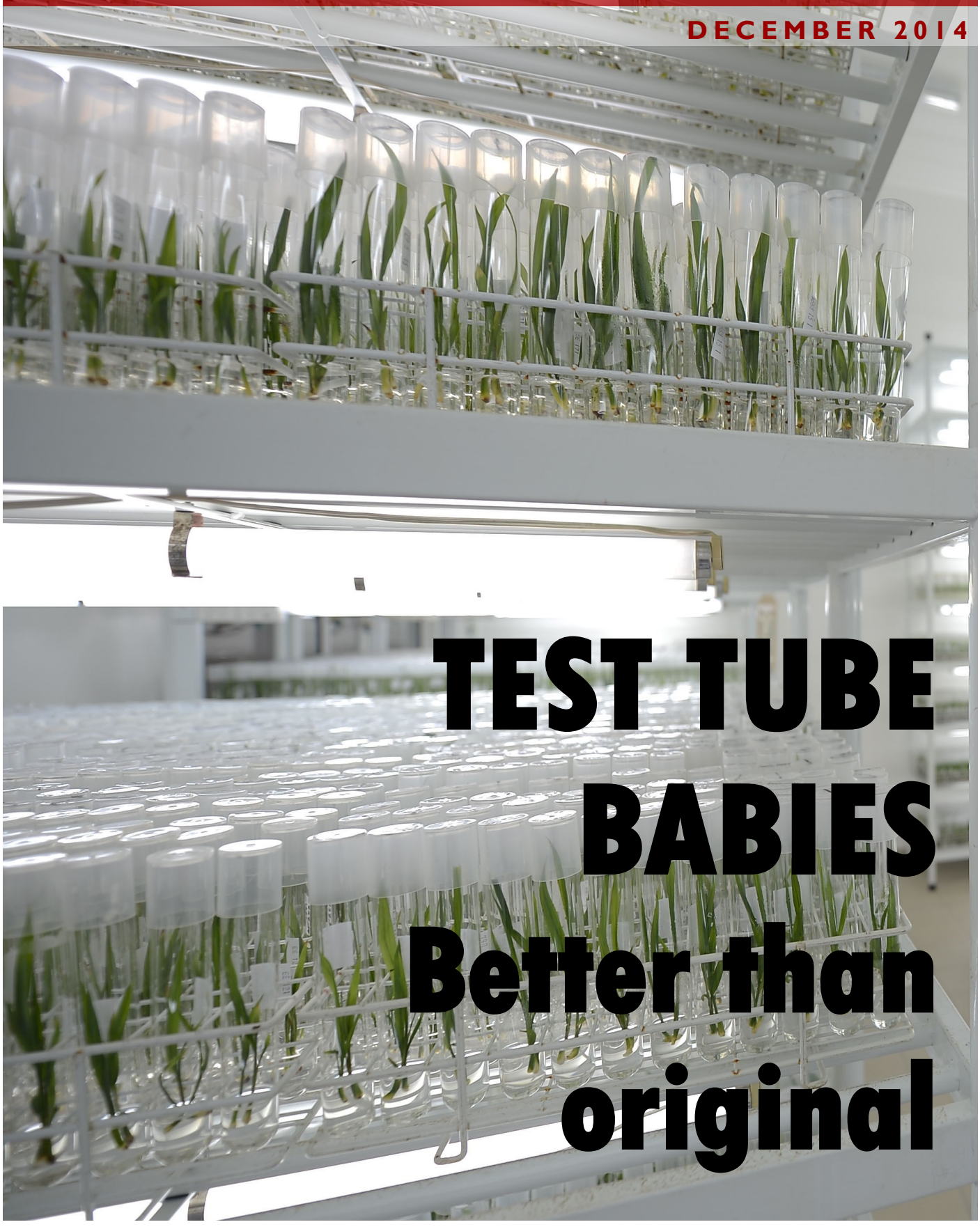
AAR NEWS

DECEMBER 2014



TEST TUBE BABIES

Better than original

INSIDE

MESSAGE FROM THE EDITOR

*"Test tube babies - better than original". This title was chosen as this issue provides highlights on the tissue culture plants which are produced through an unnatural way by manipulating the cell *totipotency to regenerate the whole plant. Well, a hundred years ago, who would have thought that these tiny little tubes can grow into something beneficial for mankind? Thanks to Jones who have pioneered the tissue culture technique for oil palm, we can now enjoy the endless benefits of cloning. "Better than original" is chosen as we can assure the quality of our tissue culture plants which gives higher yield than those raised from seeds. The activities carried out by AAR Sports Club and an introduction of our new recruits are also provided in this issue.*

Happy Reading! :)

-Ilham A.A.-

**totipotency = ability of cells to form all the cell types in a body*

EDITORIAL TEAM

Editor: Ilham Amanina Ahmad

Team Members:

Puspita Demitria, Muhammad Ezwan, Melody Malden, Tan Swee Sian and Cheah Li Wen.

"To be internationally recognized as the Premier Centre for Research and Development offering Excellent Products and Services in Tropical Plantation Tree Crops"

GENOTYPE EFFECT ON OIL PALM TISSUE CULTURE *CALLOGENESIS AND *EMBRYOGENESIS

Choo CN, Wong CK, Nur Akilla MR, Ee CC, Ilham AA, and Tan CC

Edited version of Oral Presentation Paper by Choo CN at the International Oil Palm Conference (IOPC) 2014 Bali, Indonesia

ABSTRACT

4 to 15 ramets per clone from 12 clones were recloned in order to evaluate the genotype effect on callogenesis (CI) and embryogenesis (CD). The CI percentages per clone ranged from 18.1% - 38.5% with a mean of 29.2%, while the CD percentages ranged from 1.9% - 9.9% with a mean of 5.2%. Differences in CI and CD observed among clones were statistically significant at 5% significance level. Each clonal ortet was subjected to two proprietary protocols, different only in the quantity of a plant growth regulator. The CI percentages between the two protocols did not differ significantly with means of 30.0% and 28.4%. Similarly, the CD percentages were comparable with means of 5.4% and 5.1%.

Note:

**Callogenesis = The wounding effect where the cells tend to unorganise themselves into calli with the presence of plant growth regulators*

**Embryogenesis = The developmental process where the unorganized cell differentiated and developed into organ and finally generated into a whole plant*

**Totipotency = The ability of a single cell to develop into a whole plant*

INTRODUCTION

The success of oil palm cloning via tissue culture was first reported by Jones (1974) and Rabechault *et al.* (1976) by modifying Murashige and Skoog (1962) culture medium. Today, 30 years later from the first report, the process in cloning oil palm still follows similar steps as those first developed in the 70's.

Despite a number of successful commercial oil palm tissue culture laboratories were established in Malaysia and Indonesia, limited published information on oil palm tissue culture were available, which is not difficult to comprehend. The reasons could be 1) Commercial application of oil palm tissue culture in producing *tenera* oil palm clones, 2) The rather long and tedious tissue culture process for oil palm, 3) The long period of maturity from nursery to a stable yielding palm, and 4) The dampened spirit of the researchers caused by the sporadic undesirable somaclonal variation, namely mantling of the fruits. Also, due to the said factors, information on recloning of oil palm is even scarcer.

Hence, in this manuscript, although some of the conclusions are coherent to the rather common perspectives, the data oriented investigation shall further enrich our knowledge about oil palm tissue culture specifically on the study of the genotype effect on callogenesis (CI) and embryogenesis (CD) through a recloning approach where the ortets were subjected to two proprietary protocols different in the quantity of a plant growth regulator.

METHODS AND MATERIALS

4 to 15 ramets per clone from 12 clones were recloned in 2010 and 2011. Their lineages were Deli x AVROS, Deli x Cameroon, Deli x Yangambi and Deli x Yangambi AVROS (Table 1). The 12 clones were subjected to 2 proprietary protocols which followed the frame of the first reported oil palm tissue culture process, where the two protocols were different only in the quantity of a plant growth regulator. Each protocol was tested with half of the total leaf explants from each ortet, where they totalled approximately 2000 explants per ortet. CI and CD of each ortet were observed and recorded. The contamination rate of this project averaged at 5%.

The recorded data were analyzed using the statistical software MACANOVA 5.05.

RESULTS

The mean CI percentages of each clone ranged from 18.1% – 38.5% with an overall mean of 29.2% (Table 2) while the mean CD percentages per clone ranged from 1.9% – 9.9% with an overall mean of 5.2% (Table 3). Differences of CI and CD observed among clones were statistically significant (Tables 4 and 5).

The ranges of CI of protocols 1 and 2 were 18.5% – 41.7% and 17.8% – 38.1%, respectively (Table 2). The ranges of CD of protocols 1 and 2 were 1.6% – 9.5% and 1.8% – 10.6%, respectively (Table 3). The difference in CI and CD percentages between the two protocols (Tables 2 and 3) was statistically insignificant (Tables 4 and 5).

Interactions between the protocols and the clones were also statistically insignificant.

DISCUSSIONS

Zero response was not observed from the results because the ortets were ramets where the genotypes of these palms have proven to be amenable to cloning.

The results evidently suggested that the genotype effect influencing the responses towards the two proprietary protocols for CI and CD were strong. In other words, the clones did not response equally to different protocols as suggested by the varied CI and CD percentages although they had demonstrated totipotency*; palm wise, 100% CI and CD were obtained. The immediate question arising from the finding was: should clone specific protocols be developed? Riding on the question; is there a net benefit to develop clone specific protocol? On the contrary, is there room still for improvement for these two proprietary protocols without the manipulation of plant growth regulators?

In order to address these questions, the knowledge of best observed CI and CD of these protocols and the range of standard error within clone were important to be observed as indicators and hence to deduce the possibility for further improvement of these protocols. The best observed CI and CD by these two protocols were 59.7% and 26.3% respectively (results not reported in this manuscript). The range of standard error within clone of overall CI and CD were 2.9% – 9.3% and 1.2% – 5.7% respectively. The ceiling limits of CI and CD observed were not the optimum for commercial tissue culture production given the process of tissue culture followed the frame of first reported tissue culture process; in particular to the consideration of the balance between line and quality culture management e.g. ramets produced per line for most efficient line management. The most efficient line management was subjective to the tissue culturists' experience and hence, their preference.

Most laboratories were reluctant to deviate drastically from their established protocols for fear of increasing the risk of somaclonal variation and the need for extensive field tests for clonal fidelity. To reduce this risk, the planting of more than one clone per field (polyclone approach) is recommended in commercial practices (to avoid genetic vulnerability and risk management of somaclonal variation). The effort invested to develop genotype specific protocol outweighed its benefit because the protocol...

(continued on Page 7)

TABLE 1. DETAILS OF CLONES RECLONED FROM 2010 – 2011

Clone	Lineage	No. of Ramets Recloned	Year of Recloning
A111	Deli x AVROS	6	2010 and 2011
A112	Deli x AVROS	6	2010 and 2011
A154	Deli x Cameroon	10	2010 and 2011
A174	Deli x Cameroon	8	2010 and 2011
A176	Deli x Yangambi	5	2010 and 2011
A178	Deli x Cameroon	5	2011
A183	Deli x AVROS	11	2010 and 2011
A184	Deli x Yangambi AVROS	15	2010 and 2011
A185	Deli x Yangambi AVROS	4	2010 and 2011
A200	Deli x Yangambi AVROS	5	2010 and 2011
A210	Deli x AVROS	4	2010
A212	Deli x Cameroon	9	2010 and 2011
Total		88	

TABLE 2. MEAN AND STANDARD ERROR OF PERCENTAGE OF CALLOGENESIS (CI) OF CLONES SUBJECTED TO TWO PROPRIETY PROTOCOLS

		Protocol 1		Protocol 2		Overall	
Clone	Rep	Mean	SE	Mean	SE	Mean	SE
A111	6	41.7	8.3	35.3	10.5	38.5	8.8
A112	6	31.1	9.8	28.4	9.5	29.7	9.3
A154	10	30.2	9.5	28.5	9.4	29.4	9.3
A174	8	18.5	3.1	17.8	5.4	18.1	2.9
A176	5	25.4	10.3	24.7	9.6	25.0	9.5
A178	5	27.6	10.4	22.2	6.1	24.9	6.6
A183	11	25.9	9.1	24.4	9.1	25.2	8.7
A184	15	31.9	8.2	35.3	9.2	33.6	8.0
A185	4	35.2	8.4	36.3	7.7	35.7	7.8
A200	5	36.4	5.0	38.1	12.1	37.3	8.1
A210	4	27.1	10.6	25.2	5.2	26.2	7.3
A212	9	28.8	8.8	24.3	7.8	26.6	7.4
Mean		30.0		28.4		29.2	

TABLE 3. MEAN AND STANDARD ERROR OF PERCENTAGE OF EMBRYOGENESIS (CD) OF CLONES SUBJECTED TO TWO PROPRIETY PROTOCOLS

		Protocol 1		Protocol 2		Overall	
Clone	Rep	Mean	SE	Mean	SE	Mean	SE
A111	6	6.5	3.7	7.0	5.6	6.6	4.3
A112	6	6.7	6.2	6.0	5.4	6.3	5.7
A154	10	4.4	2.0	2.0	1.2	3.2	1.4
A174	8	4.1	3.3	4.3	2.0	4.1	2.5
A176	5	5.8	2.1	6.8	4.3	6.1	2.7
A178	5	1.6	1.1	2.3	2.0	1.9	1.2
A183	11	6.8	2.8	5.5	4.3	6.2	2.9
A184	15	2.2	1.4	2.3	2.1	2.2	1.5
A185	4	2.7	1.7	1.8	1.1	2.2	1.4
A200	5	9.5	7.0	10.6	4.0	9.9	5.0
A210	4	5.4	4.0	3.1	0.4	4.0	1.5
A212	9	8.6	4.3	10.0	6.6	9.4	4.6
Mean		5.4		5.1		5.2	

TABLE 4. TWO FACTORS (CLONE & PROTOCOL) ANALYSIS OF VARIANCE ON CALLOGENESIS (CI)

Source	DF	SS	MS	F	P-value
Constant	1	1.4794E+05	1.4794e+05	1961.68746	< 1e-08
Protocol	1	67.382	67.382	0.89348	0.34604
Clone	11	5501.4	500.13	6.63165	<1e-08
ProtocolxClone	11	371.56	33.778	0.44790	0.93161
Error	152	11463	75.415		

TABLE 5. TWO FACTORS (CLONE & PROTOCOL) ANALYSIS OF VARIANCE ON EMBRYOGENESIS (CD)

Source	DF	SS	MS	F	P-value
Constant	1	4547.2	4547.2	347.86992	< 1e-08
Protocol	1	2.5057	2.5057	0.19169	0.66214
Clone	11	1146	104.18	7.96988	<1e-08
ProtocolxClone	11	66.913	6.083	0.46536	0.92202
Error	152	1986.9	13.072		

(from page 4)

...suited only to a clone with uncertainty of its variety lifespan, and the practice was meaningless as there was always a new range of ortets being cultured in order to maintain the clones' superiority against the seeds. Nevertheless, with availability of high-density molecular genotyping facilities, could this method of genetic profiling enable genotype classification that is useful for the development of group (of genotypes) specific protocol? If the approach is successfully implemented, new ortets could always be checked against its group by high-density molecular genotyping and hence determine their best protocol.

Improving CI and CD suggested little to the efficiency of the current tissue culture process because efficiency of the process is heavily reliant upon other factors in the process e.g. capability of the cultures to proliferate, to germinate (shoot and root), and to be amenable to a worker friendly system, like the liquid suspension system. Smith *et al* (2010) reported the success of a frame shift from the first reported tissue culture process, where direct somatic embryoid was obtained without callusing. The process is also being described as being very amenable to the liquid suspension system producing embryoids that behave similarly as the natural embryo where germination begin by radical growth and subsequently followed by plumule growth that leads to the possibility of automating the process. Regardless of efficiencies, all protocols must stand up to the test of fidelity, producing true-to-type ramets.

CONCLUSION

Genotype effect on oil palm tissue culture callogenesis and embryogenesis was evident in this experiment. The differences due to the two protocols were insignificant and hence, protocol x genotype interaction was not observed. Even so, genotype specific protocol was not desirable because of its complexity in implementation in commercial oil palm tissue culture laboratories that aimed to produce polyclone using continuously different genotype. Improvement of the two protocols without drastically manipulating the plant growth regulators was still expected as the random variation was still high relative to the targeted improvement of a defined optimum number of embryoid lines per clone. Since CI and CD were not the sole factors to the entire tissue culture process efficiency, continuous research is still required to improve the process without compromising on culture fidelity.

ACKNOWLEDGEMENT

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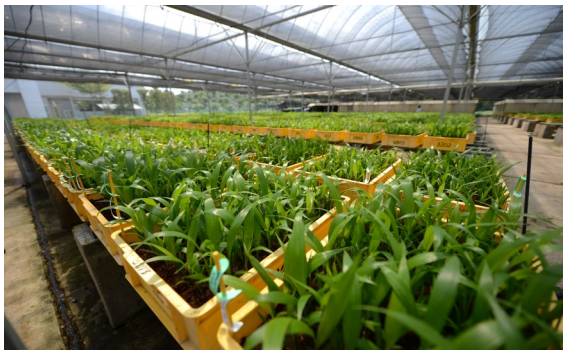
Soh AC, Wong G, Tan CC, Chew PS (2003) Oil Palm Genetic Improvement. Plant Breeding Rev. 22:165 – 220

Soh AC, Wong G, Tan CC, Chew PS, Chong SP, Ho YW, Wong CK, Choo CN, Nor Azura H and Kumar K (2011); Commercial-scale Propagation and Planting of Elite Oil Palm Clones: Research and Development towards Realization; Journal of Oil Palm Research; 23:935-952

ISO QUALITY MANAGEMENT SYSTEM IN AAR TISSUE CULTURE LABORATORY

Ilham A. A., Choo C.N., Nur Akilla M.R., Shahril N.A. and Tan C.C.

AAR Tissue Culture Laboratory was awarded the ISO 9001:2000 Quality Management System Standard in 2009 through SIRIM QAS International Sdn. Bhd. This was upgraded to ISO 9001:2008 in 2010. We are proud to be the only oil palm tissue culture laboratory in Malaysia with this certification. Entering the fifth year of the certification, we can say with absolute confidence that our tissue culture clonal ramet, AA Vitroa, is of the highest quality due to our stringent quality checking procedure at every stage, starting from selecting the best materials to be cloned, to the development of regenerated plants in the lab and their survival in the conditioning nursery.

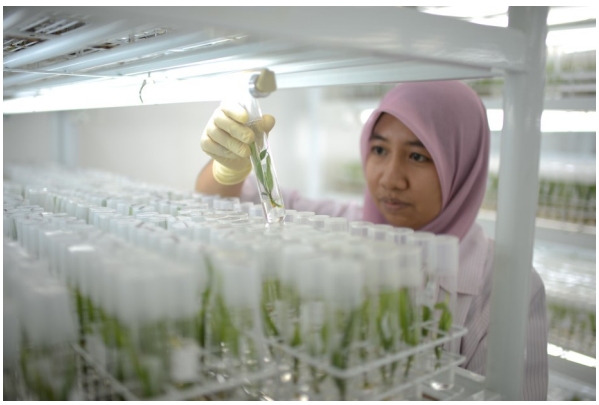


AA Vitroa, our clonal planting material in acclimatization nursery

A very important element in ISO9001:2008 is traceability, which refers to the ability to verify the source, location, history or application of an item by means of recorded documentation. The most dreaded part for all organizations under ISO is the documentations aspect through which we control our product quality and movement. ISO puts a high emphasis on controlling non-conforming products, where products that do not meet the specified criteria should either be discarded from the system or re-worked to conformity and included back into the system. For this purpose, we must have strong evidence, especially by record keeping. From the documentations that we have throughout the system in AAR Tissue Culture Laboratory, we can verify the record, starting from the customer complaint itself, back to the despatch, transplanting records, and further back to the laboratory process. ISO does not set the number of documents we should create, so we hold on to the concept of making only meaningful records to efficiently manage the process, or else, we will end up like most organizations that are trapped with documentations rather than helping to smoothen the process.

"Quality is not an act, it is a habit"-Aristotle-

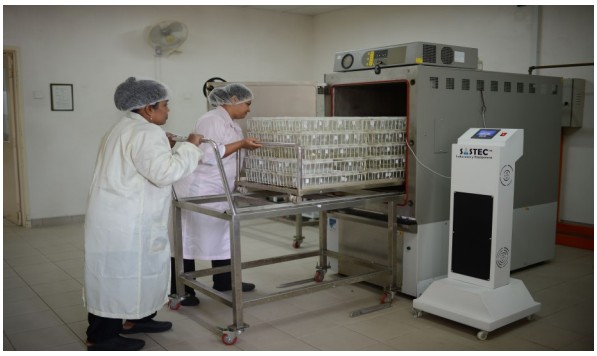
"Quality is never an accident; it is always the result of intelligent effort" - John Ruskin-



Quality check in culture room

In any production team, resources are the most crucial elements (raw material, equipment and human resources); to ensure the efficiency and continuity of the production processes. These are highlighted in ISO standards under "Resource management"- provision of resources, human resources, infrastructure and also "Product realization"-

purchasing and control of monitoring and measuring equipment. All these requirements give positive impact to our production processes, especially when we are required to only use calibrated devices, quality raw



Media ready to be sterilized in calibrated and maintained autoclave

material and quality human resources that undergo thorough continuous on-the-job training throughout the year. If anything goes wrong along the way, corrective actions would be taken and preventive measures put in place.

Our main asset is the highly dedicated local workforce

One of AAR's expectations towards its employees is "AAR expects you to make the customers satisfied and happy". This golden reminder has always been the integral part of our ISO implementation. We treat every customer's feedback positively and take prompt action where necessary. Listening to customers is definitely the best way to improve our working system to ensure maximum customer satisfaction

"Quality means doing it right when no one is looking"- Henry Ford-

Nursery technician implementing quality control

Another vital component in our ISO implementation is continual improvement. We achieve this by continually monitoring measurable and meaningful objectives. Discussions on improvements are always encouraged, be it on documentation, procedure changes, facilities, trainings, and so on – the list is endless. Our quality policy also highlights that yearly reviews will be conducted for quality policy and quality objectives to meet customer expectations.

"A dream becomes a goal when action is taken towards its achievement" -Bo Bennett-

All in all, we will never rest on our laurels and will always strive for excellence. Five years with ISO and many more years to come. Thank you for supporting us.

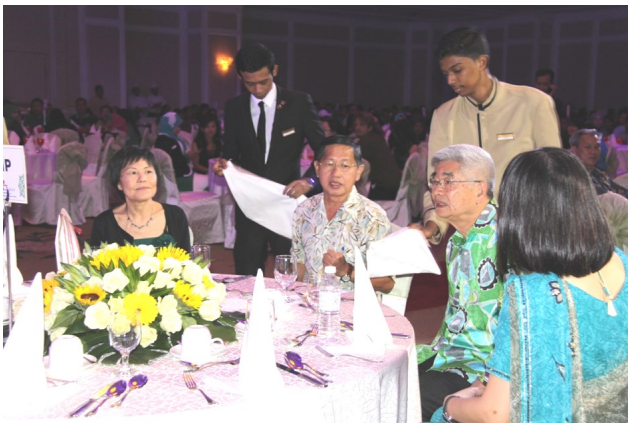
References:

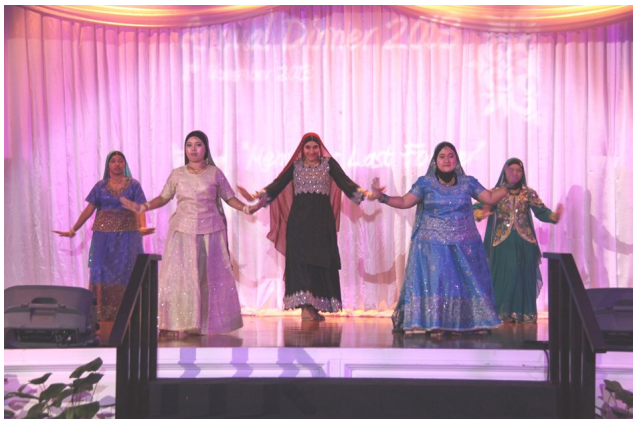
Malaysian Standard. MS ISO 9001:2008 Quality Management Systems – Requirements. Published by Standards Malaysia, 2009.

AAR TC Lab Work Instructions, Standard Operating Procedures, Quality Plan and Quality Manuals

AAR SPORTS CLUB NEWS

Annual Dinner at Royale Chulan Damansara, November 2013





Sports Day (TC Lab), December 2013

Futsal Tournament (Main Office & TC Lab), Titan Sports Centre, January 2014

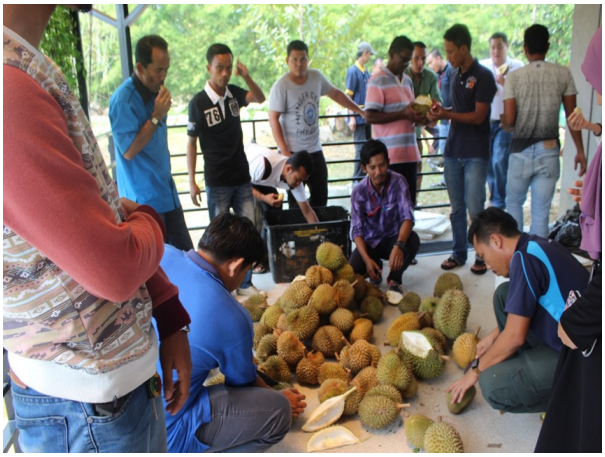
Chinese New Year Celebration (Main Office), AARKD Café, February 2014

Bowling Tournament (Main Office) at Cineleisure, The Curve, March 2014

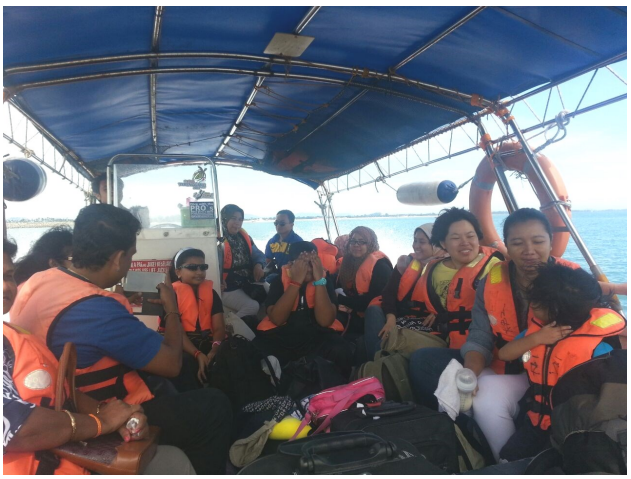
Netball Tournament (Paloh), June 2014

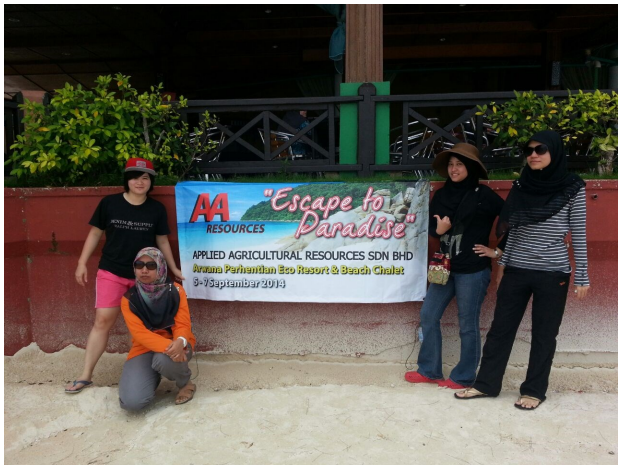
Malam Raya (Sabah), August 2014

Food & Fruit Festival (Main Office & TC Lab), August 2014



Annual Trip to Perhentian Island (Main Office, TC Lab, Paloh & Kampar), September 2014





AAR NEW RECRUITS

Siti Nuraqilah Sharudin
Human Resource Officer
(Joined April 2013)

Laura Lin Tze Chin
Graduate Research Assistant -Agronomy
(Joined August 2013)

Nik Farhana Nadiah Nik Mustapha
Graduate Research Assistant -Biotechnology
(Joined September 2013)

Cassandra Chong Yi Wen
Agronomist
(Joined April 2013)

Tan Suet Yee
Statistician
(Joined September 2013)

Kamalanathan a/l Rama-chandaran
Graduate Research Assistant - Biotechnology
(Joined April 2013)

Tan Swee Sian
Graduate Research Assistant-Microbiology
(Joined April 2013)

Shahril Naim Aminuddin
Tissue Culturist-Nursery
(Joined May 2014)

Dr. Lim Choon Kiat
Agronomist
(Joined May 2014)

PTAARI NEW RECRUITS

Leo Mualim
Agronomist
(Joined March 2014)

Rizki Darusalam
Plant Breeder / Seed Production
(Joined January 2013)