FIRST GENERATION EMBRYOGENIC MARKERS (GEM) FOR TISSUE CULTURE AMENITY

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CLONAL AMENITY

Low embryogenesis rates have long been the point of contention for large-scale ramet production ventures. This is further exacerbated by the fact that tissue culture of the oil palm to date is still a random process. This implies that laboratories will have to practise 'basket sampling' [Wallenius (1973) as cited by Kelly and Cumberland (1985)] to meet the demand for clonal plantlets. Ong-Abdullah *et al.* (2005) made a conservative estimate that tissue culture laboratories will operate at a loss of approximately RM 80 000 per year, a value that is expected to escalate, if and when abnormalities set in.

Specific gene expression patterns have been successfully used in classifying and predicting the outcomes of certain conditions. Biomarkers are molecular indicators that are highly predictive of biological processes and their development requires several stages that are depicted in the pipeline (*Figure 1*; Kushairi *et al.*, 2006).

Over the years, we have collectively isolated, characterized and where possible validated some of these biomarkers (*Figure 2*; Low *et al.*, 2006; Ong-Abdullah and Ooi, 2007). These biomarkers representing our first generation embryogenic markers (GEM) will pave the way for the production of a more robust diagnostic tool for clonal amenity in future which will make use of oil palm whole genome data.

VALIDATION STAGE

As mentioned, some markers have undergone validation, and from our studies, there is an indication that some markers can be used as early as on one-day-old explant cultures while some others can be used on 12-week-old explant cultures. The time-point of the explant culture for use in the diagnostic test varies from agency to agency (*Table 1*). Some possible reasons include the different genotypes used by the different agencies, or the different culture media/culturing conditions. Another possible reason is that the number of ortets used in our analysis was limited.

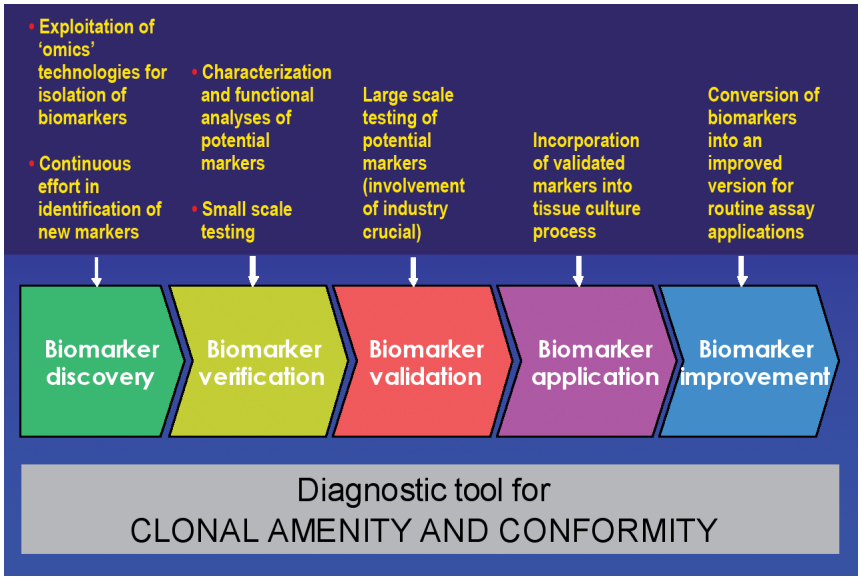

Figure 1. Biomarker development pipeline.

Source: Kushairi *et al.* (2006).

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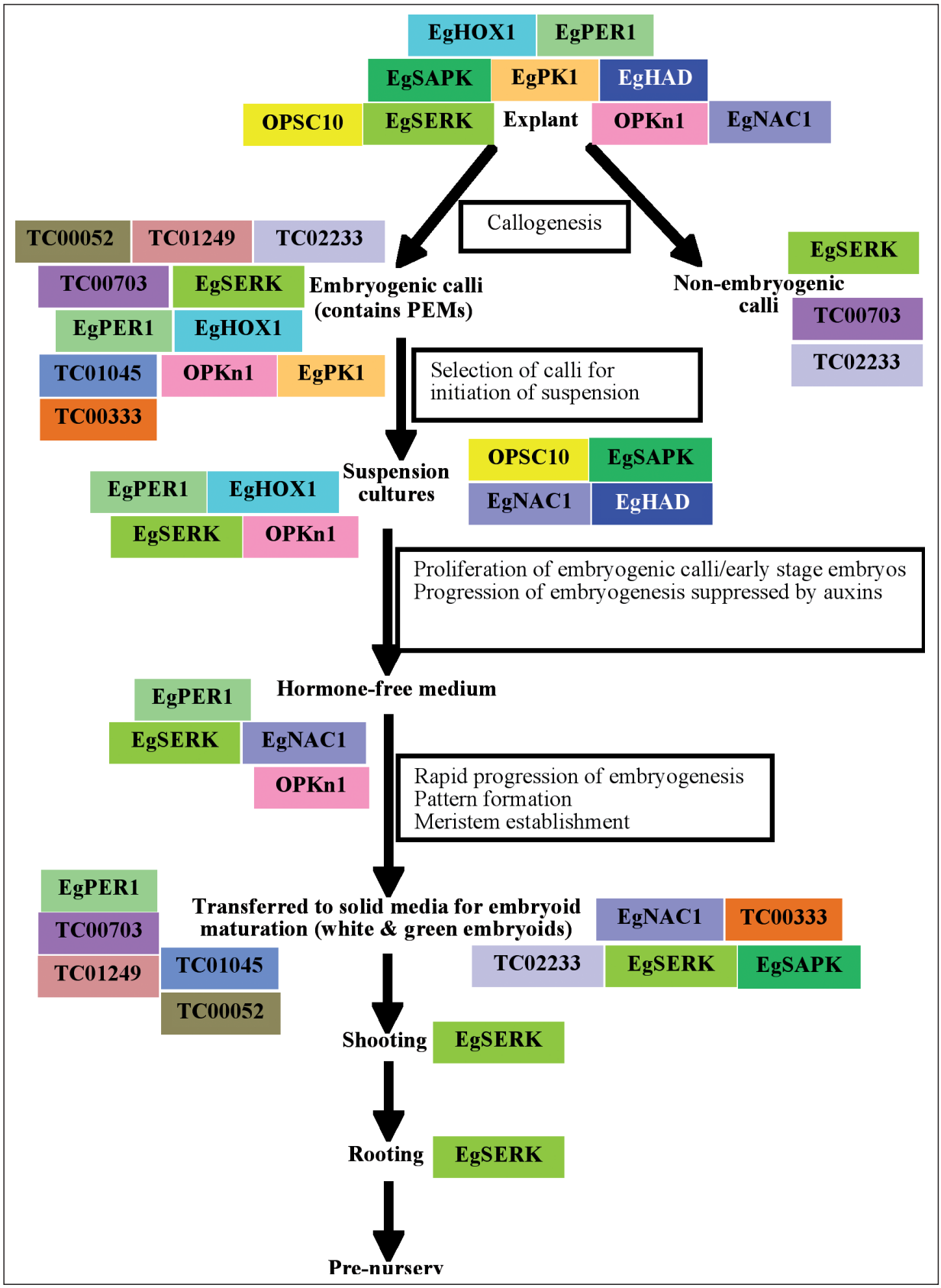


Figure 2. Schematic representation of the expression profiles of EgPER1, EgHOX1, EgPK1, EgNAC1, OPSC10, OPKN1, EgSERK, EgSAPK, EgHAD, TC00703, TC01249, TC00052, TC00333, TC01045 and TC02233 in clonal amenity (PEMs, proembryogenic masses).

Therefore, we highly recommend that agencies test these markers initially on several time-points of explants from a few ortets to determine the appropriate sampling stage for further diagnostic tests applicable to their process. As part of our validation programme to further improve the utilization of these markers, we would like to propose a collaborative effort approach.

TABLE 1. SUGGESTED TIME-POINTS FOR DIFFERENT AGENCIES ON EXPLANT SCREENING USING DIFFERENT CANDIDATE MARKERS TO PREDICT EMBRYOGENESIS

Candidate markers	Agency		
	A	B	C
EgHOX1	1d	1m/5m	1d/3m
EgPER1	1m	5m	1d
EgSERK	1d	1m	nc
EgPK1	1m	1d	Nc
OPKN1	1m	7d	2m
EgNAC1	nc	4m	nc
OPSC10	1d/1m	7d/5m	1d
EgSAPK	1m	7d	nc
EgHAD	1m	nc	1d

Note: 1d: 1-day-old explant culture; 7d: 7-day-old; 1m: 1-month-old; 2m: 2-month-old; 3m: 3-month-old; 4m: 4-month-old; 5m: 5-month-old explant cultures.

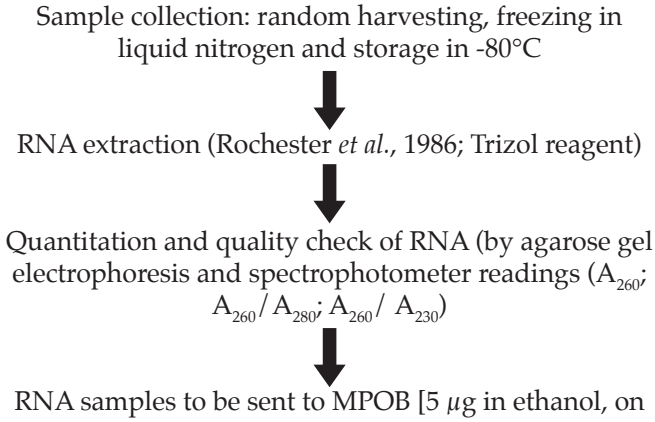
nc: no correlation to embryogenesis observed.

PROPOSED COLLABORATION

All markers should be tested on new and a wide range of samples to determine the suitability of each marker towards the samples tested based on their genetic background and culturing conditions. The samples to be tested will involve explants derived from at least 20 ortets, which are sampled at different stages of explant culture. The sampled explant stages are to include:

- Uninoculated
- 1-day-old
- 7-day-old
- 4-week-old
- 8-week-old
- 12-week-old
- 16-week-old
- 20-week-old

In this collaboration, we would require the interested party/client to prepare the samples prior to sending them to MPOB for further downstream procedures as shown in the following.



At MPOB, the RNA submitted will undergo in-house quality checks before further processing. Reverse transcription will be performed and real time PCR will be conducted. The results will be tabulated to determine which stage and which marker can be used for the set of new samples. A report will be generated and sent to the collaborator.

Alternatively, the collaborator can also choose to continue with downstream procedures if:

- a real time PCR machine is available; and
- the collaborator prefers to continue the assays with normal PCR utilizing specific primers. However, as a note of caution, this will be less sensitive than quantitative results from a real time PCR.

In addition, as a collaborator, MPOB would appreciate receiving feedback in terms of the expression data and corresponding tissue culture performance of the ortets tested. The information will contribute to the validation process of these markers.

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