

In vitro micropropagation of two local taro cultivars for large-scale cultivation

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Abstract The application of traditional taro propagation methods for large-scale cultivation would be insufficient to meet the high demand for quality planting materials. Therefore, this study aimed to develop an *in vitro* micropropagation technique for two local taro cultivars (cv.), Wangi and Putih. Taro cormels were collected from the Malaysian Agricultural Research and Development Institute (MARDI) germplasm (Serdang, Malaysia). Explants were taken from the shoot tip of cormels and initially cultured on Murashige and Skoog (MS) basal media for four weeks. The explants were then transferred to different multiplication media supplemented with different types and concentrations of cytokinins such as 6-benzylaminopurine (BAP) and Thidiazuron (TDZ). Shoot production was quantified after six weeks of culture. The highest mean number of new shoots was produced by the Wangi cultivar on MS medium supplemented with 2.0 mg/l BAP (2.10 shoots), MS medium supplemented with 0.5 mg/l TDZ (2.18 shoots), and Gamborg B5 medium supplemented with 6.0 mg/l BAP (2.43 shoots). The maximum average number of the Putih cultivar shoots was obtained on MS supplemented with 2.0 mg/l BAP (3.57 shoots). MS basal media was used for root initiation, as it produced an average of 25 roots with an 11-cm length. Various types of substrate mixtures were used during acclimatization. The best acclimatization substrate for the Wangi cultivar was 100% peat soil, whereas the Putih cultivar grew optimally in a combination of peat and perlites at a 1:1 ratio. Taro plantlets require approximately 4 to 6 weeks to acclimatize before they can be transferred to the field.

Keywords Multiplication, Microcorms, *Colocasia esculenta*, Acclimatization, Keladi

Introduction

Taro (*Colocasia esculenta* (L.) Schott) or also known as keladi in Malaysia is an edible plant primarily grown for its corm. It is immensely cultivated in the Southeast Asia, East Asia and the Pacific Islands (Macharia et al. 2014). Taro significantly contributes the people's diet in terms of the carbohydrate nourishment in many regions in developing countries. Taro can serve as food security should the production of staple food industry fails. It is ranked 14th as the most cultivated staple food around the world (Hutami and Purnamaningsih 2013; Oscarsson and Savage 2007). Either the corms or all the other parts of plant such as stalk, leaves and flower are edible depending on the cultivars (Gonçalves et al. 2013). The corms contain plenty of starch. Although it is less significant than other tropical root crops such as yam, cassava, and sweet potato, it stills a major staple in some parts of the tropical and subtropical like the African country and The Pacific Islands (Manju et al. 2017).

Taro also being a significant export commodity in numerous countries, and it is highly priced in the market. Taro has been widely used in rural agroindustrial for raw material product like syrup, gum, edible film for modified atmosphere packaging (MAP) and renewable energy source. In 2010, taro world production is approximately 9,006,116 metric tonnes (Mg). However, only 133,676 Mg of taro from the world production was exported worldwide (Joe 2012). There were demands from countries such as United States of America, Japan, China, and these are some of the world's top global importers. In 2010, production of taro in the Asia region was approximately 2.1 million Mg and about 23% of global production (Joe

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2012). In Malaysia, production of taro hugely decreased from 4,856.81 Mg in 2015 to 4,129.15 Mg in 2016 and 4,634.8 Mg in 2017 respectively (DOA 2015; 2016; 2017). The main constraints for taro production are a scarcity of disease-free planting materials (Mbong et al. 2013). This plant, which belongs to the Araceae family, is commonly propagated by suckers. It has been reported that cultivars that produce large corms produce a small number of suckers (Sivan and Liyanage 1992; Sivan 1984).

Through conventional method, the amount of taro planting materials yielded is low and probably disease-carrying. The crop also susceptible to fungal, viral, and nematode infections (Gadre and Joshi 2003). Taro leaf blight disease, which caused by *Phytophthora colocasiae* is one of the major important economic diseases of taro. It reduces corm yield of up to 50 percent (Singh et al. 2006). Other than that, pest and disease such as Dasheen Mosaic Virus Disease (DMV), the Alomae/Bobone Virus Disease Complex and the taro beetles (*Papuana woodlarkiana*, *Papuana biroi*, *Papuana huebneri*, and *Papuana trinodosa*) also reduced the yield. Plant tissue culture techniques have become a powerful tool for propagation of taro to overcome many problems from the conventional methods of propagation. The tissue culture technique provides a convenient yet phytosanitary method for international transfer of germplasm (Inno 1999).

The technique provides an economical, mass propagation, space, and labour-saving method for the preservation of germplasm. They can be stored as tissue culture in nutrient medium rather than repeatedly growing germplasm collections in the field. It only needs to be subcultured once in several months (Inno 1999). The method described in this study for *in vitro* production of two local taro cultivars is

the first report to increase productivity of mass, disease-free planting material of taro in Malaysia. Therefore, the main aim of this study is to develop an efficient *in vitro* micropropagation protocol for two local taro cultivars using shoot tip from the corm as explants.

Materials and Methods

Plant material and surface sterilization

Suckers of taro were obtained from Malaysian Agricultural Research and Development Institute (MARDI) aroids germplasm collection, number 18 (keladi wangi) and 31 (keladi putih). (Ghazali et al. 2016) (Fig. 1 and 2). Experiments were performed in the Cryopreservation laboratories of the MyGeneBank Complex in MARDI, Serdang (2°58'42.3"N 101°41'17.4"E). To obtain initial cultures of taro, suckers were cut and trimmed to about 4cm length shoot and 1.5cm width base with 3 to 4 leaf sheaths (Fig. 2). The explants were then washed with Decon 90 before rinsed with tap water. The explants were then washed under running tap water for 1 hour, soaked in 20ml dettol antiseptic liquid in 500 ml distilled water for 30 second up to 1 min and rinsed with distilled water. Explants were then soaked in 0.5 g/l Kenlate fungicide (50% benomyl) for 2 hours and rinsed with distilled water. Afterward, under the airstream horizontal laminar airflow cabinet (ESCO, USA), explants were then transferred and soaked into 50% commercial bleach Clorox® (5.65% Sodium hypochlorite) with 3 to 5 drops of tween 20 for 17 minutes under laminar airflow chamber followed by rinsing three times with double distilled water. Then, the explants were

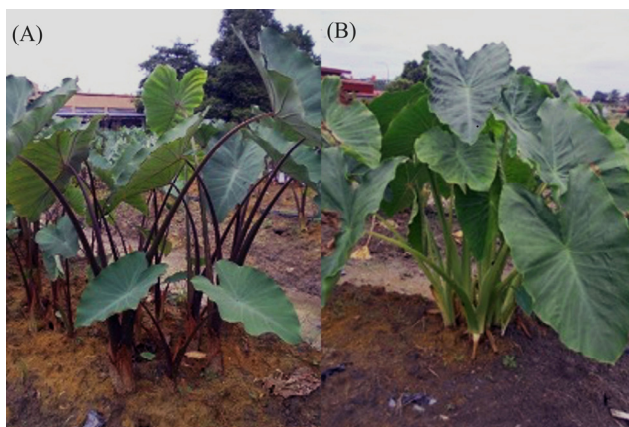


Fig. 1 The Wangi and Putih cultivars can be morphologically distinguished by their stalk color variation. (A) cv. Wangi exhibits a purplish-brown leaf stalk, whereas (B) cv. Putih has a greenish-white leaf stalk

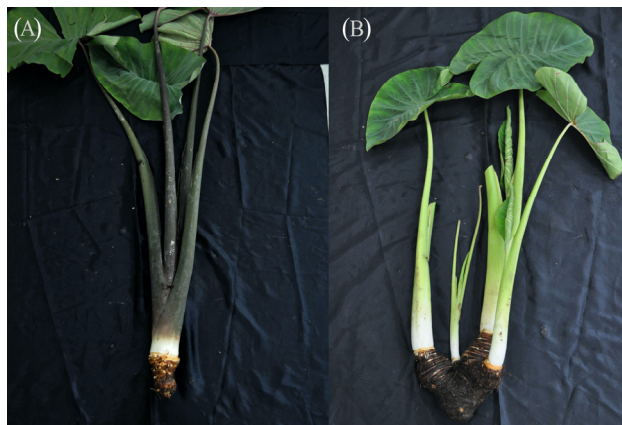


Fig. 2 Explants were taken from the cormel and trimmed to an approximately 4 cm shoot length and 1.5 cm base width with 3-4 leaf sheaths. (A) cv. Wangi, and (B) cv. Putih.

cut and trimmed into smaller pieces leaving only 1 to 2 leaf sheaths. The explants were further disinfected with 70% ethanol for 30 seconds and 10% Clorox® for 5 minutes followed by rinsing with double distilled water for four times and blotted dry on sterilized paper before cultured to Murashige and Skoog (1962) (MS) media supplemented with 30 g/l sucrose and solidified with 3 g/l Gelrite™.

Shoot Multiplication and Root Regeneration

In vitro shoot tips of cv. wangi were cultured on 2 different types of mediums which is Murashige & Skoog medium (MS) and Gamborg B5 medium (B5) supplemented with 3% (w/v) sucrose, 0.3% (w/v) Gelrite™, and different concentrations of 6-Benzylaminopurine (0, 0.5, 1, 2, 3 mg/l BAP) and Thidiazuron (0, 0.5, 1, 2, 3 mg/l TDZ) for shoot multiplication. While for cv. putih, *in vitro* shoot tips were cultured only on MS medium supplemented with different 6-Benzylaminopurine concentration (0, 0.5, 2, 4, 6, 8 mg/l BAP). Each experimental condition consisted of 10 samples and was replicated thrice. Cultures were kept under 18/6 h photoperiod at $25 \pm 2^\circ\text{C}$. Mean of shoot numbers, shoot length, root numbers and root length were recorded after 6 weeks of cultures. Sixth weeks old, regenerated shoots were cultured on MS medium for 6 weeks without any additional plant growth regulators for root induction.

Acclimatisation of plantlets

Well-developed plantlets in six weeks culture were removed carefully from the medium, washed with tap water to remove the traces of agar. Plantlets were then planted in germination trays with four different substrates mixture: T1) peat, perlite and vermiculate (1:1:1), T2) peat and vermiculate (1:1), T3) peat and perlite (1:1), and T4) peat. To maintain humidity, the plantlets were covered with transparent plastic sheets and seedling tray was located inside a plastic tray containing 1 cm height of water. Watering was done weekly. The plantlets in the trays were placed in a cool place with 50% shade. After four weeks of acclimatisation, data on the percentage of survival, plant height, root number, root length, number of micro-corms, and number of new shoots were all recorded.

Statistical Analysis

All data were analysed by analysis of variance (ANOVA) using Statistical Analysis System Software (SAS) release

9.4. The experiment was set up in a Completely Randomized Design (CRD). The significance of differences among means was done by using Duncan's Multiple Test Range (DMRT). Statistical significance was considered at $P \leq 0.05$.

Results and Discussion

Direct regeneration of taro from suckers as explants was observed in all concentrations of BAP or TDZ supplemented media. The highest multiplication of shoots for cv. wangi was found on MS media containing 2 mg/l BAP or 0.5 mg/l TDZ with a mean number of shoots of 2.10 ± 0.33 and 2.18 ± 0.36 , respectively, while 6 mg/l BAP contained in B5 media with a mean number of shoots of 2.43 ± 0.24 . Similarly, Chand et al. (1999) discovered that supplementing 1 mg/l BAP or TDZ as a single hormone resulted in 2 to 2.5 shoots of *Colocasia esculenta* cv. Niue. Supplementing higher concentration of BAP and TDZ hormones also shortens shoot and root length and reduces the number of roots produced in cv. wangi plantlets grown in both types of media.

Out of nine hormone concentration treatments in MS media and six treatments in B5, only the control treatment did not result in shoot proliferation. Table 1 and 2 showed a significant difference between treatments for all parameters observed in MS and B5 media at $P \leq 0.05$. Figure 3 and 4 showed the condition of taro cultured on MS and B5 media. After 2-3 weeks of culture in B5 media, the matured leaves from the plantlets turned brown, and senescence took over, leaving only the young shoots. This incident, however, did not occur in taro cultured on MS media. Bogale (2019) also reported that MS medium is the most suitable medium for *in vitro* production of *C. esculenta* cv. Bolosso I. compared to B5 media and Chu (N6) media.

Without any additional hormones, cv. wangi *in vitro* plantlets produced enough roots and length. In MS media without hormones, the plantlets produced an average of 21.25 ± 1.10 roots and 11.25 ± 0.69 cm root length, while in B5 media, the plantlets produced 8.23 ± 0.55 roots and 10.37 ± 1.2 cm root length. This demonstrated that cv. wangi did not require rooting or additional hormones to grow normally in MS medium. The use of high concentration of BAP and TDZ hormone suppressed root formation in taro and may result in somaclonal variation depending on the concentrations used (Jackson et al. 1977; Ko et al. 2008). The requirement for cytokinin during the multiplication stage varies depending on crop type,

Table 1 Effects of different concentrations of single hormones (BAP and TDZ) on number of shoot and root, shoot height and root length of cv. wangi after 4 weeks of culture in Murashige & Skoog medium

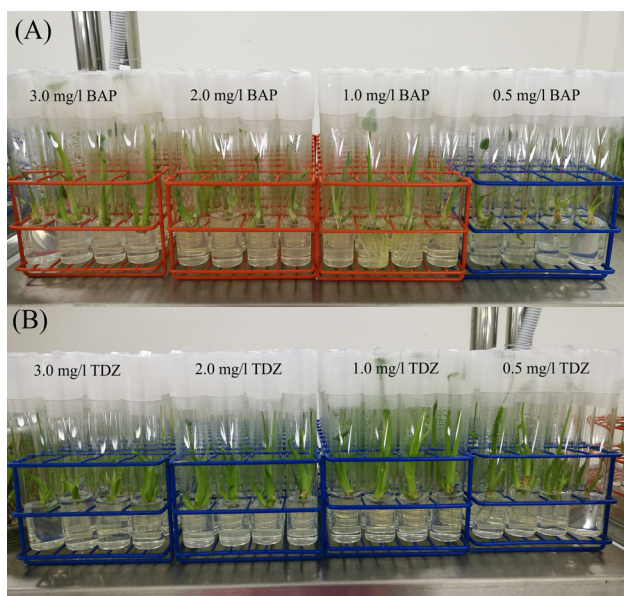
Concentrations of single hormones (BAP/ TDZ)	Number of shoots	Shoot height (cm)	Number of roots	Root length (cm)
Control	0.32 ± 0.12 ^a	13.11 ± 0.36 ^f	21.25 ± 1.10 ^g	11.25 ± 0.69 ^e
0.5 mg/l BAP	0.96 ± 0.23 ^{ab}	4.18 ± 0.61 ^a	12.14 ± 0.93 ^e	2.34 ± 0.22 ^{cd}
1.0 mg/l BAP	1.32 ± 0.24 ^{bc}	6.21 ± 0.48 ^b	14.32 ± 1.00 ^f	2.86 ± 0.21 ^d
2.0mg/l BAP	2.10 ± 0.33 ^d	6.54 ± 0.53 ^{bc}	3.46 ± 0.58 ^b	0.54 ± 0.09 ^a
3.0 mg/l BAP	2.04 ± 0.27 ^{cd}	7.61 ± 0.47 ^{cd}	5.96 ± 0.89 ^c	0.99 ± 0.14 ^{ab}
0.5 mg/l TDZ	2.18 ± 0.36 ^d	8.68 ± 0.61 ^d	9.11 ± 0.99 ^d	2.45 ± 0.17 ^d
1.0 mg/l TDZ	2.0 ± 0.20 ^{cd}	10.71 ± 0.40 ^e	2.04 ± 0.29 ^{ab}	2.11 ± 0.21 ^{cd}
2.0 mg/l TDZ	1.50 ± 0.36 ^{bcd}	5.64 ± 0.39 ^b	1.04 ± 0.24 ^a	1.14 ± 0.2 ^{ab}
3.0 mg/l TDZ	1.46 ± 0.30 ^{bcd}	5.22 ± 0.48 ^{ab}	1.64 ± 0.33 ^{ab}	1.64 ± 0.22 ^{bc}

Data followed by different letters showed significant difference at $P \leq 0.05$. Data represent mean ± standard error, n = 28.

Table 2 Effects of different concentrations of BAP on number of shoot and root, shoot height and root length of cv. wangi after 6 weeks of culture in Gamborg B5 media

BAP concentrations	No. of Shoot	Shoot height (cm)	Root numbers	Root length (cm)
0 mg/l BAP	0.03 ± 0.03 ^a	8.1 ± 1.02 ^c	8.23 ± 0.55 ^c	10.37 ± 1.20 ^b
0.5 mg/l BAP	1.47 ± 0.25 ^b	4.22 ± 0.16 ^b	0.93 ± 0.28 ^b	0.1 ± 0.03 ^a
2.0mg/l BAP	2.07 ± 0.41 ^{bc}	3.33 ± 0.15 ^{ab}	0 ^a	0 ^a
4.0 mg/l BAP	1.73 ± 0.29 ^b	2.67 ± 0.14 ^a	0 ^a	0 ^a
6.0 mg/l BAP	2.43 ± 0.24 ^c	2.97 ± 0.11 ^a	0 ^a	0 ^a
8.0 mg/l BAP	1.87 ± 0.30 ^{bc}	2.93 ± 0.16 ^a	0 ^a	0 ^a

Data followed by different letters showed significant difference at $P \leq 0.05$. Data represent mean ± standard error, n = 30.

**Fig. 3** The effects of different concentrations of single hormones (A) 6-benzylaminopurine (BAP) and (B) Thidiazuron (TDZ) on cv. wangi after 4 weeks of culture in MS medium.

explant, developmental phase, growth regulator concentration, and the interaction between growth regulators and the

**Fig. 4** The effects of different concentrations of 6-benzylaminopurine (BAP) on cv. wangi after 6 weeks of culture in Gamborg B5 medium.

environment (Yokoya et al. 1999).

Table 3 and Figure 5 revealed a highly significant difference between treatments of different concentrations of BAP for all parameters observed for cv. putih. Only the control treatment does not induce shoot proliferation among the six hormone concentration treatments. The highest multiplication of shoots was found on MS media containing 2 mg/l BAP, as with cv. wangi. Cultivar putih produced

Table 3 Effects of different concentrations of BAP on number of shoot and root, shoot height and root length of cv. putih after 6 weeks of culture in Murashige & Skoog medium

Concentrations of BAP	Number of shoots	Shoot height (cm)	Number of roots	Root length (cm)
0 mg/l BAP	0.03 ± 0.03 ^a	10.23 ± 0.31 ^d	12.27 ± 0.40 ^{bc}	12.85 ± 0.50 ^d
0.5 mg/l BAP	2.27 ± 0.28 ^b	7.40 ± 0.41 ^c	12.57 ± 0.62 ^c	7.90 ± 0.91 ^c
2.0mg/l BAP	3.57 ± 0.26 ^d	5.97 ± 0.34 ^b	10.67 ± 0.74 ^b	1.41 ± 0.19 ^b
4.0 mg/l BAP	3.10 ± 0.28 ^{cd}	4.63 ± 0.32 ^a	4.43 ± 0.67 ^a	0.53 ± 0.09 ^{ab}
6.0 mg/l BAP	3.00 ± 0.19 ^{cd}	4.43 ± 0.31 ^a	3.17 ± 0.56 ^a	0.26 ± 0.05 ^a
8.0 mg/l BAP	2.53 ± 0.20 ^{bc}	3.90 ± 0.20 ^a	2.83 ± 0.60 ^a	0.21 ± 0.04 ^a

Data followed by different letters showed significant difference at $P \leq 0.05$. Data represent mean ± standard error, n = 30.

**Fig. 5** The effects of different concentrations of 6-benzylaminopurine (BAP) on cv. putih after 6 weeks of culture in MS medium

more shoots than cv. wangi, according to the results. Cultivar putih produced 3 to 4 ($3.57b \pm 0.26$) shoots. The intensification in BAP concentrations beyond 2 mg/l has not improved the multiplication of shoots and did not affect the shoot length, root numbers and root length. In contrast to Bogale's (2018) study, taro cv. Bolosso I supplemented with BAP at concentrations ranging from 4 mg/l to 8 mg/l resulted in 3.83 to 6.13 shoots.

Root crops culture is difficult to initiate especially if the storage organ such as corm or tuber is being used as starting material. These organs may have contaminated with soil micro flora that become problematic during tissue culture process. In this study, surface sterilization technique does not include mercury (II) chloride that is very toxic to humans. It has been excessively used in explant surface sterilization due to strong sterilization efficiency to kill microbes (soil-borne, epiphytic fungi and bacteria). Hence, the sterilization used in this study gave 0% contamination. The trick behind the success of this sterilization technique is the initial size of explant taken from the corm. The smaller the size of explant, less contamination was found in the culture. If the initial explant size is big around 6-7cm length and 3-4cm width, normally bacterial infection will interfere in the culture.

Additional fungicide (benomyl) used in this study also help to reduce fungi contamination.

The survival rate of plantlets transplanted from culture jars to seedling trays was 100% for all substrate media used in this study (Table 4). The difficulties associated with tissue cultured plant survival and growth following transplantation are attributed to the plant's inability to control water due to the type of media used (Lavanya et al. 2009). Peat was the best substrate media for cv. wangi in this study, with a plant height of 26.34 cm, 12 number of roots with 19.2 cm length. While for cv. putih, a mixture of 1:1 peat and perlite found to be the best with a plant height of 28.76 cm, 11 number of roots with 21.84 cm length (Table 4 & Fig. 6). This response could be attributed to the mixture's ability to provide adequate moisture and aeration to the plants, resulting in healthy root growth. Perlite is an important component in the potting mixture when mixed with peatmoss. The addition of perlite to peatmoss increases both the amount of oxygen and the amount of water retained by the peatmoss. This obviously improves plant growth conditions (Miller and Donahue 1990). After 4 to 6 weeks planted in the nursery and acclimatized under 50% shades, the plantlets were transferred to the field. Fig. 7 showed the tissue culture plant of cv. wangi and cv. putih that had been successfully planted in the field for eight months.

Conclusion

An effective protocol needs to be established to provide a rapid technique for mass multiplication of these potential crop cultivars. Initiation of cv. wangi and putih through micropropagation begin after 1 week in culture. At multiplication stage, both cultivars multiplied when cultured in MS medium supplemented with selected cytokinin (BAP/TDZ) during this study. MS medium supplemented with 2 mg/l BAP showed the highest number of shoots produced

Table 4 The effect of different substrate media on survival percentage and growth of cv. wangi and cv. putih attained at week 4 of acclimatization. T1) peat, perlite and vermiculate (1:1:1), T2) peat and vermiculate (1:1), T3) peat and perlite (1:1), and T4) peat

Type of taro	Substrate composition	Survival (%)	Plant height (cm)	Root length (cm)	No. of roots	Plantlets producing new shoots (%)	Plantlets producing micro-corm (%)
cv. wangi	T1	100	22.38 ^a ± 1.36	17.02 ^a ± 1.40	9.72 ^a ± 0.66	0 ^{ns}	28 ^a ± 10.19
	T2	100	26.22 ^b ± 1.13	18.14 ^{ab} ± 0.33	11.16 ^{bc} ± 0.48	0 ^{ns}	56 ^b ± 11.66
	T3	100	26.74 ^b ± 2.27	18.18 ^{ab} ± 1.19	10.28 ^{ab} ± 0.77	0 ^{ns}	52 ^{ab} ± 16.25
	T4	100	26.34 ^b ± 1.44	19.2 ^b ± 1.08	12.08 ^c ± 0.41	4 ^{ns}	40 ^{ab} ± 8.94
cv. putih	T1	100	25.76 ^a ± 2.40	21.26 ^a ± 1.48	10.68 ^{ns}	28 ^{ns}	88 ^{ns}
	T2	100	25.8 ^a ± 2.24	22.88 ^a ± 1.91	10.6 ^{ns}	20 ^{ns}	100 ^{ns}
	T3	100	28.76 ^b ± 1.20	21.84 ^a ± 0.82	11.84 ^{ns}	12 ^{ns}	100 ^{ns}
	T4	100	26.04 ^{ab} ± 1.75	21.26 ^a ± 1.43	9.64 ^{ns}	16 ^{ns}	100 ^{ns}

Data followed by different letters showed a significant difference at $P \leq 0.05$. Data represent mean ± standard error, n = 25.



Fig. 6 The effect of substrate media T1) Peat, perlite and vermiculate (1:1:1), T2) Peat and vermiculate (1:1), T3) Peat and perlite (1:1), and T4) peat on the growth of (A) cv. wangi and (B) cv. putih plantlets

for both cultivars. At rooting stage, the highest number of roots was achieved on MS medium without hormone. After acclimatisation, 100% of seedlings were survived in green house. A complete cycle of initiating taro tissue culture takes around 4 to 5 months from surface sterilization to acclimatise before field planting.

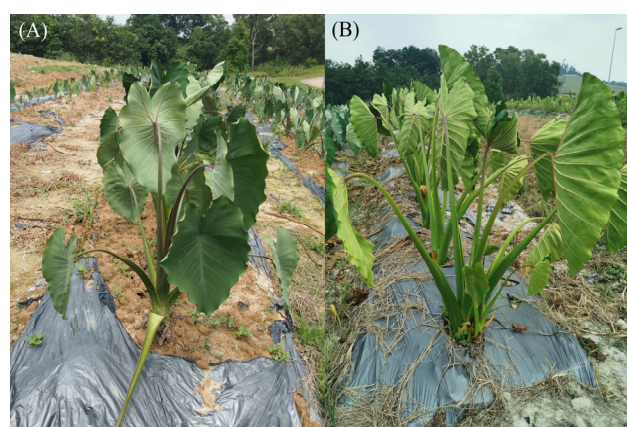


Fig. 7 Eight months old tissue culture plant of (A) cv. wangi and (B) cv. putih, successfully planted in the field

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