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Yield performance of Rice var. Sang Yod Phatthalung under field condition

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Abstract The results of yield performance of rice var. Sang Yod Phatthalung under field condition revealed that *in vitro* plants gave higher yield components (number of tillers per plant, panicles per tiller and paddy weight) than those obtained from seed-derived plants. Moreover, *in vitro* plants provided higher chlorophyll content but it was not significantly differed. Interestingly, chemical contents, anthocyanin and total phenolic compounds in dehusk seeds were significantly higher than those of seed-derived plants. It is indicated that production of Sang Yod Phatthalung planted by this technique may possible improve yield and chemical contents.

Keywords: Sang Yod Phatthalung rice, Somaclone, Vitro-plant, Seed-derived plant, Anthocyanin

Introduction

Sang Yod Phatthalung rice is indica rice and grown in Patthalung province more than one hundred years. This rice is the first cultivar which has been obtained Geographical Indications (GI) in Thailand. The specific characteristic of this cultivar is a dark-red color dehusk seed. Red rice had more than 14-15 percent of amylose content, 8.6 gram of protein per 100-gram fresh weight, 82.01 mg in gallic acid per 100-gram fresh weight of polyphenol, 15.14 mg in cyanidin-3-glucoside of anthocyanin (Yodmanee *et al*., 2011) which were higher than those of white rice. Additionally, the high antioxidant activity of pigmented rice helps lower a person's risk of developing various chronic illnesses, including diabetes, cancer, and cardiovascular disease. (Ho *et al*., 2018). However, Sang Yod Phatthalung rice is photoperiod sensitive rice which can be grown once in a year during rainy season (rainfed). In addition, yield obtained under these suitable conditions is still low (350 kg/rai) (Rice Department, 2010). Currently, highly demand of this rice for healthy food led to the high consumption of the consumers, especially aging people. Producing a specific rice variety for the

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market will boost farmers' financial gains and improve consumers' nutritional intake. Thus, in the future, Sang Yod Phatthalung rice needs to be improved both in yield and nutritional value in a short time by biotechnological method.

Using in vitro techniques, an alternative way of propagation, not only can mass multiplication of plants in shorter time (Kumar and Reddy, 2011) but also improve some characters as mentioned earlier. *In vitro* techniques can produce vitro plants which are easily induced mutation by incorporation with some plant growth regulators or mutagens. Regenerated plants (vitro plants) derived from those techniques can show genotypic and phenotypic variations (Orbovic *et al*., 2008). The occurrence of somaclonal variation obtained from these techniques provides variants or somaclonal variants (SV) and could be used as new novel lines or varieties. By this technique it will enable breeders to improve desirable traits (Leva *et al*., 2012). Somaclonal variation is a valuable source of genetic variation that can be used to identify novel variants that may enhance crop quality, high yield, and disease resistance. (Karp, 1995; Mehta and Angra, 2000; Predieri, 2001; Unai *et al*., 2004). Somaclonal variation has been related to plant growth regulators (PGRs) (Skirvin *et al*., 1994). The presence of certain PGRs like auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) also enhances the rate of this variation (Rasheed *et al*., 2005). 2,4-D has been shown to be mutagenic in barley (Khalatkar and Bhargava, 1985) and rice plants (Kumari and Vaidyanath, 1989) as well as garlic and onion root tip cells (Dolezel *et al*., 1987). It has been demonstrated that 2,4-D induces genetic variations and reduces the frequency of seedling growth in immature barley embryos cultivated on different 2,4-D concentrations. (Deambrogio and Dale, 1980). The range of genetic diversity in plants that can be expanded by somaclonal variation includes plant height, yield, number of flowers per plant, early flowering, and grain quality. (Joshi and Rao, 2009). Patnaik *et al*. (1999) reported a wide variation in quantitative traits such as plant yield and qualitative traits such as plant height, number of tillers per plant regenerated from cell suspension cultures of palmarosa grass, *Cymbopogon martinii* (Roxb). Numerous studies have also demonstrated the importance of the chosen somaclones in plant breeding, citing traits like Indian mustard's high yield and shattering resistance. (Katiyar and Chopra, 1995).

There is no reported genetic instability through tissue culture technique incorporated with BA, especially induction of shoot organogenesis directly. The rate of somaclonal variation in banana cultivars is not directly impacted by high concentrations of cytokinins. ‘Nanjanagudu Rasabale’ and ‘Cavendish’ (Venkatachalam *et al*., 2007). Conversely, high concentrations of benzyladenine (BA) caused the increment in number of chromosomes in the banana cultivar ‘Williams’ (Roles *et al*., 2005).

The objective was to compare *in vitro* plant of agronomical and physiological characteristics, anthocyanin and total phenolic compounds of rice var. Sang Yod Phatthalung under field condition.

Materials and methods

Plant material

In this experiment, two different sources of rice var. Sang Yod Phattalung were grown at Phattalung Rice Research Station (PRRS). The first source was seed plant kindly provided by PRRS and the second source was vitro-plants from somaclonal regenerant 1 (SCR1-0.5BA) seeds according to the method described by Noimusik *et al.* (2018). For seed plants they were germinated on moist tissue paper placing on Petri-dish for one week subsequented to transferred to 8-inch plastic pots covered with plastic bottle and acclimatization in the greenhouse at 28-30 °C supplied by natural light. In case of SCR1-0.5BA, the seeds were dehusked and washed with running tap water for 20 min, surface sterilized with 70% ethanol for 2 min and immersed in 20% Clorox with 3 drops Tween 20 for 6 min. Finally, the seeds were rinsed with sterile distilled water for 3 times in laminar air flow hood and blotted on sterile tissue paper. Sterilized seeds were sown on OPCM with 0.5 mg/L dicamba for 2 weeks to induce shoot. Single shoot was excised and cut the leaves above growing point to remain the shoot explant at size of 5 mm in length, then, transferred to culture in liquid MS medium with 0.5 mg/L BA to induce multiple shoots and root. The cultures were maintained on rotary shaker at speed of 100 rpm for 3 weeks. Complete plantlets (shoot with root) were acclimatized and grown at the same way as mentioned earlier in seed plants. The detail of vitro-plant preparation was demonstrated in Figure 1.

Field trial of the two different plant sources

Both sources of plants, vitro- and seed-derived plants were grown at PRRS at Phattalung with spacing of 30x30 cm, applied 16-20-0 fertilizer formula at 30 kg/rai after 20 days of growing and 50 days at booting stage. Again fertilizer, 15-15-15 was applied at the same rate after growing for 75 days at flowering stage Climatic data during growing are 27±2^{o}C, 166.9 mm rainfall and 83.7% RH. For pest management, fenpyroximate, imidacloprid and hexaconazole at concentration of 0.05% were applied to prevent red mites, brown planthopper and brown spot disease, respectively at monthly intervals. Data on agronomical and physiological characteristics and chemical contents containing dehusk seeds were statistically compared.

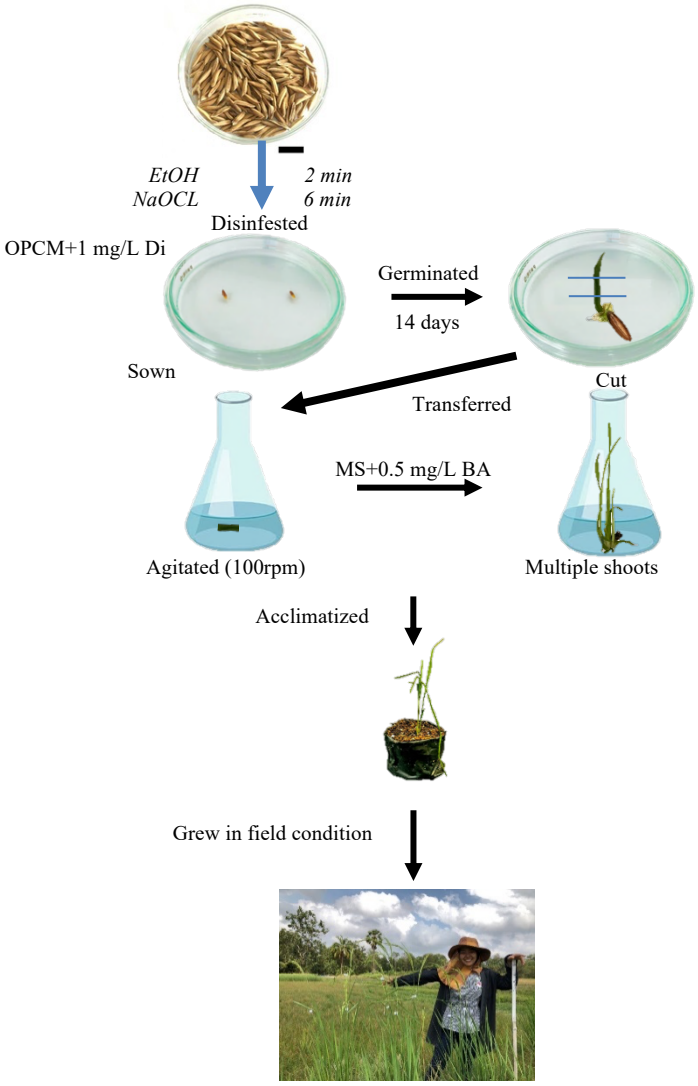

Figure 1. Preparation of vitro plants of *Sang Yod Phattalung* rice for studying yield performance in PRRS: 1. Seeds were dehusked and surface sterilized with 70% ethanol (EtOH) for 2 min, and immersed in 20% Clorox with 3 drops Tween 20 for 6 min. Finally, the seeds were rinsed with sterile distilled water for 3 times in laminar air flow hood and blotted on sterile tissue paper. 2. Sterilized seeds were sown on OPCM with 0.5 mg/L dicamba for 2 weeks to induce shoot. 3. Single shoot was excised and cut the leaves above growing point to remain the shoot explant at size of 5 mm in length. 4. Then, transferred to culture in liquid MS medium with 0.5 mg/L BA to induce multiple shoots and root. The cultures were maintained on rotary shaker at speed of 100 rpm for 3 weeks. 5. Complete plantlets (shoot with root) were acclimatized and grown at PRRS.

Agronomical characteristic determination

Some key parameters of yield components such as number of tillers/plant, number of panicles/plant and 100 paddy weight between the two different types of plants were recorded from samples of those plants after 150 days of growing.

Physiological characteristic determination

Sample of plants from the two different types of plants were subjected to measure chlorophyll content by digital chlorophyll meter SPAD 502 plus after growing for 120 days.

Anthocyanin content determination

Anthocyanin extraction was carried out according to modified method from Yamuangmorn and Prom-u-thai, (2016). Briefly, 0.75 g of fine powder of seeds were transferred to glass test tube (PYREX® NO. 9820) to which 3 ml deionized water was added, heated in water bath at 50 °C for 30 min, mixed every 5 min and centrifuged at 13,000 rpm for 10 min. The supernatant was obtained and used for anthocyanin analysis. A 0.7 ml of supernatant was transferred to glass test tube (PYREX® NO. 9820) for preparing two dilutions of the sample. One tube was adjusted with potassium chloride buffer (pH 1.0) to final volume of 2.8 ml and another tube was adjusted with sodium acetate buffer (pH 4.5) to the same final volume. These dilutions were equilibrated by leaving at room temperature for 30 min. Each dilution was measured at absorbance of 520 and 700 nm compared to blank cell filled with deionized water. The absorbance of the diluted sample (A) was calculated as the following formula.

$A = (A_{510\ nm,\ pH\ =\ 1} - A_{700\ nm,\ pH\ =\ 1}) - (A_{510\ nm,\ pH\ =\ 4.5} - A_{700\ nm,\ pH\ =\ 4.5})$

For monomeric anthocyanin pigment concentration, the original sample was calculated using the following formula.

$$[\text{monomeric anthocyanin pigment}]\ \text{mg/L} = A \times MW \times DF \times 1000 / (\varepsilon \times 1)$$

where A = absorbance, MW = molecular weight (449.2 g/mol), DF = dilution factor,

ε = extinction molar coefficient (26,900 L/cm mol).

and it was converted to mg of total anthocyanin content /100 g sample.

Total phenolic content determination

Phenolic extracts were modified by method from Koufan *et al*. (2020). Briefly, 0.05 g of fine powder of seeds were added with 5 ml of 95% ethanol,

leave at 4 °C for 2 h and filtered through filter paper (Whatman No. 1). The samples were adjusted to 5 ml using absolute ethanol then stored at -20 °C for further uses. Analysis of total phenolic contents were determined according to Koufan *et al*. (2020). For 20 µl of the phenolic extract, 1.6 ml of ultrapure water, and 0.1 ml of Folin-Ciocalteu reagent were combined to create the ethanolic extracts. After allowing the mixture to stand for 8 minutes at room temperature, 0.3 ml of a 20% sodium carbonate solution was added. The reaction mixture to stand for 2 hours at room temperature in the dark, the absorbance at 760 nm was measured using a spectrophotometer.

The total phenolic content was expressed as gallic acid (GAE) equivalents (mg per gram of dry extract) using the following formula, based on the calibration curve:

$y = 0.0037 x - 0.0002$

where y is the absorbance and x is total phenolic content

Statistical analysis

The comparison of those parameters in all characteristics between the two different treatments consisted of *in vitro* plant and seed-derived plants which were analyzed according to completely randomized design (CRD) with 40 replicates per treatment. Data were tested by using one-way analysis of variance (ANOVA) and the significant differences between means were separated by Duncan's multiple range test (DMRT) (p <0.01) using the program R statistical package version 2.14.2.

Results

Agronomical characteristics

Yield components in rice consist of number of tillers per plant, panicles per tiller and paddy weight. Rice cultivar which has the higher of those components must give the higher yield. The present study clearly showed that vitro-derive plants gave higher results in both number of tillers (34.00 tillers/plant) and panicles per plant (34.00 panicles/plant) but significantly different was not found. Whereas paddy weight (1.765 g) was significantly different (p<0.01) (Figure 2). The results from this study are clearly indicated that rice plant obtained from tissue culture technique give higher yields than those grown by seed (conventional method).

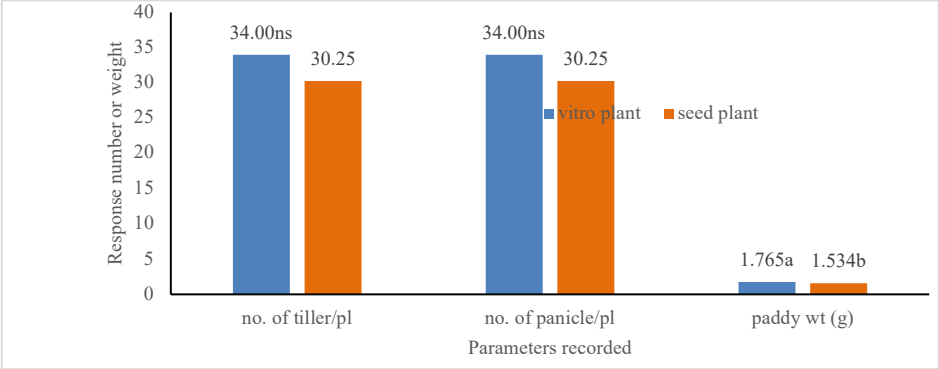

Figure 2. Yield component between vitro plant and seed-derived plants grown at PRRS for 120 days

ns: not significantly different; means showing different letters between histogram are significantly different by DMRT

Physiological characteristic

In vitro plants of rice var. Sang Yod Phattalung obtained from multiplication shoot tips in 0.5 BA containing liquid OPCM gave higher chlorophyll content (39.65 SPAD unit) than seed-derived one (36.07 SPAD unit) as measured by SPAD technique. However, significantly different was not obtained (Figure 3).

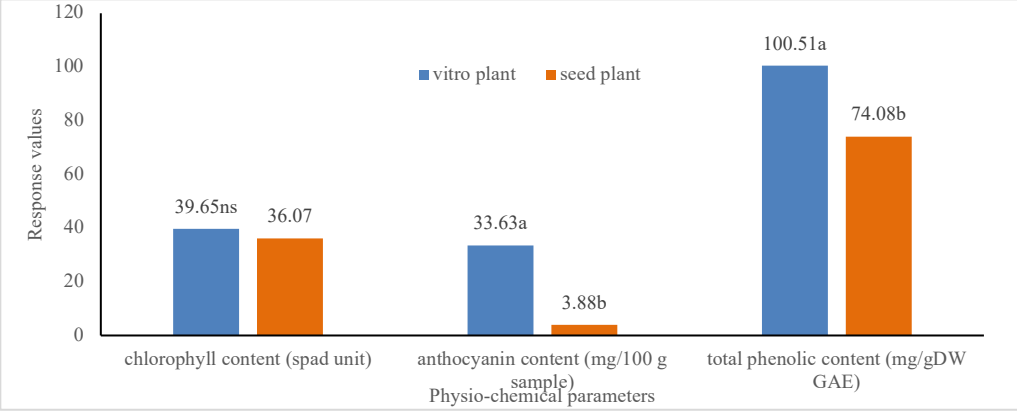

Figure 3. Physio-chemical characters between vitro- and seed-derived plants grown at PRRS for 150 days

ns: not significantly different, means showing different letters between histogram are significantly different by DMRT

Anthocyanin and total phenolic compounds

Interestingly, *in vitro*-derived plants gave far significantly different (p<0.01) of both anthocyanin (33.63 mg/100 g sample) (Figure 4) and total phenolic compounds (100.51 mg/gDW GAE) than those of seed-derived plants (3.88 mg/100 g sample, 74.08 mg/gDW GAE, respectively) (Figure 3).

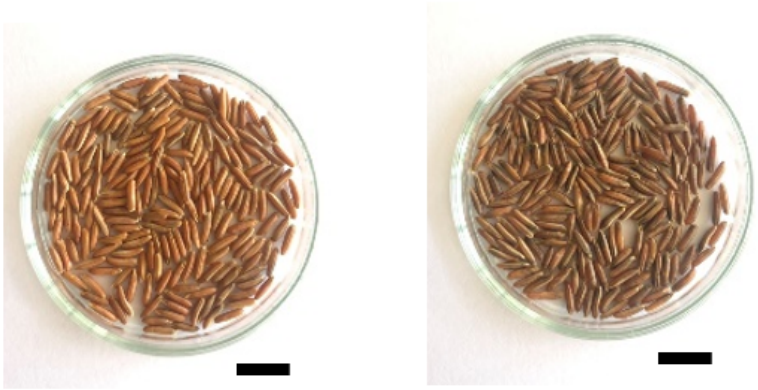

Figure 4. Dehusked seeds of *Sang Yod Phatthalung* rice obtained from seed-derived plants (left) and vitro plants (right) after growing at PRRS for 150 days (bars = 1 cm)

Discussion

Agronomical characteristics

In the present study revealed that rice plant obtained from tissue culture technique give higher yield than growing by seed. The most important factor involved in this phenomenal is plant growth regulators containing in culture medium during culture period, especially benzyladenine or BA. Wang *et al*. (2020) examined in inferior rice tillers and discovered that applying 6-BA raised the inferior tillers' grain counts and improved tiller yields even more. Additionally, they reported that 6-BA decreased the possibility of oxidative damage to chloroplasts by means of excessive excitations. Additionally, adding 6-BA increased the activities of catalase, ascorbate peroxidase, and leaf peroxidase and significantly reduced the build-up of H_2O_2 and malondialdehyde.

One of the main factors influencing rice yield is the number of tillers; it has been found that nitrogen and cytokinin have a significant regulatory effect on rice tillering. Nevertheless, little is understood about cytokinin regulating tillering bud growth and on the relationship of them during this process. BA is a

kind of synthetic plant growth regulator belonging to cytokinin group. It has been shown to stimulate nitrate reductase (NR) activity in the leaf and root of two rice cultivars, Yangdao 6 and Nanjing 44, as well as the germination of tillering buds and a significant difference in their subsequent outgrowth. This is because it significantly increases the cytokinin (Z+ZR and iP+iPR) content in the tillering node. (Liu *et al*., 2011). Similar results were also reported by many authors for the positive effects of BA on agronomical results of both vegetative and reproductive stages of cultivated cereals e.g., wheat (Gupta *et al*., 2003; Zheng *et al*., 2016), barley (Hosseini *et al*., 2008) and maize (Gao *et al*., 2017).

Physiological characteristic

From result, vitro-derived plant gave higher chlorophyll content than seed-derived one. Similar result was reported in detached cotyledons of zucchini (*Cucurbita pepo*) treated with a solution of 45 µM BA for 72 hours. Based on dry weight of the cotyledon, chlorophyll content increased 27% compared to that of control treatment (soaking in distilled water at the same time) (Ananiev *et al*., 2004). Increase in chlorophyll content improves photosynthesis and accelerate crops grain productivity. Thus, this evidence supported the fact that vitro-derived plants promoted the higher results in agronomical characteristics than seed-derived plants. Like agronomical characteristics, this effect is also conferred by BA. Gao *et al*. (2017) reported in *Zea mays* L. that adding BA improve photosynthesis by increasing chlorophyll content. Similar results were also obtained in flowering plant, *Gerbera jamesonii* by Cioc *et al*. (2019) who reported that adding BA in the culture medium resulted in higher total chlorophyll contents in the leaves. Higher concentrations of BA applied to indoor ornamental plants improved the content of chlorophyll in the leaves of *Schefflera arboricola*, *Ficus benjamina*, and *Dizigotheeca elegantissima* foliage plants.

Cytokinin BA activates genes involved in chloroplast development in in vitro cultures, as described by Dobranszki and Mendler-Drienyovszki (2014), and plays a significant role in the development and structural differentiation of chloroplasts. Moreover, this cytokinin inhibited leaf senescence due to hamper chlorophyll break down, control plant morphometry and photosynthetic pigment content. However, application at high concentration may cause a reduction of those phenomena.

Anthocyanin and total phenolic compounds

In this study vitro-derived plants gave far significantly different (p<0.01) of both anthocyanin and total phenolic compounds than those of seed-derived plants. Similar result was reported by Siatka (2019) in callus culture of angelica

in the presence of 1 mg/L BA alone. However, in the present study, BA at lower concentration of 0.5 mg/L is effective. The different result might be due to different plant species and target organs that anthocyanin accumulation. In some cases, the optimum production of anthocyanin was reported in the presence of both BA and auxin, especially 2,4-D. In callus culture of *Sesbania grandiflora* L. (Red Katuray), the highest anthocyanin formation was obtained with the addition of equal concentration (2.5 mg/L) of both 2,4-D and BA in the culture medium after 21 days of culture. BA or 2,4-D alone containing the medium gave far lower production of that pigment (Largado-Valler, 2016). This evidence was supported by Diekman and Hammer (1995) who found that cytokinin (0.5 mg/L or 2.2 μM BA sprayed to leaves) stimulates a large accumulation of anthocyanin in *Arabidopsis* and that this increase is due to the coordinate increased accumulation of mRNAs encoded by four genes in the anthocyanin biosynthesis pathway.

Higher plants include secondary metabolites called anthocyanins, which provide flowers and fruits with their color. These are water-soluble vacuolar pigments, glucosides of anthocyanidins, which can have varying pH-related colors, such as red, purple, or blue. They belong to a class of molecules called flavonoids synthesized via phenylpropanoid pathway. They occur in all tissues of higher plants, including leaves, stems, roots, flowers and seeds. While the present study revealed that vitro-derived plants (obtained from 0.5 mg/L BA containing medium) gave the highest anthocyanin content and total phenolic content (Figure 3 and 4), significantly different ($p<0.01$) with seed-derived plants (without treatment of BA). Thus, propagation of Sang Yod Phattalung rice through in vitro technique in the presence of 0.5 mg/L BA is beneficial for chemical contents in dehusk seeds served to the demand of consumers in the future like those reported in Red Katuray by Largado-Valler (2016).

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