breeding stock plants. Also for example, in some embodiments the method is carried out with the purpose of generating improved *tenera* agricultural production plants, in which case the parental *dura* breeding stock plant is crossed with a *pisifera* breeding stock plant to obtain a high yielding oil palm plant directly among the progeny, which will be *tenera* agricultural production plants.

20 Alternatively, in some embodiments the parental oil palm plant is a *tenera* breeding stock plant, the progeny comprises an oil palm plant selected from the group consisting of a *tenera* breeding stock plant, a *pisifera* breeding stock plant, and a *tenera* agricultural production plant, and the high-yielding oil palm plant is selected from the group consisting of a *tenera* breeding stock plant and a *tenera* agricultural production
25 plant. For example, in some embodiments the method may be carried out with the purpose of generating improved *tenera* breeding stock, in which case the parental *tenera* breeding stock plant is crossed with another *tenera* breeding stock plant, to obtain a *tenera* high yielding palm plant directly among the progeny, of which 25% will be *dura*, 50% will be *tenera*, and 25% will be *pisifera*. Also for example, in some embodiments
30 the method is carried out with the purpose of generating improved *tenera* agricultural production plants, in which case the parental *tenera* breeding stock plant is crossed with a

pisifera breeding stock plant, to yield progeny corresponding to 50% *tenera* and 50% *pisifera*. The *pisifera* resulting from this cross can in turn be used as *pisifera* breeding stock for generation of *tenera* agricultural production plants.

Progeny plants may be cultivated by conventional approaches, e.g. seedlings may
5 be cultivated in polyethylene bags in pre-nursery and nursery settings, raised for about 12 months, and then planted as seedlings, with progeny that are known or predicted to exhibit high yields chosen for further cultivation.

As will be apparent from the foregoing, the step of selecting progeny of the parental oil palm plant may also be based on differences between levels of more than one
10 of the first metabolites, i.e. malic acid and pyruvic acid, and/or more than one of the second metabolites, i.e. 2-oxoglutaric acid, isocitric acid, and citramalic acid, in the mesocarp tissue of the fruit of the parental oil palm plant and the levels of the metabolites in mesocarp tissue of a fruit of a reference oil palm plant to obtain the high-yielding oil palm plant. For example, in some embodiments the step of selecting is based on
15 differences with respect to two of the first metabolites and one of the second metabolites, or one of the first metabolites and two of the second metabolites. Also for example, in some embodiments the step of selecting is based on differences with respect to combination of one or two of the first metabolites, and one, two, or three of the second metabolites, e.g. each possible combination.

20

Method for obtaining palm oil

A method for obtaining palm oil from a high-yielding oil palm plant is also disclosed. The method includes the steps of obtaining a high-yielding oil palm plant as explained above; and isolating palm oil from a fruit of the high-yielding oil palm plant.
25 The step of isolating palm oil may be carried out by conventional approaches, e.g. harvesting of fruit bunches followed by extraction of oil, within 24 hours, from the fresh and non-wounded fruits thereof.

Method for Predicting Oil Yield of a Test Oil Palm Plant

30 As noted above, also provided is a method for predicting oil yield of a test oil palm plant. The method comprises (i) determining the level of a first metabolite and the

level of a second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof, (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (iii) predicting the oil yield of the test oil palm plant based on the difference.

The metabolites noted above as being useful in the method for obtaining a high-yielding oil palm plant, i.e. a first metabolite selected from the group consisting of malic acid and pyruvic acid, and a second metabolite selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid, are also useful in the method for predicting oil yield of a test oil palm plant.

The step of determining the level of the above-noted first metabolite and the level of the above-noted second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof may also be carried out similarly as described above, e.g. by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay, and/or as a mean level, and/or with respect to more than one of the first and/or second metabolites, except that the levels of the first and second metabolites in the mesocarp tissue of the fruit is determined with respect to a fruit of a test oil palm plant rather than a parental oil palm plant.

The step of determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant may also be carried

out as described above, based for example on the difference being that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, wherein the p value for the comparison based on a T-test is less than 0.1, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, wherein the p value for the comparison based on a T-test is also less than 0.1. Moreover, the difference may be based on any of the specific differences noted above with respect to each of the first and second metabolites, e.g. in some embodiments the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to 17 weeks after pollination thereof, and the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 50% to 69% lower than the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof. In addition, differences may be determined with respect to levels of more than one each of the first and second metabolites.

The predicting step may be carried out, for example, based on the amount of the difference in the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant, and/or based on correlations between levels of the metabolites and yield of palm oil. The predicting step also may be carried out, for example, based on differences with respect to the levels of more than one each of the first and second metabolites.

Kit for Obtaining a High-Yielding Oil Palm Plant

As also noted above, also provided is a kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant. The kit comprises: (i) a first reagent for quantitative detection of a first metabolite; (ii) a second reagent for quantitative
5 detection of a second metabolite; (iii) an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof; and (iv) instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second
10 metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant, wherein the difference is that the level of the first metabolite in the mesocarp
15 tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental or
20 test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The metabolites described above as being useful in the method for obtaining a high-yielding oil palm plant, i.e. a first metabolite selected from the group consisting of malic acid and pyruvic acid, and a
25 second metabolite selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid, are also useful in the kit for obtaining a high-yielding oil palm plant. As one of ordinary skill will appreciate, the first reagent for quantitative detection of the first metabolite and the second reagent for quantitative detection of the second metabolite may be any of numerous reagents useful for specific and quantitative detection of a given
30 metabolite that are known in the art. Moreover, the extract of the mesocarp tissue may be any of numerous extract forms that are known in the art, e.g. a lipid phase extract based on lyophilization followed by isopropanol extraction and recovery of the lipid phase in chloroform, or an aqueous phase extract based on mechanical homogenization followed by recovery of the aqueous phase in water, as discussed above.

Another Method for Obtaining a High-Yielding Oil Palm Plant

As noted above, also provided is another method for obtaining a high-yielding oil palm plant. The method comprises: (i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof; (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and (iii) selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

Metabolites

In accordance with this other method, the first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and the second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate. These metabolites are associated with glycolytic pathways, which convert glucose into glycolytic intermediates and then pyruvate or related compounds, as well as sources of energy. Like for the above-noted metabolites of the citric acid cycle, although fatty acid biosynthesis and triacylglycerol use glycolytic intermediates and require sources of energy, it would not have been apparent whether levels of each of the first metabolites, i.e. fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and of each of the second metabolites, i.e. 3-phosphoglyceric acid and ribulose-5-phosphate, in mesocarp tissue of a fruit of a parental oil palm plant exhibiting increased production of fatty acids and triacylglycerols would have been higher, the

same, or lower, than levels in mesocarp tissue of a fruit of a reference oil palm plant, e.g. whether higher levels of the first and second metabolites might drive production of fatty acids and triacylglycerols, or whether the levels may have no effect on, and be unaffected by, production of fatty acids and triacylglycerol, or whether the levels may be reduced
5 because of increased production of fatty acids and triacylglycerols, or some combination thereof.

Determining level of metabolite

In accordance with this other method, the level of the first metabolite, i.e.
10 fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and the level of the second metabolite, i.e. 3-phosphoglyceric acid and ribulose-5-phosphate, in mesocarp tissue of a fruit of a parental oil palm plant may also be determined as described above. For example, the levels may be determined in a preparation of metabolites from mesocarp tissue, e.g. a crude preparation, a minimally
15 purified preparation, or a highly purified preparation of mesocarp metabolites. The preparation may include total metabolites or a subset of total metabolites. The mesocarp tissue of the fruit of the parental oil palm plant may be obtained and tested at a particular developmental stage of the fruit from which it is derived, e.g. 11 to 21 weeks after pollination thereof, for example, 11-19 weeks, 11-17 weeks, 11-15 weeks, 11-13 weeks,
20 13-21 weeks, 13-19 weeks, 13-17 weeks, 13-15 weeks, 15-21 weeks, 15-19 weeks, 15-17 weeks, 17-21 weeks, 17-19 weeks, 19-21 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or 21 weeks after pollination thereof.

Moreover, in some embodiments the step of determining the level of the first
25 metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a parental oil palm plant may be carried out by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay.

30 In addition, in some embodiments the step of determining the levels of the first and second metabolite in mesocarp tissue of a fruit of a parental oil palm plant may be

carried out by determining (i) the mean level of the first metabolite in mesocarp tissue of at least one fruit of at least one parental oil palm plant, and (ii) the mean level of the second metabolite in mesocarp tissue of the at least one fruit of the at least one parental oil palm plant. The mean levels of the first and second metabolites may be determined, for example, by determining the levels of the first metabolite in mesocarp tissue of each of a plurality of fruits of at least one parental oil palm plant, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. Alternatively, the mean level of the first metabolite may be determined by determining the levels of the first metabolite in mesocarp tissue of at least one fruit from each of a plurality of parental oil palm plants, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. As another alternative, the mean levels of the first and second metabolites may be determined by determining the levels of the first metabolite in mesocarp tissue of a plurality of fruits from each of a plurality of parental oil palm plants, summing the levels, and then dividing the sum by the total number of fruits tested to obtain the mean level of the first metabolite, and applying the same approach with respect to the second metabolite to obtain the mean level of the second metabolite. As a further alternative, the mean levels of the first and second metabolites may be determined by combining mesocarp tissue from at least one fruit of at least one parental oil palm plant and then determining the levels of the first and second metabolites in the combined mesocarp tissue to obtain the mean levels of the first and second metabolites. As a still further alternative, the mean levels of the first and second metabolites may be determined by various combinations of the above-noted approaches.

Furthermore, in some embodiments, levels are determined for more than one of each of the first and second metabolites. For example, in some embodiments levels are determined for more than one of the first metabolites, i.e. fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, as well as for at least one of the second metabolites, i.e. 3-phosphoglyceric acid and ribulose-5-

phosphate. Also for example, in some embodiments levels are determined for at least one of the first metabolites, i.e. for at least one of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, as well as for more than one of the second metabolites, i.e. for both 3-phosphoglyceric acid and
5 ribulose-5-phosphate.

In any case, as noted above, determining the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is necessarily transformative of the mesocarp tissue, and the levels of the metabolites can be expressed in absolute quantitative terms or in relative terms.

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Determining difference between levels of metabolite

In accordance with this other method, the step of determining whether there is a difference between the level of the first metabolite, i.e. fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and
15 the level of the second metabolite, i.e. 3-phosphoglyceric acid and ribulose-5-phosphate, in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant may also be carried out as described above. For example, the step of determining whether there is a difference may be carried out by comparing the
20 respective levels of the metabolites, for example by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay, as described above, and checking for a difference therebetween. Moreover, like for the mesocarp tissue of the
25 fruit of the parental oil palm plant, the mesocarp tissue of the fruit of the reference oil palm plant may be obtained and tested at a particular developmental stage of the fruit from which it is derived, e.g. 11 to 21 weeks after pollination thereof, for example 11-19 weeks, 11-17 weeks, 11-15 weeks, 11-13 weeks, 13-21 weeks, 13-19 weeks, 13-17 weeks, 13-15 weeks, 15-21 weeks, 15-19 weeks, 15-17 weeks, 17-21 weeks, 17-19
30 weeks, 19-21 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or 21 weeks after pollination thereof. Like for the

levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant, the levels of the first and second metabolites in the mesocarp tissue of the fruit of the reference oil palm plant may be determined as mean levels.

The levels of the first and second metabolites in mesocarp tissue of a fruit of a reference oil palm plant may be determined before, during, or after determination of the levels of the first and second metabolites in mesocarp tissue of the fruit of the parental oil palm plant.

In some embodiments the step of determining whether there is a difference is considered to reveal a biologically and/or statistically significant difference based, for example, on the difference being that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and wherein the p value for the comparison based on a T-test is less than 0.1, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and wherein the p value for the comparison based on a T-test is also less than 0.1.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 33% to 82% higher than the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in

mesocarp tissue of the fruit of the reference oil palm plant is that the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 33% to 82% higher than the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof, and the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 30% to 75% higher than the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 30% to 75% higher than the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof, and the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 22% to 190% higher than the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 22% to 190% higher than the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof, and the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to

31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof, and the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 47% to 75% higher than the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

In some embodiments, the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 47% to 75% higher than the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after

pollination thereof, and the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

5 Selecting progeny

In accordance with this other method, the step of selecting progeny of the parental oil palm plant based on the difference between the level of the first metabolite, i.e. a metabolite selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and the level of
10 the second metabolite, i.e. a metabolite selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate, in the mesocarp tissue of the fruit of the parental oil palm plant and the levels of the first and second metabolites in mesocarp tissue of the fruit of the reference oil palm plant to obtain the high-yielding oil palm plant may be carried out as described above. For example, the step of selecting progeny may
15 be carried out by choosing a parental oil palm plant for propagation based on the difference and crossing the plant with another oil palm plant, e.g. another oil palm plant also exhibiting the same or a similar difference with respect to one of the metabolites, by conventional breeding techniques to obtain progeny corresponding to the high-yielding oil palm plant. In some embodiments, the parental oil palm plant is a *dura* breeding stock plant, the progeny comprises an oil palm plant selected from the group consisting of a
20 *dura* breeding stock plant and a *tenera* agricultural production plant, and the high-yielding oil palm plant is selected from the group consisting of a *dura* breeding stock plant and a *tenera* agricultural production plant. Alternatively, in some embodiments the parental oil palm plant is a *tenera* breeding stock plant, the progeny comprises an oil
25 palm plant selected from the group consisting of a *tenera* breeding stock plant, a *pisifera* breeding stock plant, and a *tenera* agricultural production plant, and the high-yielding oil palm plant is selected from the group consisting of a *tenera* breeding stock plant and a *tenera* agricultural production plant. Moreover, progeny plants may be cultivated by conventional approaches and then planted as seedlings, with progeny that are known or
30 predicted to exhibit high yields chosen for further cultivation. In addition, the step of

selecting progeny of the parental oil palm plant may also be based on differences between levels of more than one each of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant and the levels of the first and second metabolites in mesocarp tissue of a fruit of a reference oil palm plant to obtain the high-yielding oil palm plant.

Method for obtaining palm oil

A method for obtaining palm oil from a high-yielding oil palm plant is also disclosed. The method includes the steps of obtaining a high-yielding oil palm plant as explained above; and isolating palm oil from a fruit of the high-yielding oil palm plant. Again, the step of isolating palm oil may be carried out by conventional approaches, e.g. harvesting of fruit bunches followed by extraction of oil, within 24 hours, from the fresh and non-wounded fruits thereof.

Another Method for Predicting Oil Yield of a Test Oil Palm Plant

As noted above, also provided is another method for predicting oil yield of a test oil palm plant. The method comprises: (i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof; (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and (iii) predicting the oil yield of the test oil palm plant based on the difference.

The metabolites noted above as being useful in the other method for obtaining a high-yielding oil palm plant, i.e. a first metabolite selected from the group consisting of

fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and a second metabolite selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate, are also useful in the method for predicting oil yield of a test oil palm plant.

- 5 The step of determining the levels of the first and second metabolites in mesocarp tissue of a fruit of a test oil palm plant may also be carried out similarly as described above, e.g. by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining
10 assay, and a chromatographic assay, and/or as a mean level, and/or with respect to more than one each of the first and second metabolites, except that the level of the metabolite in the mesocarp tissue of the fruit is determined with respect to a fruit of a test oil palm plant rather than a parental oil palm plant.

- The step of determining whether there is a difference between the level of the first
15 metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant may also be carried out as described above. For example, the step of determining whether there is a difference may be carried out by comparing the respective levels of the metabolites.
- 20 Moreover, like for the mesocarp tissue of the fruit of the parental oil palm plant, the mesocarp tissue of the fruit of the reference oil palm plant may be obtained and tested at a particular developmental stage of the fruit from which it is derived, e.g. 11 to 21 weeks after pollination thereof, for example 11-19 weeks, 11-17 weeks, 11-15 weeks, 11-13 weeks, 13-21 weeks, 13-19 weeks, 13-17 weeks, 13-15 weeks, 15-21 weeks, 15-19
25 weeks, 15-17 weeks, 17-21 weeks, 17-19 weeks, 19-21 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or 21 weeks after pollination thereof. Like for the levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant, the levels of the first and second metabolites in the mesocarp tissue of the fruit of the reference oil palm plant may
30 be determined as mean levels. Moreover, the levels of the first and second metabolites in mesocarp tissue of a fruit of a reference oil palm plant may be determined before, during,

or after determination of the levels of the first and second metabolites in mesocarp tissue of the fruit of the parental oil palm plant. In addition, in some embodiments the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and wherein the p value for the comparison based on a T-test is less than 0.1, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and wherein the p value for the comparison based on a T-test is also less than 0.1. Moreover, the difference may be based on any of the specific differences noted above with respect to each specific metabolite, e.g. in some embodiments the difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant is that the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 33% to 82% higher than the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof, and the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

As also described above, the predicting step may be carried out, for example, based on the amount of the difference in the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of the fruit of the reference oil palm plant, and/or based on correlations between levels of the metabolites and yield of palm oil. The predicting step also may be carried out, for example, based on differences with respect to the levels of more than one each of the first and second metabolites.

Another Kit for Obtaining a High-Yielding Oil Palm Plant

As also noted above, also provided is another kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant. The kit comprises: (i) a first reagent for quantitative detection of a first metabolite; (ii) a second reagent for quantitative detection of a second metabolite; (iii) an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof; and (iv) instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant, wherein the difference is that the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. The metabolites described above as being useful in the other method for obtaining a high-yielding oil palm plant, i.e. a first metabolite selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and a second metabolite selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate, are also useful in the kit for obtaining a high-yielding oil palm plant. As noted above, the first reagent for quantitative detection of the first metabolite and the second reagent for quantitative detection of the second metabolite may be any of numerous reagents useful for specific and quantitative detection of a given metabolite that are known in the art. Moreover, the extract of the mesocarp tissue may be any of numerous extract forms that are known in the art, e.g. a lipid phase extract based on lyophilization followed by isopropanol extraction and recovery of the lipid phase in chloroform, or an aqueous phase extract based on mechanical homogenization followed by recovery of the aqueous phase in water, as discussed above.

The following examples are for purposes of illustration and are not intended to limit the scope of the claims.

Example 1

Initial Metabolite Profiling of Mesocarp Tissue

5 Objectives

Objectives included identifying metabolites that are present at different levels in mesocarp tissue of fruits of high-yielding palms versus reference palms at a single time point at the onset of lipid production, for purposes of obtaining high-yielding oil palm plants and predicting palm oil yields of test oil palm plants.

10 Methods

Screening populations:

Two screening populations of oil palm plants, a high-yielding screening population and a low-yielding screening population, each consisting of five individual palm plants, were used. The screening populations were derived from crosses of Serdang Avenue *dura* (at least 75% of Serdang Avenue *dura*) and AVROS *pisifera* (at least 75% of AVROS *pisifera*) to yield *tenera* progeny. More specifically, the high-yielding screening population was derived from a population of oil palm plants that had previously been determined to yield relatively high amounts of palm oil, specifically 10 to 12 tonnes of palm oil per hectare per year, and thus to have a high-yielding phenotype, also termed an HY phenotype. The low-yielding screening population was derived from a population of oil palm plants that had previously been determined to yield relatively low amounts of palm oil, specifically 4 to 7 tonnes of palm oil per hectare per year, and thus to have a low-yielding phenotype, also termed an LY phenotype. Both populations were derived from a Carey Island oil palm plantation. The yield determinations were based on historical oil yield data for each sample.

Collection of oil palm mesocarp tissue:

Fruits were harvested from plants of the high-yielding and low-yielding screening populations at week 16 post-pollination.

Profiling methods:

As a preliminary matter, extracts were profiled in a non-targeted manner using liquid chromatography-mass spectrometry ("LC-MS") to investigate global differences between the populations. Where possible, metabolites were identified by comparing their accurate masses and MS/MS fragmentation data as acquired by a mass spectrometer with those of authentic standards. Subsequently, a group of selected metabolites were profiled in the extracts using capillary electrophoresis-mass spectrometry ("CE-MS") by comparison with data for authentic standards in order to identify specific metabolite markers.

(1) Liquid Chromatography–Mass Spectrometry Method

Extraction for LC-MS profiling:

Mesocarp tissue from each fruit was lyophilized. Approximately 100 mg of each lyophilized mesocarp tissue was weighed into a 15 mL FALCON tube. Then 2 mL of 75% (v/v) isopropanol with 0.01% BHT was added to the tissue. The mixture was heated and shaken in a thermo-mixer for 15 minutes at 750 rpm. A mixture of (i) 0.2 mg/mL phenanthrene dissolved in 2 mL chloroform, (ii) 0.2 mg/mL ribitol dissolved in methanol, and (iii) 1.6 mL water was used for internal standards for lipids and polar metabolites. The mixture was shaken for 30 seconds using a vortex mixer. Incubation was carried out for 30 minutes at 250 rpm and 4° C in a thermo-mixer. Then 4 mL of chloroform and water at a ratio 1:1 was added to the mixture. The mixture was shaken 30 seconds using a vortex mixer. The mixture was then centrifuged at 4° C and 4000 rpm for 10 minutes to separate the lipid layer (in chloroform, at the bottom) and the polar layer (in methanol and water, at the top).

LC-MS profiling:

LC-MS profiling was conducted on the polar (top) layer as follows. LC-MS data were acquired using Accela-LTQ Orbitrap brand instrument (Thermo Fisher, Germany).

Sample analysis was carried out in positive and negative ion modes of detection. The mass scanning range was 100 to 2000 m/z, while capillary temperature was 300° C and sheath gas auxiliary gas flow rates were 35 and 15 arbitrary units (“arb”), respectively. The sweep gas flow rate was set at 1 arb I-spray voltage at 4.5 kb. The resolution was 30,000 at 1 microscan and maximum injection time at 500 ms. The capillary voltage and tube lens were set at 40 V and 80 V, respectively for positive ion modes. Both respective parameters were set at -2.00 V and -47.44 V for negative ion mode. The MS/MS spectra of metabolites were obtained by collision energy ramp at 35 V. Autosampler temperature was set at 10° C with 3.0 µL injection volume. The LC/MS system (controlled by Xcalibur brand software version 2.0, Thermo Fisher Corporation) was run in binary gradient mode. Solvent A was 0.1 % v/v formic acid/water and solvent B was acetonitrile containing 0.1% v/v formic acid. The flow rate was 0.2 mL/min. An Acquity UPLC HSS T3 chromatography column (1.8 µm, 2.1 x 100 mm; Waters, Malaysia) set at 45° C was used for analyses. The gradient was as follows: 1% B (0 - 1.8 min), 10% B (3 min) to 40% B at 20 min and hold for 3 min, 90% B at 26-28 min and 1% B at 29-35 min. The raw data were processed and compared using Sieve version 1.2 (Thermo Fisher, Alpha Analytical, Malaysia) with the frame time and m/z width set at 1.5 min and 0.002, respectively.

(2) Capillary Electrophoresis–Mass Spectrometry Method

Mesocarp samples (55 to 70 mg) were added to 500 µL of methanol including an internal standard (50 µM methionine sulfone and D-camphor-10-sulfonic acid) in tubes and were frozen with liquid nitrogen. The samples were homogenized using a cell breakage machine with beads (TOMY, MS-100R). Chloroform (500 µL) and Milli-Q water (200 µL) were added to the homogenates, mixed thoroughly and centrifuged (2,300 × g, 4° C, 5 min). After centrifugation, the water layer (400 µL) was recovered and then passed through a 5-kDa cut-off filter (MILLIPORE, Ultrafree MC UFC3LCC). The resulting filtrates were desiccated and then dissolved with 50 µL of Milli-Q water. The products were then subjected to preliminary analysis to determined peak shapes and intensities. Based on these results, the products were diluted by 50% or 20% and subjected to CE-TOFMS analysis in cation or anion modes, respectively.

Samples were analyzed using an Agilent CE-TOFMS system (Agilent Technologies) equipped with a fused silica capillary (i.d. 50 μm $\times$ 80 cm). For cation mode, the run buffer was Cation Buffer Solution (p/n: H3301-1001), and the rinse buffer was Cation Buffer Solution (p/n: H3301-1001). Sample injection pressure was 50 mbar (10 sec). The MS parameters were as follows: CE voltage : Positive, 27 kV; MS ionization : ESI Positive; MS capillary voltage : 4,000 V; MS scan range : m/z 50-1,000; Sheath liquid : HMT Sheath Liquid (p/n : H3301-1020). For anion mode, the run buffer was Anion Buffer Solution (p/n: H3302-1021), and the rinse buffer was Anion Buffer Solution (p/n: H3302-1022). Sample injection pressure was 50 mbar (25 sec). The MS parameters were as follows: CE voltage : Positive, 30 kV; MS ionization : ESI Negative; MS capillary voltage : 3,500 V; MS scan range : m/z 50-1,000; Sheath liquid : HMT Sheath Liquid (p/n: H3301-1020).

Data Acquisition and Analysis:

Peaks detected in CE-TOFMS analysis were extracted using automatic integration software (MasterHands ver. 2.1.0.1). Details of peak information including m/z , migration time ("MT"), and area were obtained. Peak area was converted into relative peak area according to the following equation: Relative Peak Area = (Metabolite Peak Area)/(Internal Standard Peak Area $\times$ Sample Amount).

Results and Discussion

Principle component analysis ("PCA") and Orthogonal Partial Least Square-Discrimination Analysis ("OPLS-DA") were used to identify metabolites that reflect the differences between high and low yielding oil palm plants. PCA and OPLS-DA were performed with SPSS ver. 18 and SIMCA-P ver. 12.

The PCA results of metabolome data are shown in FIG. 1. PCA of the five high-yielding palms (designated H3, H5, H7, H8, and H9) and five low-yielding palms (designated L3, L4, L5, L7, and L8) batch shows clear clustering. This method focuses on the variation between specified groups. Accordingly, the difference between the two groups can be comprehensively detected with the areas or relative intensities of the peaks in the chromatograms, as shown for example in FIG. 2 with respect to spermine. OPLS-

DA S-plot supervised analysis can be used to more clearly identify compounds of interest and to determine which compounds are most responsible for the clustering of high and low yielding samples, as shown in FIG. 3.

Thus, for example, spermidine and spermine were identified among various metabolites that are present at significantly higher levels in mesocarp tissues of fruits of high-yielding oil palm plants compared to low-yielding oil palm plants. Specifically, spermidine was identified by comparing MS/MS fragmentation data (peak ID 255, m/z 146.16571(M+H)⁺, time 1.27, based on LC-MS positive mode) with the accurate mass of spermidine. Spermine was also so identified (peak ID 14, m/z 203.22334 (M+H)⁺, time 1.278, also based on LC-MS positive mode). The differences of m/z of spermidine and spermine with their accurate masses were 3.7 ppm and 1.97 ppm, respectively. Moreover, both spermidine, m/z 146.16571(M+H)⁺, and spermine, m/z 203.22334 (M+H)⁺, gave the product ion m/z 129.17, which corresponds to the loss of an amino group and diaminopropane, respectively. In addition, MS fragmentation patterns of spermidine and spermine practically identical to those observed here have been reported previously. See, for example, Ippolito, J.E. et al., An Integrated Functional Genomics and Metabolomics Approach for Defining Poor Prognosis in Human Neuroendocrine Cancers, 102 Proc. Natl. Acad. Sci. USA 9901–9906 (2005).

Based on these analyses, seven metabolites were identified as being present at higher levels in mesocarp tissue of fruits of high-yielding oil palm plants in comparison to low-yielding oil palm plants, specifically spermidine, spermine, ornithine, glutamine, adenine, tyramine, and glycerol-3-phosphate, as indicated in TABLE 1. Moreover, seven metabolites were identified as being present at higher levels in mesocarp tissue of fruits of low-yielding oil palm plants in comparison to high-yielding oil palm plants, specifically tryptophan, 2-phosphoglyceric acid, 3-phosphoglyceric acid, gluconic acid, cytosine, uridine triphosphate (“UTP”), and adenosine triphosphate (“ATP”), as also indicated in TABLE 1.

In accordance with these results, it is believed that palm oil contents of mesocarp tissue of fruits of oil palm plants can be enhanced by use of these identified metabolites as metabolite-based markers to obtain high-yielding oil palm plants and to predict oil yield of test oil palm plants, as described herein.

Example 2Extensive Metabolite Profiling of Mesocarp Tissue

Extensive metabolite profiling was carried out essentially as described above in Example 1 in order to identify additional metabolite-based markers useful for obtaining high-yielding oil palm plants and predicting palm oil yield of test oil palm plants. Specifically, eight palms were selected from each of the high- and low-yielding screening populations as described in Example 1, i.e. a high-yielding population previously determined to yield 10 to 12 tonnes of palm oil per hectare per year, and a low-yielding population previously determined to yield 4 to 7 tonnes of palm oil per hectare per year. Fruits were harvested from plants at weeks 12, 14, 16, 18, 20, and 22 post-pollination to represent fruit development before, during, and after lipid biosynthesis and ripening occurs. Profiling of the two populations was carried out using CE-MS with the same extraction and acquisition parameters described in Example 1. Secondary profiling was carried out using LC-MS (LTQ-Orbitrap as in Example 1, and LC-triple quadrupole MS as described below) as well as GC-MS in order to investigate the instrument-specificity of the results.

Methods

1. CE-MS method: As described in Example 1.
2. LC-MS (LTQ-Orbitrap) method: As described in Example 1.
3. LC-MS (Xevo Triple Quad) method:

Mesocarp sample extraction was performed in the same manner as used for LC-MS (LTQ-Orbitrap) analysis. An Acquity UPLC system coupled to a detector Xevo TQs was used for analytical determination of the targeted metabolites in the mesocarp samples. Chromatography was conducted with an HSS T3 (2.1 x 100 mm, 1.7 μ m) column. The column and samples were maintained at 45° C and 4° C, respectively. The LC mobile phases used were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The flow rate was 0.3 mL/min. The elution gradient was as

follows: initial hold at 95% solvent A; 0 to 3 min linear gradient to 60% solvent A; 3 to 5 min 5% solvent A; 5 to 5.1 min linear gradient to 95% solvent A and hold on to 7 min.

Injection volume was 3 μ L.

The mass spectrometry was operated in both positive and negative mode with multiple reaction monitoring using ESI. The capillary voltage was set at 2.9 kV, and desolvation gas was set at 800 L/hr at temperature of 350° C. The collision gas flow was set at 0.15 mL/min. The MRM settings in the MS/MS function with corresponding cone voltage and collision energy were optimized for each standard compound. Auto dwell times were set for positive mode and negative mode, respectively. Total acquisition duration for both UPLC and MS was set at 15 min. Data were acquired and processed using MassLynx V4.1 and TargetLynx, respectively.

Standard compounds were weighed and dissolved with 5% (v/v) acetonitrile in MilliQ water to make a stock solution with final concentration of 1 mg/mL (1000 ppm). The stock solutions were diluted to 1 ppm as working stock solutions. A mix standard at concentration of 1 ppm was prepared and injected into LC-MS daily to check system sensitivity and reproducibility.

4. GC-MS method:

Mesocarp sample extraction was performed in the same manner as used for LC-MS (LTQ-Orbitrap) analysis. Derivatization of the polar metabolites was then conducted to enable detection using GC-MS. Samples were taken out from storage and placed in a SpeedVac concentrator for 30 minutes prior to derivatization. This was to ensure that all water content is dried off as moisture will hinder the derivatization process. Then 120 μ L of 20 mg/mL methoxyamine hydrochloride in pyridine was added and samples incubated at 60° C for 4 hours. Following this, 120 μ L of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane was added. This was followed with further incubation at 60° C for 1 hour. Derivatized samples were then analyzed using an Agilent 6890N Gas Chromatograph coupled with an Agilent 5973i Mass Detector and 6890 series autosampler. Volatiles were separated on a DB-5ms column, 30 m x 0.25 mm i.d. x 0.25 μ m film thickness. The temperature programme was set at 80° C, held for 3 minutes, followed by a 5° C per minute ramp to 315° C, and

holding at this temperature for 5 minutes. The injection port was splitless at 280° C while column flow was maintained constant at 1 mL/min of Helium gas. The MS source was set at 230° C with a scanning range of m/z 50 to m/z 650. Sample of 1 µL was injected into a programmable injector. The total run time is 55 minutes.

5 Data analysis:

Data were analyzed using the same methods as described in Example 1.

Results and Discussion

Results are shown in FIGS. 4-13. Specifically, FIGS. 4 and 5 provide heat map data and graphs of relative concentration versus time post-pollination for various
10 metabolites of the citric acid cycle metabolic pathway, as determined for mesocarp tissue of fruits of high-yielding palms plants versus mesocarp tissue of fruits of low-yielding palm plants. Similarly, FIGS. 6A-B and 7 provide heat map data and graphs of relative concentration versus time post-pollination for various metabolites of amino acid metabolic pathways, FIGS. 8 and 9 provide heat map data and graphs of relative
15 concentration versus time post-pollination for various metabolites of purine and pyrimidine metabolic pathways, FIGS. 10 and 11 provide heat map data and graphs of relative concentration versus time post-pollination for various metabolites of oxidative phosphorylation metabolic pathways, i.e. electron transport chain and ATP synthesis, and FIGS. 12 and 13 provide heat map data and graphs of relative concentration versus time
20 post-pollination for various metabolites of glycolytic pathways, all as determined for mesocarp tissue of fruits of high-yielding palms plants versus mesocarp tissue of fruits of low-yielding palm plants. For reference, in the heat maps of FIGS. 4, 6A-B, 8, 10, and 12, increasing darkness of shading indicates increasingly higher levels of a given metabolite. Also, in the graphs of FIGS. 5, 7, 9, 11, and 13, time is indicated on the x-
25 axis as weeks post-pollination, data for high-yielding palms are indicated as solid lines, and data for low-yielding palms are indicated as dashed lines.

Results are also summarized in TABLES 2 and 3. Specifically, TABLE 2 lists thirty-six metabolites useful for identifying high-yielding palms and predicting palm oil yields of test palms based on the metabolites being present at higher levels in mesocarp

tissue of fruits of high-yielding oil palm plants in comparison to low-yielding oil palm plants. As can be seen, the results indicate that levels of 2-aminobutyric acid, 2-hydroxyvaleric acid, adenine, asymmetric dimethyl arginine, alanine, alanyl-alanine, arginine, asparagine, cadaverine, cyclohexylamine, cytidine, fructose-1,6-diphosphate, gamma-aminobutyric acid, glucose-1-phosphate, glycerol-3-phosphate, glyoxylic acid, guanosine, homo-L-arginine, glutamine, indole-3-acetaldehyde, lysine, malic acid, myo-inositol-phosphate (i.e. myo-inositol-1-phosphate and/or myo-inositol-3-phosphate), myo-inositol-2-phosphate, nicotinamide, ω -N-methylarginine, octylamine, ophthalmic acid, pyruvic acid, ribose-5-phosphate, serine, spermidine, triethanolamine, uracil, uridine, and beta-alanine were at least 15% higher in mesocarp tissue of fruits of high-yielding palms compared to mesocarp tissue of fruits of low-yielding palms, for one or more weeks from 12 to 22 weeks post-pollination.

Similarly, TABLE 3 lists thirty-five metabolites useful for identifying high-yielding palms and predicting palm oil yields of test palms based on the metabolites being present at lower levels in mesocarp tissue of high-yielding oil palm plants versus low-yielding oil palm plants. As can also be seen, the results indicate that levels of 1-methyladenosine, 2-oxoglutaric acid, 3-phosphoglyceric acid, 4-aminophenylsulfone, 4-guanidinobutyric acid, 6,8-thioctic acid, ascorbic acid, adenosine triphosphate, chelidonic acid, citramalic acid, cytosine, etidronic acid, glucaric acid, glutathione disulfide, imidazole-4-acetic acid, imidazolelactic acid, isocitric acid, methyl sulfate, metronidazole, mucic acid, N6-acetyllysine, N6-methyladenine, N-acetylhistidine, N-methylproline, pantothenic acid, phosphorylcholine, quinic acid, ribulose-5-phosphate, symmetric dimethyl arginine, stachydrine, tartaric acid, tetrahydrouridine, thiamine, tryptophan, and uridine triphosphate were at least 5% lower in mesocarp tissue of fruits of high-yielding palms compared to mesocarp tissue of fruits of low-yielding palms, also for one or more weeks from 12 to 22 weeks post-pollination.

In accordance with these results, it is believed that palm oil contents of mesocarp tissue of fruits of oil palm plants can be enhanced by use of these identified metabolites as metabolite-based markers to obtain high-yielding oil palm plants and to predict oil yield of test oil palm plants, as described herein.

Example 3Use of Metabolites as Markers for Obtaining High-Yielding Oil Palm Plants

A high-yielding oil palm plant can be obtained from a *dura* x *pisifera* cross, e.g. a *dura* x *pisifera* cross that is likely to segregate in terms of palm-oil yield performance or
5 for commercial production of palm-oil producing plants, as described in the following prophetic example.

A *dura* x *pisifera* cross is carried out by standard methods, and progeny are obtained. The progeny are field planted and grown, until fruiting, for at least 18 months. Fruit bunch specimens are taken from each progeny plant for testing at one or more time
10 points from 11 to 21 weeks post-pollination. Testing at multiple time point is preferable to testing at a single time point, as the former provides a higher number of comparative points. Fruitlets are separated immediately from the bunch and ten fruits samples are randomly selected therefrom. Mesocarp tissue is obtained from the ten fruit samples and combined into one mesocarp tissue sample per progeny plant. The resulting mesocarp
15 tissue samples are frozen under liquid nitrogen until further processing.

Upon resuming processing, the mesocarp tissue samples are subjected to extraction as described in Example 1 above. Alternatively, other mild polar extraction conditions known to those skilled in the art can be used, e.g. methanol or ethanol
20 extraction, with or without a proportion of water. Non-polar components are removed by subsequent solvent partitioning with a non-polar solvent, e.g. chloroform, dichloromethane, or ethyl acetate. Alternatively, non-polar components can be removed by solid phase extraction using a non-polar stationary phase, e.g. C18. Also alternatively, non-polar components can be left in the mixture. The samples are then evaporated to dryness.

25 Samples are then prepared for analysis by any of various standard approaches that are well known in the art by reconstituting the samples to the same concentration in a solvent system appropriate for the approach. For example, HPLC and LC-MS can be used for analysis, in which case samples can be reconstituted in methanol-water mixtures or dimethyl sulfoxide, with or without pH modifier added. Alternatively, GC-MS
30 derivatization can be used, in which case samples can be reconstituted as described in Example 1 or by various approaches known in the art. Also alternatively, NMR can be

used, in which case samples can be reconstituted in a deuterated solvent such as methanol- d_4 or dimethyl sulfoxide- d_6 .

The level of a first metabolite and the level of a second metabolite in the samples are then determined. The first metabolite is selected from the group consisting of malic acid and pyruvic acid, and the second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid. Alternatively, the first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate, and the second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate. Thus, for example, the level of malic acid and the level of isocitric acid in the sample can be determined.

It is then determined whether there is a difference between the level of the first metabolite and the level of the second metabolite in the samples in comparison to the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof. The difference can be that the level of the first metabolite in the sample is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and that the level of the second metabolite in the sample is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant. Thus, for example, the difference can be that the level of malic acid is at least 15% higher in the sample in comparison to the level of malic acid in the mesocarp tissue of the fruit of the reference oil palm plant, and that the level of isocitric acid is at least 5% lower in the sample in comparison to the level of isocitric acid in the mesocarp tissue of the fruit of the reference oil palm plant.

Based on these comparisons, the progeny plants are classified in terms of palm oil yields, resulting in a distribution of progeny spanning a spectrum of high-yielding palm plants to low-yielding palm plants. The set of high-yielding progeny are then selected for further breeding stock development. Thus, for example, progeny for which the level of malic acid is at least 15% higher in the sample in comparison to the level of malic acid in the mesocarp tissue of the fruit of the reference oil palm plant and for which the level of isocitric acid is at least 5% lower in the sample in comparison to the level of isocitric acid

in the mesocarp tissue of the fruit of the reference oil palm plant can be classified as having a high oil palm yield. Moreover, such progeny can then be selected for further breeding stock development.

Example 4

5 Use of Metabolites as Markers to Identify Parental Oil Palm Plants Suitable for Producing High-Yielding Progeny

Parental oil palm plants that are suitable for producing high-yielding progeny can be identified by use of metabolites as markers for high oil-palm yields as described in the following prophetic example. Two *dura* x *pisifera* crosses are carried out by standard
10 methods, and progeny are obtained. The two crosses can include one parent in common, e.g. a particular individual *dura* plant is used for the two crosses, or alternatively a particular individual *pisifera* plant is used for the two crosses. Alternatively, the two crosses can include no parent in common. The progeny are field planted and grown, fruit bunch specimens are taken, and mesocarp tissue samples are obtained and tested, as
15 described in Example 3, above. The concentrations of one or more of a first metabolite and one or more of a second metabolite are then determined in the mesocarp tissue samples obtained from the progeny of both crosses, and compared with respect to the concentrations of the first and second metabolites in the mesocarp tissue of the fruit of the reference oil palm plant, also as described in Example 3, above. The parental plants
20 that yield the cross that exhibits relative metabolite concentrations consistent with higher yields of palm oil, e.g. higher levels of malic acid and lower levels of isocitric acid, can then be selected for further crosses, e.g. with other parental plants that also yield crosses that exhibit relative metabolite concentrations consistent with higher yields of palm oil, in order to obtain high-yielding oil palm plants.

25 Example 5

Additional Uses of Metabolites

The metabolites and methods described in the Examples above can also be used for breeding stock improvement (e.g. comparisons or selections), estate management practices, selection of high yielding wild material with other potentially useful traits for

inclusion into breeding programmes, and selection of high yielding ortets for tissue culture production markers, as described in the following prophetic example. Fruit mesocarp samples are collected from groups of palm-oil producing palm plants of similar age and are analyzed as described in Example 3. The groups of palm samples can be
5 selected to compare, for example, two different commercial palm-oil producing populations, a commercial palm-oil producing population and wild material, or palms grown from tissue culture processes. Metabolite markers are analyzed and used to compare the potential palm-oil yields of materials, as described in Example 3. Based on the results, individual palm plants exhibiting high yields are selected and used for
10 breeding stock improvement, for estate management practices, inclusion in breeding programmes, and/or selection of high yielding ortets for tissue culture production.

Example 6

Detection and Comparison of Selected Metabolite Markers

15 A high-yielding oil palm plant can be obtained by use of the methods described in the Examples above, wherein one or more metabolites are detected by, for example, CE-MS, LC-MS, GC-MS, simple HPLC analysis, NMR analysis, or an enzyme-based biosensor, with the choice of detection depending on the metabolite, as described in the following prophetic example.

20

Collection of fruit mesocarp samples:

Oil palm plants are obtained for analysis as described in the Examples above. In this regard, oil palm plants can be classified as high-yielding in comparison to reference plants, with the oil palm plants and the reference plants each being considered either
25 individually or in groups. In order to reduce experimental error, in the case of individual comparisons, i.e. comparisons involving single plants, multiple fruit bunch and fruit samples should be collected and analyzed, e.g. preferably greater than four samples per individual, and more preferably greater than seven samples per individual. In the case of group samples, i.e. comparisons involving multiple plants, multiple plant specimens
30 should also be collected within each group.

Extraction of mesocarp samples:

Extraction can be conducted using any of the various extraction methods described in the Examples above. Alternatively, additional methods known to those skilled in the art can be used, e.g. super critical fluid extraction.

5 Quantitation of selected metabolites:

One or more of each of the first and second metabolites noted in Example 3 can be used to identify parental oil palm plants suitable for producing high-yielding progeny or to classify high-yielding oil palm plants, both relative to reference oil palm plants.

Multiple metabolites can be used in particular to reduce the potential effect of
10 experimental error.

As noted above, the levels of the one or more metabolites in the mesocarp tissue of the fruit of a parental or test oil palm plant can be determined by, for example, CE-MS, LC-MS, GC-MS, simple HPLC analysis, NMR analysis, or an enzyme-based biosensor.

Malic acid and isocitric acid

15 As noted above, parental oil palm plants suitable for producing high-yielding progeny can be identified, and test oil palm plants that are high-yielding can be classified, both relative to reference oil palm plants, based on the level of malic acid in the mesocarp tissue of the fruit of the parental or test oil palm plant being at least 15% higher, e.g.
20 between 43% and 100% higher, than the level of malic acid in the mesocarp tissue of the fruit of the reference oil palm plant, 11 to 21 weeks, e.g. 15 to 17 weeks, after pollination thereof, and the level of isocitric acid in the mesocarp tissue of the fruit of the parental or test oil palm plant being at least 5% lower, e.g. between 13% and 35% lower, than the level of isocitric acid in the mesocarp tissue of the fruit of the reference oil palm plant, 11 to 21 weeks, e.g. 11 to 19 weeks, after pollination thereof.

25 Malic acid and isocitric acid can be detected and quantified efficiently using CE-MS as described above. Malic acid and isocitric acid also can be detected and quantified by simple HPLC analysis and spectrophotometric detection, e.g. using a column of strong cation exchange resin, eluting with dilute mineral acid, and monitoring of the column effluent by UV spectroscopy at 210 nm, as taught for example by Turkelson et al., 50

Analytical Chemistry 1420 (1978). NMR analysis can also be used to efficiently compare malic acid and isocitric acid in biological samples, e.g. based on identification and quantitative analysis of plant metabolites in tissue extracts, as taught for example by Krishnan et al., Metabolite Fingerprinting and Profiling in Plants Using NMR, 56 Journal of Experimental Botany 255-265 (2005). Malic acid also can be detected and quantified by use of an enzyme-based biosensor, e.g. an amperometric biosensor based on malate quinone oxidoreductase, with phenazine methosulfate as an electrochemical mediator, as taught for example by Bucur et al., 21 Biosensors & Bioelectronics 2290-2297 (2006). The combination comparisons of malic acid and isocitric acid can be carried out based on these methods by direct comparison of extract profiles at a consistent concentration.

Industrial Applicability

The methods and kits disclosed herein are useful for obtaining high-yielding oil palms and for predicting oil yields of test oil palm plants, and thus for improving commercial production of palm oil.

TABLE 1. Subset of metabolites of interest identified as present at relatively high levels in mesocarp tissue of fruits of high-yielding oil palm plants ("high in high-yielding plants"), or alternatively low-yielding oil palm plants ("high in low-yielding plants"), at 16 weeks post-pollination.

High in High-Yielding Plants	High in Low-Yielding Plants
Spermine	Tryptophan
Spermidine	2-Phosphoglyceric acid
Ornithine	3-Phosphoglyceric acid
Glutamine	Gluconic acid
Adenine	Cytosine
Tyramine	Uridine triphosphate ("UTP")
Glycerol-3-phosphate	Adenosine triphosphate ("ATP")

TABLE 2. Metabolites useful for identifying high-yielding palms based on the metabolites being present at higher levels in mesocarp tissue of fruits of high-yielding oil palm plants in comparison to low-yielding oil palm plants.

Metabolite*	Week Range	Ratio Range (Low/High)	Max p-value	Percentage Range (% Higher in High-Yielding Plants)	Primary Metabolic Pathway(s)
2-Aminobutyric acid	14	0.58	3.6E-02	47-110%	Misc.
2-Hydroxyvaleric acid	12	0.54	4.8E-02	56-130%	Misc.
Adenine	12-20	0.34-0.43	1.4E-02	110-240%	Nucleosides
Asymmetric dimethyl arginine (ADMA)	14	0.62	2.3E-02	39-92%	Amino acids
Alanine (Ala) [†]	14	0.50	1.1E-02	67-150%	Amino acids
Alanyl-alanine (Ala-Ala)	18	0.64	3.1E-02	35-85%	Amino acids
Arginine (Arg) ^{†, §}	12-14	0.3-0.55	4.8E-03	67-300%	Amino acids
Asparagine (Asn)	14-16	0.34-0.48	1.7E-02	89-240%	Amino acids
Cadaverine	14, 18-20	0.62-0.72	4.8E-02	30-75%	Polyamines
Cyclohexylamine	12-20	0.61-0.69	4.2E-03	35-79%	Misc.

Cytidine	14-20	0.42-0.58	2.4E-02	59-170%	Nucleosides
Fructose-1,6-diphosphate	20	0.65	4.7E-02	33-82%	Glycolytic pathway - Lipid biosynthesis
g-Aminobutyric acid (GABA) [†]	12-16, 20	0.50-0.67	3.5E-02	39-120%	Misc.
Glucose-1-phosphate	20	0.67	3.8E-02	30-75%	Glycolytic pathway - Lipid biosynthesis
Glycerol-3-phosphate	12-18	0.40-0.77	5.5E-02	22-190%	Glycolytic pathway - Lipid biosynthesis
Glyoxylic acid	12-14	0.60-0.66	1.6E-02	41-82%	Misc.
Guanosine	12-20	0.41-0.65	4.8E-02	43-180%	Nucleosides
Homo-L-arginine [†]	12-14	0.3-0.4	5.0E-03	120-300%	Amino acids
Glutamine [†]	12-14	0.18-0.35	4.8E-03	150-670%	Amino acids
Indole-3-acetaldehyde	20	0.72	1.4E-03	22-61%	Misc.
Lysine (Lys) [§]	12-14	0.53-0.66	5.0E-02	41-110%	Amino acids
Malic acid [†]	16	0.60	3.4E-02	43-100%	TCA cycle
myo-Inositol-1-phosphate; myo-Inositol-3-phosphate	12-14	0.51-0.61	1.5E-02	52-120%	Misc.
myo-Inositol-2-	12-14, 18-20	0.56-0.66	4.1E-02	41-96%	Misc.

phosphate					
Nicotinamide	12, 16-20	0.37-0.61	3.7E-02	52-210%	Energy
N ω -Methylarginine	12-14	0.58-0.75	2.6E-02	25-89%	Amino acids
Octylamine	12, 16-20	0.58-0.67	1.5E-03	39-89%	Misc.
Ophthalmic acid	14	0.62	4.7E-02	39-92%	Misc.
Pyruvic acid	12	0.65	4.3E-02	33-82%	Glycolytic pathway - Lipid biosynthesis
Ribose-5-phosphate	12-14	0.62-0.63	3.2E-02	47-75%	Glycolytic pathway - Lipid biosynthesis
Serine (Ser) ^{†,‡,§}	12-14, 20	0.2-0.69	2.7E-02	35-570%	Amino acids
Spermidine	16-18	0.64-0.77	3.5E-02	22-69%	Polyamines
Triethanolamine	14	0.67	2.5E-02	30-75%	Misc.
Uracil	12-16, 20	0.39-0.63	1.3E-02	47-190%	Nucleosides
Uridine	14, 18, 20	0.48-0.69	3.2E-02	35-130%	Nucleosides
Beta alanine (β -Ala)	18	0.65	4.1E-02	33-82%	Amino acids

* All metabolites listed in TABLE 2 were detected by CE-MS.

† Results for alanine, malic acid, and serine were also validated by GC-MS.

‡ Results for arginine, g-aminobutyric acid, homo-L-arginine, glutamine, and serine were also validated by LC-MS (LTQ-Orbitrap).

§ Results for arginine, lysine, and serine were also validated by LC-MS (Xevo Triple Quad).

TABLE 3. Metabolites useful for identifying high-yielding palms based on the metabolites being present at higher levels in mesocarp tissue of fruits of low-yielding oil palm plants in comparison to high-yielding oil palm plants.

Metabolite*	Week Range	Ratio Range (Low/High)	Max p-value	Percentage Range (% Lower in High-Yielding Plants)	Primary Metabolic Pathway(s)
1-Methyladenosine	14-16	1.48-1.68	4.9E-02	28-44%	Nucleosides
2-Oxoglutaric acid	12-14	2.11-3.16	6.3E-03	50-69%	TCA cycle
3-Phosphoglyceric acid [†]	14-16	1.30-1.34	4.7E-02	17-31%	Glycolytic pathway - Lipid biosynthesis
4-Aminophenylsulfone	14	1.31	5.6E-03	9.9-34%	Misc.
4-Guanidinobutyric acid	12,16	1.38-1.41	7.8E-03	22-34%	Misc.
6,8-Thioctic acid	14-20	1.45-1.70	1.5E-02	26-44%	Misc.
Ascorbic acid	14-20	1.38-2.20	3.4E-02	22-57%	Misc.
Adenosine triphosphate (ATP) [†]	12-20	1.60-2.12	9.0E-03	33-55%	Energy
Chelidonic acid	12, 16, 20	1.30-1.69	7.5E-02	17-44%	Misc.
Citramalic acid	18	1.62	4.1E-02	30-45%	Misc.
Cytosine	14-16	1.36-1.58	1.5E-02	21-40%	Nucleosides
Etidronic acid	16	1.40	4.5E-02	17-38%	Misc.
Glucaric acid	14	1.53	1.3E-02	25-42%	Misc.

Glutathione disulfide (GSSG)_divalent	14	1.50	2.0E-02	23-41%	Misc.
Imidazole-4-acetic acid	20	2.10	7.4E-03	47-57%	Misc.
Imidazolelactic acid	18-20	1.52-2.04	3.5E-02	30-53%	Misc.
Isocitric acid	12, 16-18	1.25-1.43	1.7E-02	13-35%	TCA cycle
Methyl sulfate	14, 20	1.58-1.86	3.2E-02	32-49%	Misc.
Metronidazole	14-16	1.40-1.46	1.4E-02	23-36%	Misc.
Mucic acid	14-16	1.44-1.60	4.6E-02	25-41%	Misc.
N6-Acetyllysine	14	1.65	9.7E-03	31-46%	Amino acids
N6-Methyladenine	14	1.79	7.5E-02	37-50%	Amino acids
N-Acetylhistidine	20	1.55	2.1E-02	26-43%	Amino acids
N-Methylproline	20	1.57	1.1E-03	27-44%	Amino acids
Pantothenic acid	14	1.42	2.2E-03	18-38%	Misc.
Phosphorylcholine	14	1.58	3.1E-02	28-44%	Misc.
Quinic acid	14, 16, 20	1.32-1.73	1.6E-02	18-45%	Misc.
Ribulose-5-phosphate	14	1.27	4.8E-02	6.5-32%	Glycolytic pathway - Lipid biosynthesis
symmetric dimethyl arginine (SDMA)	20	1.57	4.5E-02	27-44%	Amino acids
Stachydrine	18	2.58	4.6E-02	58-64%	Misc.

Tartaric acid	20	1.93	2.4E-02	42-53%	Misc.
Tetrahydrouridine	12-14	1.30-1.42	4.2E-02	17-34%	Nucleosides
Thiamine	18	1.64	1.8E-02	31-46%	Misc.
Tryptophan (Trp)	16	3.31	1.8E-02	68-72%	Amino acids
Uridine triphosphate (UTP)	12-20	1.35-2.52	4.9E-02	20-62%	Nucleosides

*All metabolites listed in TABLE 3 were detected by CE-MS.

†Results for 3-phosphoglyceric acid and adenosine triphosphate were also validated by LC-MS (Xevo Triple Quad).

Claims

1. A method for obtaining a high-yielding oil palm plant comprising:

(i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof, wherein (a) the first metabolite is selected from the group consisting of malic acid and pyruvic acid; and (b) the second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid;

(ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and

(iii) selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

2. The method of claim 1, wherein the difference is that:

the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to 17 weeks after pollination thereof; and

the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 50% to 69% lower than the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof.

3. The method of claim 1, wherein the difference is that:

the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to

5 17 weeks after pollination thereof; and

the level of the isocitric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 13% to 35% lower than the level of the isocitric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof.

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4. The method of claim 1, wherein the difference is that:

the level of the malic acid in the mesocarp tissue of the fruit of the parental oil palm plant 15 to 17 weeks after pollination thereof is 43% to 100% higher than the level of the malic acid in the mesocarp tissue of the fruit of the reference oil palm plant 15 to

15 17 weeks after pollination thereof; and

the level of the citramalic acid in the mesocarp tissue of the fruit of the parental oil palm plant 17 to 19 weeks after pollination thereof is 30% to 45% lower than the level of the citramalic acid in the mesocarp tissue of the fruit of the reference oil palm plant 17 to 19 weeks after pollination thereof.

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5. The method of claim 1, wherein the difference is that:

the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13

25 weeks after pollination thereof; and

the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 50% to 69% lower than the level of the 2-oxoglutaric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof.

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6. The method of claim 1, wherein the difference is that:

the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof; and

- 5 the level of the isocitric acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 13% to 35% lower than the level of the isocitric acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof.

- 10 7. The method of claim 1, wherein the difference is that:

the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof; and

- 15 the level of the citramalic acid in the mesocarp tissue of the fruit of the parental oil palm plant 17 to 19 weeks after pollination thereof is 30% to 45% lower than the level of the citramalic acid in the mesocarp tissue of the fruit of the reference oil palm plant 17 to 19 weeks after pollination thereof.

- 20 8. The method of any one of claims 1 to 7, wherein the levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant are determined by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining
25 assay, and a chromatographic assay.

9. The method of any one of claims 1 to 8, wherein:
the parental oil palm plant is a dura breeding stock plant;
the progeny comprises an oil palm plant selected from the group consisting of a
30 dura breeding stock plant and a tenera agricultural production plant; and

the high-yielding oil palm plant is selected from the group consisting of a dura breeding stock plant and a tenera agricultural production plant.

10. The method of any one of claims 1 to 8, wherein:

5 the parental oil palm plant is a tenera breeding stock plant;

the progeny comprises an oil palm plant selected from the group consisting of a tenera breeding stock plant, a pisifera breeding stock plant, and a tenera agricultural production plant; and

10 the high-yielding oil palm plant is selected from the group consisting of a tenera breeding stock plant and a tenera agricultural production plant.

11. The method of any one of the claims 1 to 10, wherein the difference is that (a) the mean level of the first metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the parental oil palm plants is at least 15% higher than the mean level of the first metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the reference oil palm plants, and (b) the mean level of the second metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the parental oil palm plants is at least 5% lower than the mean level of the second metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the reference oil palm plants.

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12. A method for obtaining palm oil from a high-yielding oil palm plant comprising:

(i) obtaining the high-yielding oil palm plant by the method of any one of claims 1 to 11; and

(ii) isolating palm oil from a fruit of the high-yielding oil palm plant.

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13. A method for predicting oil yield of a test oil palm plant comprising:

(i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination thereof, wherein (a) the first metabolite is selected from the group consisting of malic acid and pyruvic acid; and (b) the second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid;

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- (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and
- (iii) predicting the oil yield of the test oil palm plant based on the difference.

14. A kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test oil palm plant comprising:
- (i) a first reagent for quantitative detection of a first metabolite, wherein the first metabolite is selected from the group consisting of malic acid and pyruvic acid;
- (ii) a second reagent for quantitative detection of a second metabolite, wherein the second metabolite is selected from the group consisting of 2-oxoglutaric acid, isocitric acid, and citramalic acid;
- (iii) an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof; and
- (iv) instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 5% lower than the level

of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant.

15. A method for obtaining a high-yielding oil palm plant comprising:

5 (i) determining the level of a first metabolite and the level of a second metabolite in mesocarp tissue of a fruit of a parental oil palm plant 11 to 21 weeks after pollination thereof, wherein (a) the first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate; and (b) the second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate;

10 (ii) determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant and the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks after pollination thereof, wherein the difference is that (a) the level of the first metabolite
15 in the mesocarp tissue of the fruit of the parental oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant; and

20 (iii) selecting progeny of the parental oil palm plant based on the difference to obtain the high-yielding oil palm plant.

16. The method of claim 15, wherein the difference is that:

25 the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 33% to 82% higher than the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof; and

the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than

the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

17. The method of claim 15, wherein the difference is that:

5 the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 33% to 82% higher than the level of the fructose-1,6-diphosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof; and

10 the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

18. The method of claim 15, wherein the difference is that:

15 the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 30% to 75% higher than the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof; and

20 the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

19. The method of claim 15, wherein the difference is that:

25 the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 19 to 21 weeks after pollination thereof is 30% to 75% higher than the level of the glucose-1-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 19 to 21 weeks after pollination thereof; and

the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than

the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

20. The method of claim 15, wherein the difference is that:

5 the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 22% to 190% higher than the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof; and

10 the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

21. The method of claim 15, wherein the difference is that:

15 the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 19 weeks after pollination thereof is 22% to 190% higher than the level of the glycerol-3-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 19 weeks after pollination thereof; and

20 the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

22. The method of claim 15, wherein the difference is that:

25 the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof; and

the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than

the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

23. The method of claim 15, wherein the difference is that:

5 the level of the pyruvic acid in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 13 weeks after pollination thereof is 33% to 82% higher than the level of the pyruvic acid in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 13 weeks after pollination thereof; and

10 the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

24. The method of claim 15, wherein the difference is that:

15 the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 47% to 75% higher than the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof; and

20 the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 17 weeks after pollination thereof is 17% to 31% lower than the level of the 3-phosphoglyceric acid in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 17 weeks after pollination thereof.

25. The method of claim 15, wherein the difference is that:

25 the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 11 to 15 weeks after pollination thereof is 47% to 75% higher than the level of the ribose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 11 to 15 weeks after pollination thereof; and

the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the parental oil palm plant 13 to 15 weeks after pollination thereof is 6.5% to 32% lower than

the level of the ribulose-5-phosphate in the mesocarp tissue of the fruit of the reference oil palm plant 13 to 15 weeks after pollination thereof.

26. The method of any one of claims 15 to 25, wherein the levels of the first and second metabolites in the mesocarp tissue of the fruit of the parental oil palm plant are
5 determined by an analytical technique selected from the group consisting of gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, a biochemical assay, a chemical assay, a staining assay, and a chromatographic assay.

27. The method of any one of claims 15 to 26, wherein:
10 the parental oil palm plant is a dura breeding stock plant;
the progeny comprises an oil palm plant selected from the group consisting of a dura breeding stock plant and a tenera agricultural production plant; and
the high-yielding oil palm plant is selected from the group consisting of a dura breeding stock plant and a tenera agricultural production plant.

15 28. The method of any one of claims 15 to 26, wherein:
the parental oil palm plant is a tenera breeding stock plant;
the progeny comprises an oil palm plant selected from the group consisting of a tenera breeding stock plant, a pisifera breeding stock plant, and a tenera agricultural production plant; and
20 the high-yielding oil palm plant is selected from the group consisting of a tenera breeding stock plant and a tenera agricultural production plant.

29. The method of any one of the claims 15 to 28, wherein the difference is that (a)
the mean level of the first metabolite in the mesocarp tissue of a plurality of the fruits of a
plurality of the parental oil palm plants is at least 15% higher than the mean level of the
25 first metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the reference oil palm plants, and (b) the mean level of the second metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the parental oil palm plants is at least 5%

lower than the mean level of the second metabolite in the mesocarp tissue of a plurality of the fruits of a plurality of the reference oil palm plants.

30. A method for obtaining palm oil from a high-yielding oil palm plant comprising:
(i) obtaining the high-yielding oil palm plant by the method of any one of claims
5 15 to 29; and
(ii) isolating palm oil from a fruit of the high-yielding oil palm plant.
31. A method for predicting oil yield of a test oil palm plant comprising:
(i) determining the level of a first metabolite and the level of a second metabolite
10 in mesocarp tissue of a fruit of a test oil palm plant 11 to 21 weeks after pollination
thereof, wherein (a) the first metabolite is selected from the group consisting of fructose-
1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-
phosphate; and (b) the second metabolite is selected from the group consisting of 3-
phosphoglyceric acid and ribulose-5-phosphate;
(ii) determining whether there is a difference between the level of the first
15 metabolite and the level of the second metabolite in the mesocarp tissue of the fruit of the
test oil palm plant and the level of the first metabolite and the level of the second
metabolite in mesocarp tissue of a fruit of a reference oil palm plant, also 11 to 21 weeks
after pollination thereof, wherein the difference is that (a) the level of the first metabolite
in the mesocarp tissue of the fruit of the test oil palm plant is at least 15% higher than the
20 level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm
plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the
test oil palm plant is at least 5% lower than the level of the second metabolite in the
mesocarp tissue of the fruit of the reference oil palm plant; and
(iii) predicting the oil yield of the test oil palm plant based on the difference.
- 25 32. A kit for obtaining a high-yielding oil palm plant or predicting oil yield of a test
oil palm plant comprising:

- (i) a first reagent for quantitative detection of a first metabolite, wherein the first metabolite is selected from the group consisting of fructose-1,6-diphosphate, glucose-1-phosphate, glycerol-3-phosphate, pyruvic acid, and ribose-5-phosphate;
- (ii) a second reagent for quantitative detection of a second metabolite, wherein the second metabolite is selected from the group consisting of 3-phosphoglyceric acid and ribulose-5-phosphate;
- (iii) an extract of a mesocarp tissue of a fruit of a reference oil palm plant 11 to 21 weeks after pollination thereof; and
- (iv) instructions indicating use of the first reagent and the second reagent for determining whether there is a difference between the level of the first metabolite and the level of the second metabolite in mesocarp tissue of a fruit of the parental or test oil palm plant, also 11 to 21 weeks after pollination thereof, and the level of the first metabolite and the level of the second metabolite in the extract of the mesocarp tissue of the fruit of the reference oil palm plant, wherein the difference is that (a) the level of the first metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 15% higher than the level of the first metabolite in the mesocarp tissue of the fruit of the reference oil palm plant, and (b) the level of the second metabolite in the mesocarp tissue of the fruit of the parental or test oil palm plant is at least 5% lower than the level of the second metabolite in the mesocarp tissue of the fruit of the reference oil palm plant.