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Welcome Message

With great pleasure, the Rajamangala University of Technology Phra Nakhon (RMUTP) welcomes you to "The 10th RMUTP International Conference on Science, Technology and Innovation for Sustainable Development: Turning Digital Disruptions into Opportunities 2019 (10th RMUTP ICON SCi-2019)". This event will be held on the 4-5 June, 2019 at the Sukosol, Bangkok, Thailand. We also welcome participants from overseas to Thailand and look forward to giving you a taste of Thailand's culture.

Our conference provides an outstanding international forum to present and discuss progress in research, development, standards, and applications of the topics related to Science, Technology and Innovation for Sustainable Development.

The 10th RMUTP International Conference will offer high quality activities including research and poster sessions. We feel sure to provide you an engaging environment with an excellent opportunity to exchange new research results, major ideas and start fruitful collaborations. International visitors are also encouraged to experience the Thai culture and attractions around Bangkok. We take this opportunity to thank you for your participation, we hope you enjoy your time and take advantage of our conference. We look forward to seeing you.

Sincerely Yours,

Supatra Kosaiyakanont

Assoc. Prof. Supatra Kosaiyakanont
President of Rajamangala University of Technology Phra Nakhon
Conference Chair, The 10th RMUTP International Conference

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Content

SUSTAINABILITY SCIENCE

Screening of Candidate Mutants of KDML105 Rice using Salinity Tolerance Score and Physiological Indices

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Keywords: Salinity stress, Rice, Mutants, KDML105, Soluble sugars, Photosynthesis

Abstract. Salt tolerance abilities of KDML105 and its mutants were evaluated using "Salinity Tolerance Score" (SS) based on the standard evaluation system of IRRI. Seedlings of candidate mutants, treated with 150 mM NaCl for 5 days in hydroponic system, were screened for salt-tolerance using both scores of SS (SS = 3) and physiological indices. Three lines of mutants MT4, MT5 and MT 6 scoring 30.65%, 46.0% and 34.0% respectively at SS = 3 were selected. Selected lines were further evaluated for yield component under salt stress which proved better performance of MT5 over MT4 and MT6 lines. On the other hand, treatment of salt at 250 mM NaCl at booting stage showed different responses of all tested mutants in terms of physiological indices. Photosynthetic pigments (Chl_a, Chl_b and carotenoids) and net photosynthesis rate (NPR) invariably reduced in all mutants and KDML105 due to NaCl stress. Despite of relatively lower contents of pigments, mutant lines displayed higher NPR compared to KDML105 under salinity stress. Soluble sugars (glucose, fructose and sucrose) were examined in root, leaf blade and leaf sheath of KDML105 and mutant lines under salinity stress. Endogenous contents of glucose and fructose dropped significantly in roots and leaf sheath of KDML105 and mutants, whereas salt stress caused considerable increase in the contents of glucose and fructose in leaf blade of all lines. Further, candidate salt-tolerant mutants had more hexose contents in leaf blade than KDML105. Growth and morphological performances of salt-tolerant mutants were examined on the basis of shoot length, number of tillers, panicle length, number of spikelets, fertility percentage and grain weight. In conclusion, selected mutants displayed promising salt-tolerant abilities, growth and yield.

1. Introduction

Salinity is considered as one of the major abiotic constraints for agricultural production mostly in arid and semi-arid regions. High concentration of salts can interfere potential of water uptake in plant, and high salt ions such as Na^+ and Cl^- are toxic to plant cells [1]. Salt stress cause various consequences in plant physiological responses such as membrane leakage, ion imbalance, increased production of reactive oxygen species (superoxide radicals, hydrogen peroxide and hydroxyl radicals), unbalancing osmotic pressure, etc. These physiological stresses can also cause a progressive loss in amount of chlorophyll, leading to a corresponding reduction of light absorption by leaves [2] and finally lead to suppression in plants growth and development. Salinity stress occurs mostly in the Northeastern part of Thailand where almost 35% of the land area faces a varying degrees of salt stress

problems which limits the crop production [3]. Rice is considered as glycophytic species, which is sensitive to salt stress at both vegetative [4] and reproductive stages [5]. In order to meet the increasing demand of rice, there is an urgent need to develop rice varieties for saline prone areas with higher yield potential and greater yield stability. Strategies to improve salt-stress tolerance in crops have been widely used through conventional breeding. However, this approach can be costly, space and time consuming, labor intensive, and requires large population. Mutation breeding method is one of the good alternative method where the mutants are created using different kinds of mutagen to produce improved characteristic such as photoperiod insensitivity, early flowering, and short in stature and anthocyanin accumulation in pericarp. Many interesting lines of KDML 105 have been derived using these techniques namely PKOS1, TKOS4, and BKOS6 [6]. Previously, KDML 105 mutants have been produced by exposing the seeds of KDML 105 with the combination of gamma irradiation and ethyl methanesulfonate (EMS) with different concentrations [7]. In the present study, M4 mutant generation was used to screen for salt tolerance based on Salinity Tolerance Score at the seedling, and early reproduction (booting) stage. The phenotypic expressions such as growth and development in responses to salt stress, tiller number, no. of spikelets, photosynthetic capability, soluble sugars etc. were investigated in candidate mutant lines.

2. Material and methods

2.1 Plant material and screening for salt tolerance

KDML 105 seeds were obtained from Pathumthani Rice research Center, Thailand. Along with this, M4 generation of KDML 105 mutants were obtained from Laboratory of System Plant Biotechnology, Department of Biotechnology, Faculty of Science, Mahidol University [7]. The M4 generation mutants included 3 lines namely MT4, MT5 and MT6. Seeds of M4 generation KDML105 mutants along with KDML 105 were dehusked and selected for the complete shape of grain with characters like long grain, clear endosperm without chalkiness and large and healthy embryo. Dehusked seeds were briefly surface sterilized with 70% ethanol for few seconds prior to washing in solution of 15% Clorox for 20 min, kept in a water bath for 20-30 min and then rinsed 3 times with sterile distilled water. Sterilized seeds were allowed to germinate on a Petri dish for 4-5 days and were transferred to a tray containing half-strength Yoshida solution for 8 days. Then, the seedlings were transferred to the Hydroponic system (Hydrowork 200, Higreen Hydroponics Farm) containing half strength Yoshida solution for 10-15 days. The Yoshida solutions were replaced every 2 or 3 days and pH was maintained around 5.8 during the entire growth period. The salt treatments were imposed by supplying the nutrient solution with NaCl at the rate 150 mM for 5 days and the mutant lines were evaluated for salt tolerance. Survived seedlings (from salt treatment of 150 mM NaCl at the seedling stage in hydroponic system) were recovered and transferred to green house into pots having holes on all side for the inlet and outlet of water or salinity solution. The seedlings were regularly supplied with Yoshida solution and allowed to grow until booting stage. At booting stage, mutant lines were again treated with 250 mM NaCl and evaluated for salt tolerance. Evaluation of the salt stress symptoms was performed using the scoring system known as salinity tolerance score (SS) based on the standard evaluation system (SES) with some modifications [8]. Scoring was based on visible symptoms numbered from 1 as highly tolerant, 3 as tolerant, 5 as moderately tolerant, 7 as susceptible and 9 as highly susceptible. Plants were randomly sampled and observed for morphological characteristic after fourteen days of 250 mM NaCl treatment. The parameters included number of tiller, number of

panicle, panicle length, number of spikelet per panicle, their percentage of fertility and grain weight per plants.

2.2 Analysis photosynthetic pigments and net photosynthetic rate

Mutant lines treated with 250 mM NaCl were examined for chlorophyll content and the total carotenoids (Cx+c) concentration [9, 10]. The leaves of the randomly sampled mutant lines were weighed about 100 mg and ground with liquid nitrogen. The samples were placed in a glass vial along with 5 ml of 95.5% acetone. Then the glass vials were sealed to prevent evaporation and then incubated at 4 °C in darkness for at least 42 hr. The concentrate solution was filtered through Whatman's filter paper and 1 ml of it was used to measure OD at spectrum of 661, 644 and 470 nm respectively in a UV-visible spectrophotometer (DR/4000, HACH, Colorado, USA) using acetone as blank. The chlorophyll content were calculated according to the following equations: [Chla] = 9.784D662 - 0.99D644; [Chl_b] = 21.42D644 - 4.65D662 Total chlorophyll = [Chl_a] + [Chl_b]; carotenoids = (1000 A_{470}−2.270 Chl_a – 81.4 Chl_b)/214. Net photosynthetic rate was measured at the second leaves ranked from the top of the rice shoot and were measured between 9:00-15:00 h using Portable Photosynthesis System with an Infrared Gas Analyzer (IRGA; Model LI 6400, LI–COR® Inc, Lincoln, Nebraska, USA) according to the method of Cha-um et al. (2006). The net photosynthetic rate was examined at day 0, 1, 3,7,10 and 14 of salt treatment.

2.3 Soluble sugar analysis

Determination of soluble sugar extraction (sucrose, glucose and fructose), the flag leaf of mutants were analysed according to the modified method of [11]. 50 mg of the leaf samples were ground in liquid nitrogen followed by addition of 1ml nano-pure water, shaken vigorously for 15s and sonicated for 15 minutes. Then sample were centrifuged at 12,000 rpm for 15 min. The supernatant containing the soluble sugar were filtered through a 0.45 µm membrane filter (VertiPrure™, Vertical®) and were stored at -20 °C. Further, the sugar analysis process was carried out using HPLC. Crude extracts with a volume of 15 µl were automatically injected into the HPLC system. The analytical column used in the HPLC was a MetaCarb 87C, equipped with a cartridge holder and was analysed online with a 410 differential refractometer detector. Deionized water was used as the mobile phase with a 0.4 ml min^{-1} flow rate. Sucrose, glucose and fructose (Fluka) were used as standards [12]. The presence of soluble sugars in leaf blade, leaf sheath and roots were analysed qualitatively by comparing the retention time of the peak area in samples and standard (Appendix G, H and I). Standard sucrose, glucose and fructose (Sigma-Aldric) were used to prepare standard curve.

3 Result and discussion

3.1 Screening KDML 105 and its mutants for salt tolerance

3.1.1 Evaluation at seedling stage under hydroponic solution

KDML105 and its mutant lines MT4, MT5 and MT6 were screened from population of the clump no. (CN) as CN_1 - CN_{10} and CN_{11} - CN_{20}, with each clump no. having 5 replications. M_4 generation mutants showed normal growth under hydroponic solutions in the absence of salt stress. The seedlings of mutant lines were grown in hydroponic solution and treated with 150 mM NaCl for 5 days. Visual scoring of salt-injury symptoms of the seedling was determined using salinity tolerant

score (SS) and the survival percentages in each mutant line (Fig. 1). The symptoms of salt stress based on the SS score with some modification on visual aspect were recorded as - score 1: normal growth with no leaf symptom; score 3: nearly normal growth with leaf tips of few leaves whitish and rolled; score 5: mostly rolled leaves or few yellow leaves, score 7: growth severely retarded, most leaved dry with only a few leaves elongating; score 9: some plants dying, almost all plant dying or dead. The Salt Tolerance Score (SS) has been approved to be a good practice to evaluate the plant line under any stresses depending on many environmental factors.

The CN_1 to CN_{10} mutants showed the highest percentage of survival at score 3. Among the MT4, MT5 and MT6 lines, MT5 showed the highest percentage of survival of 58% in score 3, which concluded that MT5 mutants are more tolerant to stress of 150 mM NaCl as compared to KDML 105. The average percentage of survival of MT4, MT5 and MT6 lines were 78%, 86% and 82%, respectively. Similarly, in 2nd repeat group with code no. CN_{11}- CN_{20}, MT5 lines showed the highest survival percentage (34%) when compared to KDML 105, MT4 and MT6 lines. This indicated that MT5 mutant was able to tolerate the stress more than MT4 and MT6 in both groups. The average survival percentage of MT4, MT5 and MT6 lines were 82%, 86% and 72%, respectively. However, among all the tested mutant lines, no candidate mutant line showed highly tolerant score which is SS 1.

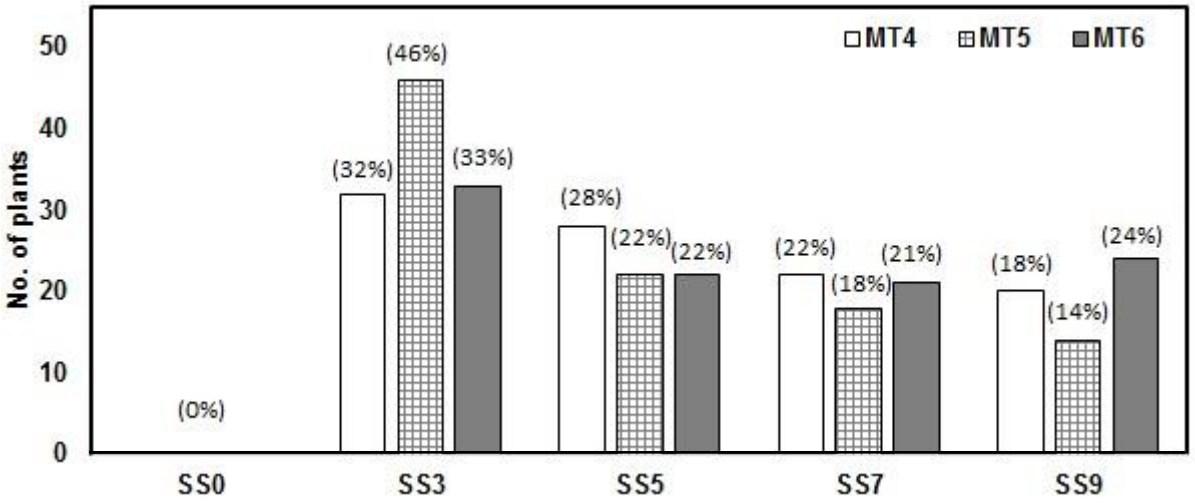

Fig. 1 Screening of salt-stress tolerance among seedlings of KDML105 mutant using M4 generation of mutant code MT4, MT5 and MT6. There were total 20 clumps in each mutant line which were sampling from different clump numbers (CN1-CN20), each CN with 5 replications. Evaluation was performed by Salinity Tolerance Score (SS) ranking from less tolerance (SS9) to most tolerance (SS1) based on the standard evaluation system (SES) after treated seedlings with 150 mM NaCl. The Fig. shows no. of plants of each mutant line (MT4-MT6) scoring at SS3, SS5, SS7, and SS9 but none of these lines show SS1 score.

3.1.2 Evaluation at booting stage under field conditions

Rice being usually sensitive at the seedling and early reproductive stages, it is important to screen the mutant lines not only at seedling but also at early reproductive stages [5, 13]. Henceforth,

evaluation of salt tolerance was done at the fully vegetative /early reproductive stage (booting stage) in order to confirm the reliability of screening at the seedling stage (20-day after germination) and to examine the physiological and biochemical responses of the mutant lines under salinity stress. The mutant seedlings which scored as SS3, SS5 and SS7 for their salt-tolerant property were chosen to recovery in no salt condition and further grown in pot for 45-50 days till booting stage (approximately 84 days after germination). The rice clumps were then separated into two sets; one to gain the seeds while another set was to test with salt stress at 250 mM NaCl. They were allowed to adapt in the green house for 5 days and further treated with 250 mM NaCl solution along with KDML 105. The EC of the salt water was maintained about 12 dSm during experimental period. The mutants subjected to salt stress for 5 days with 250 mM NaCl showed normal growth. On the other hand, experiment under non-salinized solution were also set up for comparison. The candidate mutants exhibited 100% of survival after 5 days of treatment. The relative percentages of survival and screening of the salt-stress tolerance among the tested mutants at the seedling stage and fully vegetative stage on day 5 after salt treatment were compared (Fig, 2). These comparisons were made in order to check the mutants treated with the single stress (150 mM NaCl at seedling stage) and the double stress (150 mM at seedling stage, recovering and then followed with treatment of 250 mM NaCl at fully vegetative stage). Comparison between the scores during seedling and booting stage showed that both treatments showed no candidate mutant that scored as SS 1 (highly salt tolerant). However, seedlings with 150 mM NaCl ranged the SS score from 3 to 9, which were not higher than 35%. On the other hand, the group of seedlings that pretreated with NaCl for 5 days and then selected only those that got SS3 and SS5 to further treated again with 250 mM NaCl at booting stage showed better percentages of SS3 in all tested lines. Different proportions of SS score were observed among mutant lines and stages of development. When exposed to 250 mM NaCl, MT5 and MT6 lines exhibited more salt-tolerant score at score 3 compared to MT4 line. All candidate mutants exhibited 100% survival and no plant scored SS7 and SS9 (salt sensitive) even at higher concentration of 250 mM NaCl. The highest proportion of mutants was observed in SS3 (79.9-86.6%). Therefore, the mutant lines showed more salt-tolerant score (SS3) with high percentages of survival at the early reproductive stage, which obtained from first screening at the seedling stage at 150 mM NaCl. At day 5 of salt treatment, most of tested mutants showed average 84.4% of SS 3 and 15.5% of SS 5. Among tested lines, MT5 and MT6 exhibited relatively more percentages of survival (86.6%) at SS3.

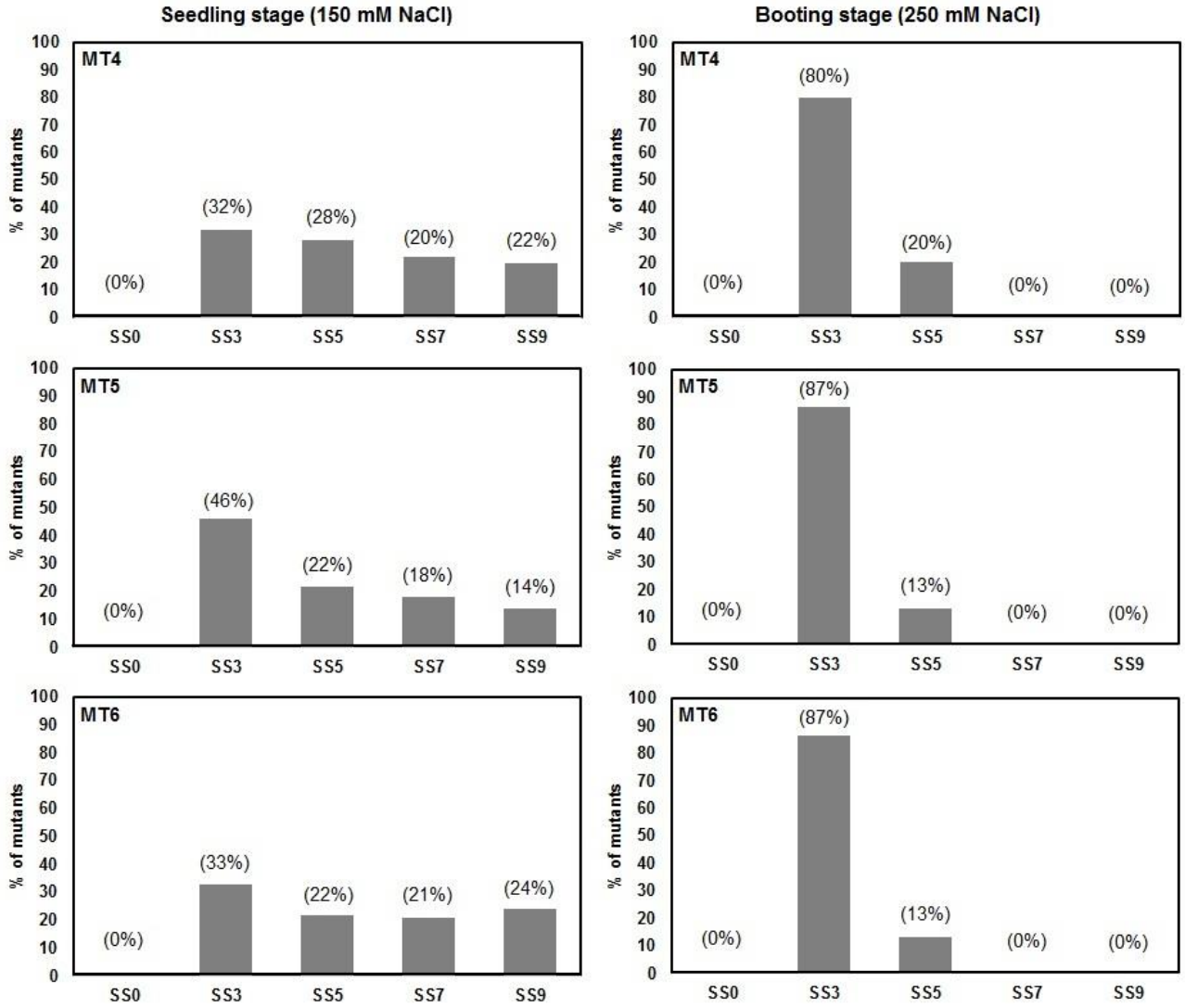

Fig. 2 Comparison of the salt tolerance score (SS) evaluated among mutants of KDML105 (MT4, MT5 and MT6) between the group of seedlings exposed with single salt stress (150 mM NaCl, 5 days) and those treated twice with salt stress at seedling stage (150 mM NaCl, 5 days) and later treated again with 250 mM NaCl at booting stage (5 days).

Further extension of 250 mM salt treatment period until 14 days resulted in stress symptom on the flag leaves. None of the mutant lines survived at SS3 whereas they scored 36.8%, 35.9% and 24.4% at SS5, SS7 AND SS9 respectively when exposed with salt for 14 days. Highest percentage of survival (46.7%) at SS5 was detected in MT4, whereas MT5 and MT6 scored only 33.3% and 26.6% survival at SS5 respectively. The percentages of rice mutants that exhibited severe stress symptoms (SS7) on day 14 were 33.3% in MT4, 26.6% in MT5 and 46.6% in MT6 lines. Further, an average of 24.4% mutant population demonstrated SS9 for severe symptom to salt stress. MT4, MT5 and MT6 finally exhibited 6.7%, 5.2% and 8.9% of plant death, respectively after 14 days of 250 mM NaCl treatment. The SS score and the survival percentage in each mutant line at day 5 and day 14 were recorded. The SS evaluation of salinity tolerance based on the standard evaluation system was able to determine the responses of mutant seedling as moderately tolerant, susceptible and highly susceptible

for 14 days period of salt stress at booting stage of development. It can be suggested that the selection system did not rely only on the SS score but also on environmental factors such as period of salt treatment, cultivation season, relative humidity (% RH) and temperature. 250 mM NaCl stress exposure up to 14 days affected growth of leaves and clump size and panicle development of KDML105 rice (Fig. 3). Comparison of spikelet development between each candidate mutant treated with salt stress was done. The spikelet of mutant treated with 250 mM NaCl showed incomplete seed set when compare to KDML105 grown in normal condition. The rice panicles showed severe damage caused by stress, especially at the top part of panicle (Fig. 4). The mutant line MT4-CN16 and MT-6CN22 showed early setting of flowers which might either be caused by poor fertility or poor grain filling capability. This indicated that salt stress affected panicle and spikelet development, which further affect the grain filling process. These damages finally end up leading to a reduction in grain yield [13, 14]. Conclusively, mutant lines treated with 250 mM NaCl for 14 days at booting stage suffered from severe stress symptoms.

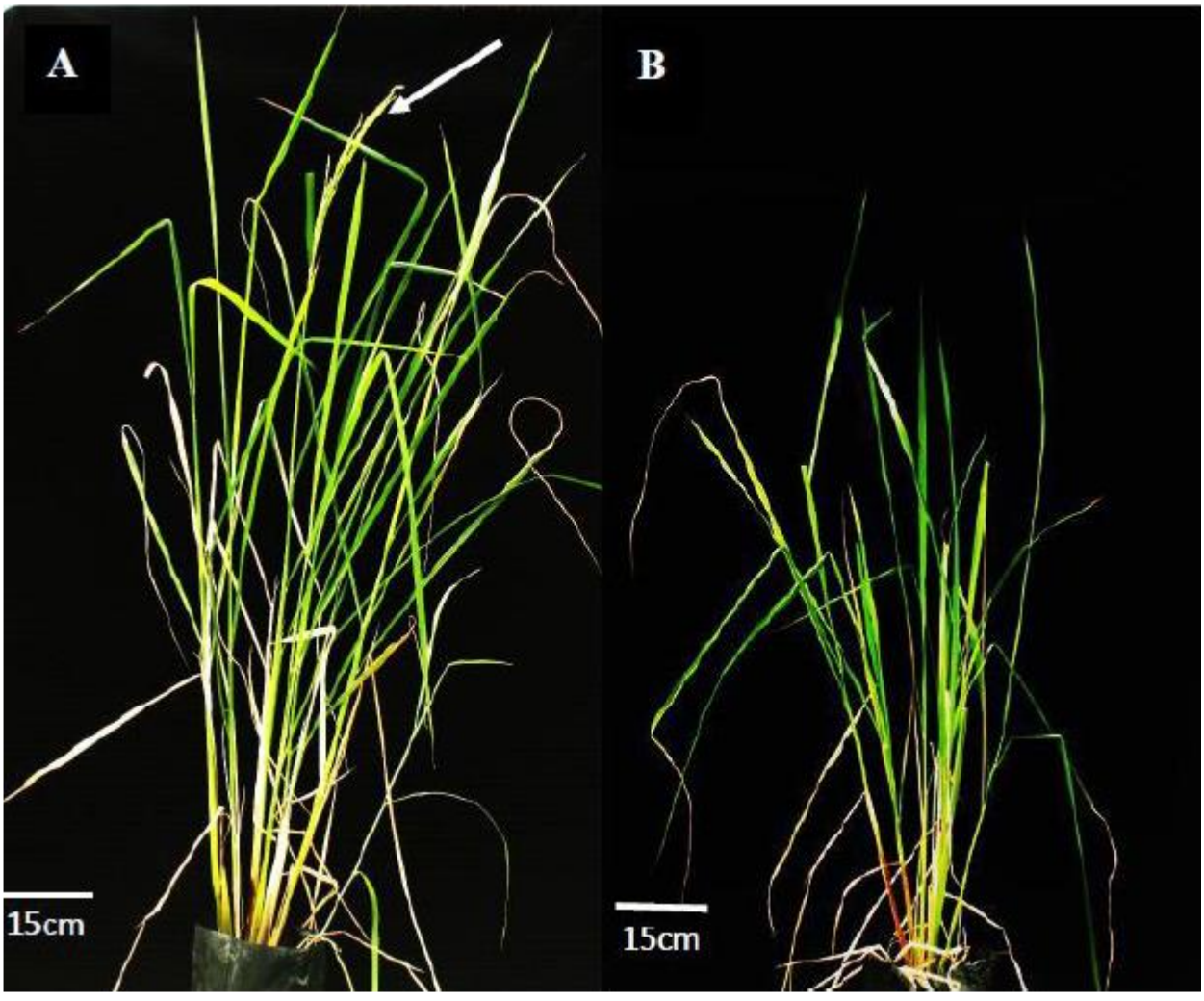

Fig. 3 The morphology of KDML105 rice clump at 84-day-old grown in pots that treated with 0 mM NaCl (A) and 250 mM NaCl (B) for 14 days. The KDML105 without salt stress showed development of complete spikelet (arrow).

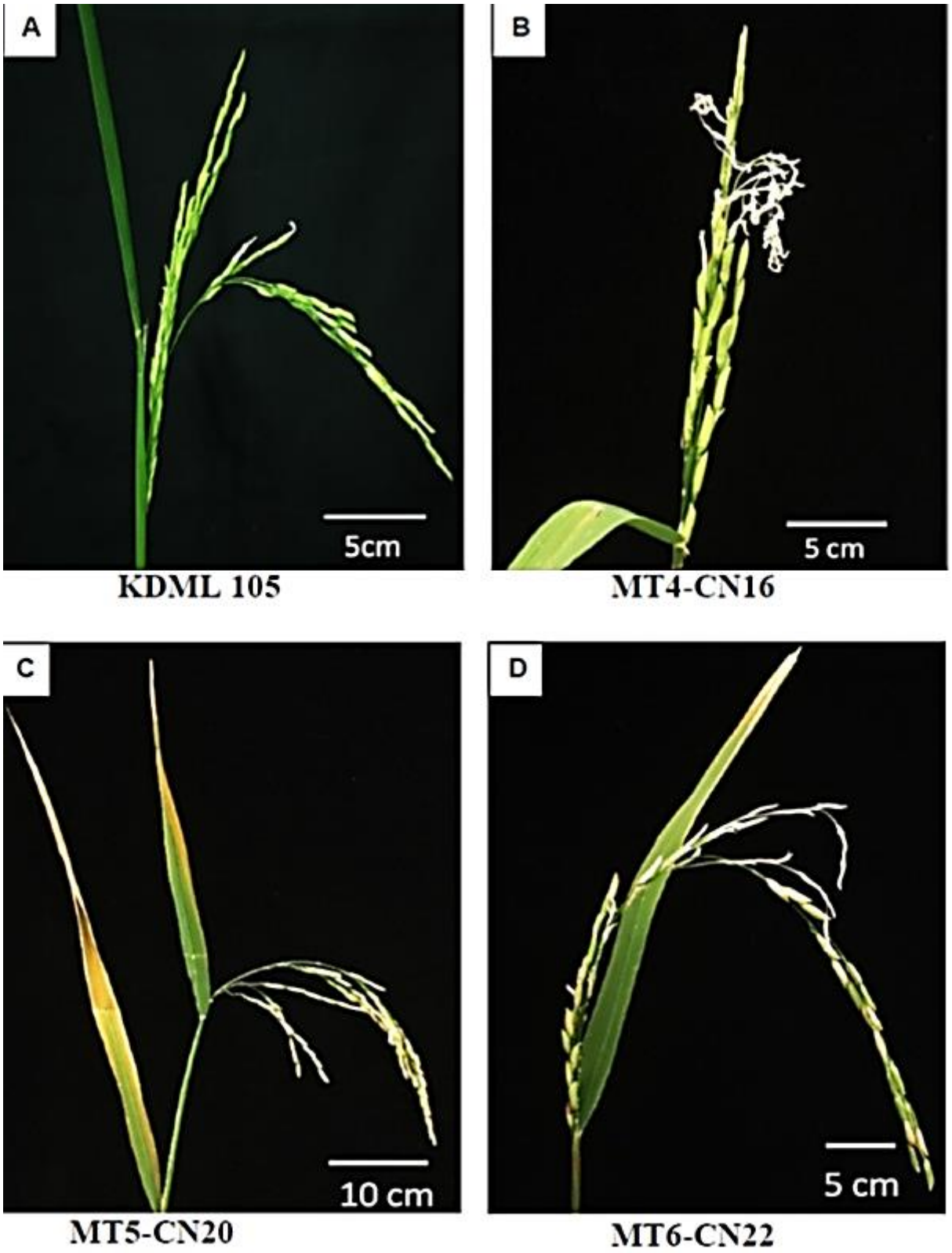

Fig. 4 The characteristics of spikelet KDML105 in condition without salt (A) shows normal spikelet and seed set (control), whereas (B), (C) and (D) illustrated its candidate mutants (MT4-CN16, MT5-CN20, MT6-CN22) when exposed with 250 mM NaCl for 14 days.

3.2 Evaluation of the morphological responses in selected KDML 105 mutants

Selected KDML 105 mutants affected by double stress of 150 mM at seedling stage, followed with 250 mM NaCl at booting stage were analysed for morphological responses. The candidate mutants were selected from CN11-CN24 based on their SS scores recorded at both seedling stage and booting stage. Under the stress of 250 mM NaCl, the mutant population showed reduction in the shoot length and numbers of tiller (Table 1). Among the mutant lines, MT5 showed more reduction in the shoot length than MT4 and MT6 when compared to the KDML 105 (with NaCl). The mutant lines also showed significant reduction in the panicle length, no. of spikelet per plant and fertility rate. Interestingly, MT5CN11 displayed highest fertility rate compared to other tested mutants and KDML 105 (with NaCl). However, the total grain weight of all mutants drastically reduced under salt stress. KDML 105 (with NaCl) showed 14%, 34% and 25% reduction in the shoot length, panicle length and numbers of spikelet per panicle. The total numbers of spikelet, fertility rate and the total grain weight were also dramatically reduced to 58%, 63% and 90%. These results indicated that the stress of 250 mM NaCl at booting stage significantly affected morphological characters and yield of mutants.

Table 1 Morphological determination of KDML105 and its candidate mutants selected from seedlings of CN11-CN20 screened with 250 mM NaCl. The different letters within the columns indicate significant differences at $p < 0.05$ (*) or highly significant differences at $p < 0.01$ (**) of mean as analyzed by DMRT.

Plant line	Shoot length (cm)	No. of tiller	Panicle length (cm)	No. of spikelet per panicle	No. of spikelet per plant	Fertility (%)	Grain weight per plant (g)
KDML105 (Control, without NaCl)	131.0±1.0 a	9.6±1.5 a	38.6±2.5 a	133.6±9.3 a	451.0±2.6 a	72.5±1.6 a	3.3±0.1a
KDML105	113.3±1.0 b	8.0±1.0 b	25.4±1.6 b	100.6±8.3 b	187.6±1.5 b	26.5±1.3 b	0.3±0.0 c
MT4 CN12	57.3±1.0 d	2.3±0.6 c	21.1±0.8 cd	42.6±2.1 f	83.3±1.5 e	16.8±0.5 c	0.4±0.0 b
MT4 CN16	61.2±0.9 c	2.6±0.6 c	19.5±0.8 d	47.0±1.0 ef	91.3±2.1 d	18.1±0.9 b	0.4±0.0 b
MT4 CN20	62.6±0.6 c	2.6±0.6 c	20.0±0.5 cd	40.6±2.1 f	84.0±2.0 e	16.9±0.2 c	0.3±0.1 c
MT5 CN11	53.3±0.9 e	2.0±0.0 c	20.0±0.2 cd	56.3±1.5 cd	102.0±1.0 c	21.1±1.0 b	0.4±0.0 b
MT5 CN14	54.2±0.9 e	2.3±0.6 c	20.0±0.8 cd	62.0±2.0 c	93.0±1.0 d	18.8±0.1 b	0.4±0.0 b
MT5 CN20	53.6±0.5 e	2.0±0.0 c	19.2±0.2 d	56.0±1.0 cd	94.0±1.0 d	19.1±0.1 b	0.4±0.0 b
MT6 CN15	62.8±0.5 c	2.6±0.6 c	20.0±0.2 cd	52.6±2.0 de	64.0±2.0 f	13.2±0.1 d	0.3±0.0 c
MT6 CN17	61.1±0.1 d	2.3±0.6 c	21.9±0.6 c	55.0±1.0 cd	66.6±1.5 f	13.9±0.4 d	0.4±0.0 b
MT6 CN18	63.0±0.7 c	2.3±0.6 c	20.3±0.2 cd	51.0±1.0 de	65.0±1.0 f	13.2±0.2 d	0.3±0.0 c
Significant level	**	*	*	**	**	*	*

The initial osmotic effect of NaCl stress inhibits growth and development. The ionic effect causes premature leaf senescence, leaf rolling, chlorosis or necrosis at the tips and in marginal parts of older leaves and stunted shoot growth. The remaining green leaf area and younger leaves are thus maintained by the stressed plants, probably to cope with the stress [15]. In the present study, the mutant

lines exhibited the salt-stress symptoms caused by 250 mM NaCl such as leave necrosis, sterility, incomplete spikelet and low seed set. It was previously reported that salt stress affects pollen viability, stigma receptivity and seed setting, leading to reduction in grain yield [13, 14]. In our experiment, it was found that salt stress at 250 mM NaCl reduced the number of primary branches, panicle length, panicle weight and spikelet numbers of mutants. The low grain weight observed in mutant lines is possibly caused by the reduction of cellular metabolic activities which might restrict the cell wall elasticity, thus making it rigid and consequently decreasing the cell turgor pressure. The other probable cause could be due to the shrinkage of cell contents, reduced development and differentiation of tissues, imbalanced nutrition and damage to the membranes [16, 17].

3.3 Analysis of photosynthetic pigments and net photosynthesis rate in selected mutants

Determination in the contents of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll and carotenoids was done in KDML 105 mutants when exposed to 250 mM NaCl for 14 days (Table 2). The mutants showed significant reduction in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids when compared to KDML 105 (+ NaCl). On exposure of KDML105 to 250 mM NaCl for 14 days, chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids were reduced to 25%, 44%, 25% and 24%, respectively. Chlorophyll degradation could be induced by salt stress, which may directly or indirectly affects the net photosynthetic rate in rice [13]. Mutants also showed reduction in the total chlorophyll and carotenoids but some lines were able to sustain the photosynthetic capability. Among the selected mutants for salt tolerance based on SS3, some mutant lines performed better tolerant capability to salt stress in terms of morphological index while others were better at physiological indices. For example the mutant line MT5CN-11 showed highest percentage of fertility, whereas MT4CN 12 line displayed highest rate of net photosynthesis under salt stress. Chlorophyll degradation could be induced by many stress factors, leading to changes of certain enzyme activities, photosynthetic electron transport, carbon metabolism, and photophosphorylation in photosynthesis [18]. Salt stress induced damages of photosynthetic pigments are coupled with destruction of various enzymatic pathways responsible for CO_2 harvesting during photosynthesis. In this experiment, candidate mutants clearly showed chlorophyll degradation and growth reduction. Present results too agreed with previous reports indicating reduction in the chlorophyll contents of rice under salt stress depending on the level of salt tress, length of exposure and salt tolerance capability of rice [19].

Net photosynthetic rate (NPR) was measured in the flag leaf of KDML 105 mutants exposed to 250 mM NaCl (Fig. 5). It was found that KDML 105 (+NaCl) showed more severe reduction in NPR when compared to all the mutants. The ability of the M4 generation mutant to maintain the net photosynthesis under salt stress compared to KDML 105 may indicate that the mutant lines accumulated less Na+ in their flag leaves, leading to enhanced photosynthesis rate. Chla, Chlb, total content of chlorophyll and carotenoid contents were compared in correlation with NPR. It was found that there was no apparent correlation between NPR and pigmentation. Each selected mutant line showed reduction in the total chlorophyll and carotenoids but had higher NPR than KDML 105 (+NaCl). Among the mutant lines, MT5-CN19 and MT5-CN19 could retain adequate photosynthetic capability even being very low in photosynthetic pigments. This result indicated that these mutant lines had different responses to cope with the salt-stress where they were able to retain the photosynthetic capability even the photosynthetic machinery components such as chlorophyll was

reduced. The alternative defence responses might be due to the osmoregulation system by producing osmoprotective agents which are involved in stabilizing membrane integrity, some enzymes and proteins. These osmoprotectants include some compatible solutes such as sugars, polyols, amino acids and proline. Generally, sugars play significant role in plant defence mechanisms, therefore the soluble sugar were studied further to know the distribution of sugars in the different parts of rice mutants.

Table 2 Contents of Chlorophyll a (Chl_a), Chlorophyll b (Chl_b), total chlorophyll, and carotenoid in KDML 105 and selected mutants treated with 250 mM NaCl for 14 days. The different letters within the columns indicate significant differences at $p < 0.05$ (*) or highly significant differences at $p < 0.01$ (**) of mean as analyzed by DMRT.

Plant line	**Chl_a (µg g^{-1} FW)**	**Chl_b (µg g^{-1} FW)**	**Total Chl (µg g^{-1} FW)**	**Carotenoids (µg g^{-1} FW)**
KDML105 (Control, without NaCl)	545.5±3.2 a	237.2±3.3 a	782.7±6.5 a	162.8±1.6 a
KDML105	407.8±4.6 b	133.6±2.5 b	541.4±7.1 b	121.2±1.6 b
MT4 CN12	335.7±3.0 c	109.1±1.4 c	444.8±4.4 c	80.2±4.3 g
MT4 CN13	272.3±1.7 g	105.7±2.4 c	378.0±4.1 e	88.6±3.1 f
MT4 CN16	324.9±1.7 de	109.1±2.7 c	434.0±4.4 cd	98.4±1.0 de
MT4 CN19	287.2±2.5 f	98.5±1.1 d	385.7±3.6 e	84.0±5.2 f
MT4 CN20	323.2±2.6 de	98.0±0.6 d	421.2±2.3 cd	96.5±3.4 e
MT5 CN11	325.8±2.9 de	138.5±1.5 b	464.3±4.4 c	94.7±4.7 ef
MT5 CN14	331.1±2.8 cd	96.8±2.8 d	427.9±5.6 c	91.1±7.8 ef
MT5 CN16	289.0±1.3 f	136.6±3.5 b	425.6±4.8 c	96.4±3.3 e
MT5 CN19	288.0±3.1 f	110.8±1.8 c	398.8±4.9 cd	94.5±3.2 ef
MT5 CN20	277.0±3.7 f	97.7±0.5 d	374.7±4.2 g	77.5±2.9 h
MT6 CN15	286.5±4.0 g	107.8±4.6 c	394.3±8.6 f	98.9±1.2 de
MT6 CN17	334.0±5.0 c	136.0±1.8 b	470.0±6.8 c	107.1±5.8 c
MT6 CN18	330.1±3.8 c	132.9±1.9 b	463.0±5.7 c	105.2±4.6 cd
MT6 CN22	328.2±5.8 cd	136.2±2.4 b	464.4±8.2 c	106.6±7.0 c
MT6 CN24	332.4±2.5 cd	135.5±3.1 b	467.9±5.6 c	110.1±1.2 c
Significant level	**	**	*	*

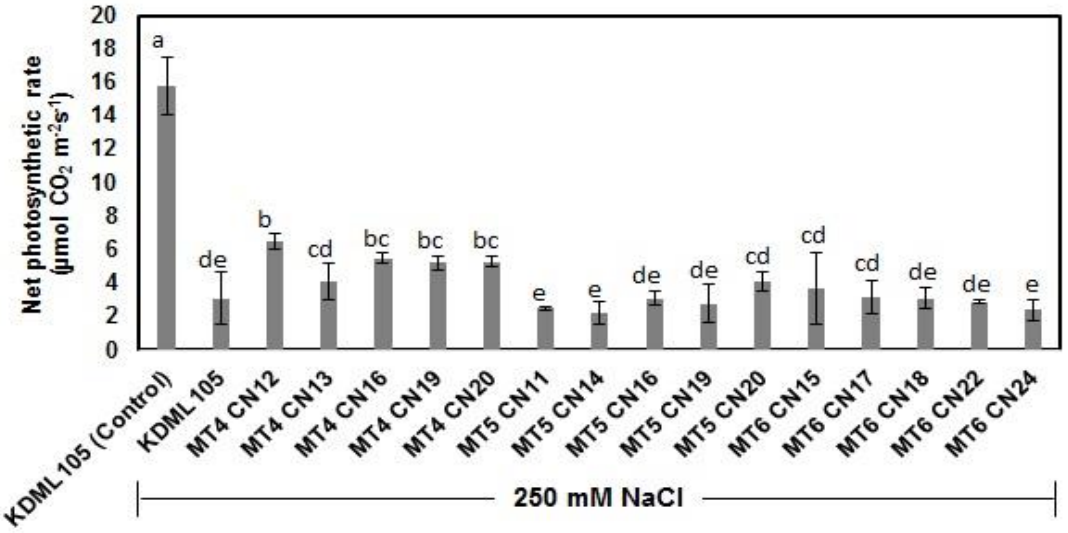

Fig 5 Net photosynthetic rate or Pn (µmol CO_2 $m^{-2}s^{-1}$) of 15 candidate mutants compared with KDML105 after treated with 250 mM NaCl for 14 days. The Pn of salt treated samples were compared with KDML105 that was cultured without stress (Control). The different letters on top of each bar graph, indicate highly significant differences of mean as analyzed by DMRT at $p < 0.01$.

3.4 Analysis of soluble sugars in selected mutants

The distribution of reducing sugars (sucrose, glucose and fructose) were measured in root, leaf blade and leaf sheath (Fig. 6) of the mutant lines treated with 250 mM NaCl. Three mutant lines that exhibited SS as moderately tolerant line (score 5) and a highly susceptible line (score 9) were selected in order to compare the changes of reducing sugar with KDML105. The different concentrations of each reducing sugar were detected in different parts. As root is first part that exposed to salt-stress, the contents of soluble sugar, especially fructose were expressively more in KDML 105 compared to its mutants, whereas KDML105 cultured without salt showed low contents of sugars. Generally, when the plants is under the salt or water stress, they try to maintain the osmotic potential in cytoplasm by accumulating more compatible solute such as proline, sorbitol, trehalose, etc. [20]. There was not much difference in the sugar contents in leaf blade between KDML105 with and without NaCl stress. Only sucrose content elevated up to 3 folds in salt stressed KDML105 compared to control, whereas glucose and fructose contents were slightly dropped. Interestingly, sucrose contents were very low in leaf blade of all tested mutant lines subjected to salt stress. In contrast with root tissues, glucose and fructose content in the leaf blade of salt stressed mutants increased considerably. The concentrations of glucose and fructose under salt stress in leaf blade of mutants were higher than KDML105. Lines. The high amounts of hexose (glucose and fructose) in the leaf blade may be due to a breakdown of disaccharide sucrose into glucose and fructose, caused by osmotic stress [21]. On the other hand, all mutant lines had less contents of soluble sugar in leaf sheath compared to KDML 105 with and without salt stress. However, sucrose contents were not much affected in the leaf sheath of all treated mutants baring few exceptions. The mutant lines showed reduction in the accumulation of the soluble sugars, in particular, the contents of hexose sugars (glucose and fructose). It has been reported that both salt-sensitive cultivar (PTT1) and salt-tolerant cultivar (Hj) when exposed to 342 mM NaCl at seedling stage were enriched in soluble sugar in most parts of young leaves.

Soluble sugars have been reported as osmoprotectants which are transported from older tissues to newly growing tissues during salt stress [12, 19, 22].

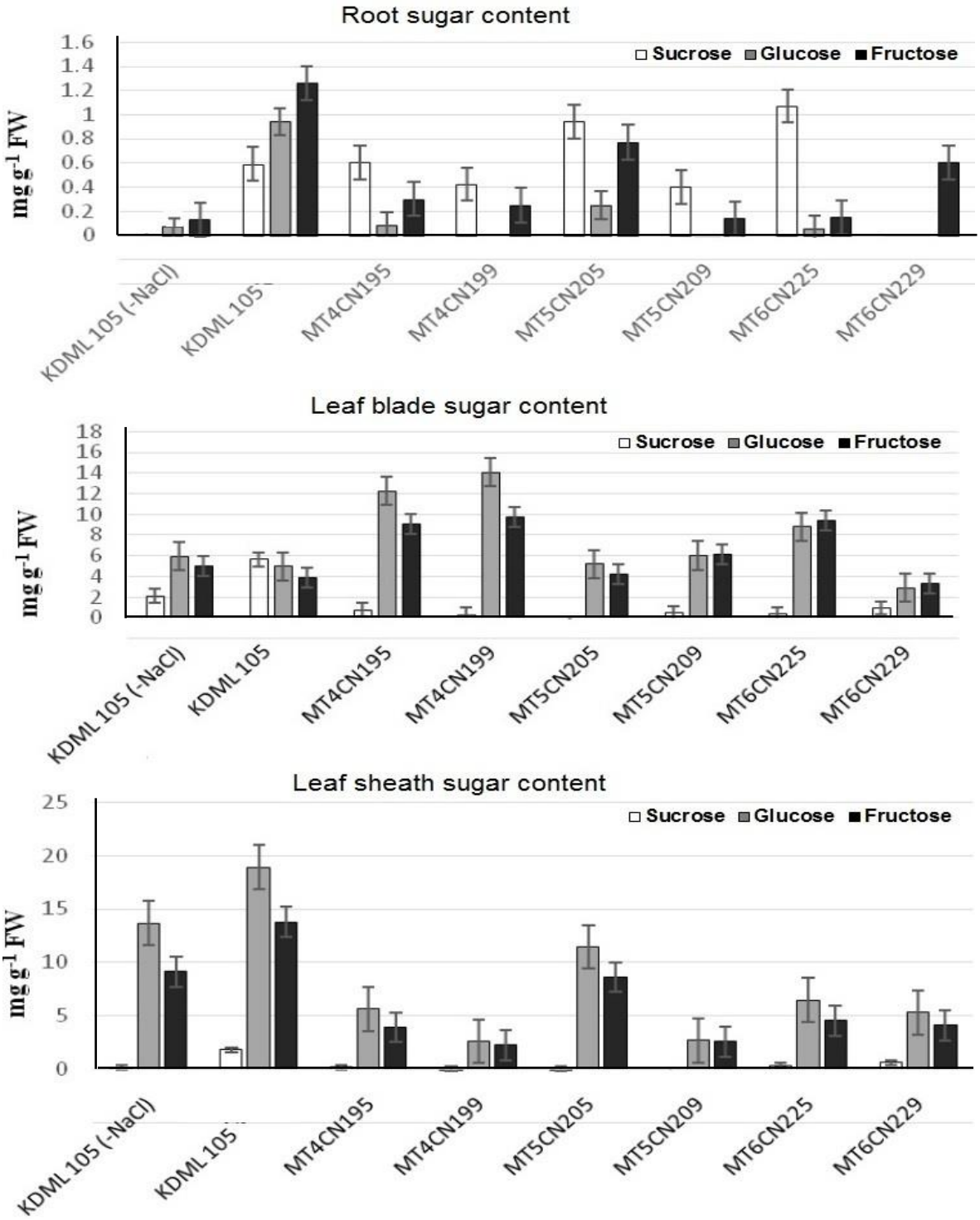

Fig 6 Content analyses of sucrose, glucose and fructose examined in the root, leaf blade, and leaf sheath of KDML105, MT4CN19. MT5CN20 and MT6CN22 after exposed to 250 mM NaCl for 14 days. KDML105 was cultured without stress (-NaCl) was used as control. Error bars indicate standard deviation.

4. Conclusion

Accumulation of Na^+ in extracellular compartment of plant under salt stress creates the osmotic stress that inhibits difficulties in the water uptake potential by the cell. This effect of sodium stress on plant nutrition causes the suppression of K^+ uptake which is as an essential activator for many enzymes located in the cytosol [23]. Moreover, salt stress results in ion toxicity [20] which occurs when Na^+ is accumulated in cytoplasm and interferes with the cell metabolism. When, salt tolerance rice cultivars and salt sensitive rice were exposed to same level of salinity, salt-tolerant rice cultivars accumulated less Na^+ in their tissue than salt sensitive rice [24] which indicated salt sensitive cultivars may be more vulnerable to ion toxicity than salt tolerance cultivar. Our study not only focused on screening the mutants based on salt tolerance score, but also examined the morphological parameters of moderately tolerant mutant lines under salinity stress. As salt stress directly affects plant growth and metabolism and has an effect on cellular components, analysis of photosynthetic pigments and net photosynthetic rate under salinity stress clearly indicated that there is no correlation between them. The mutant lines having relatively lower concentration of photosynthetic pigments also showed improved photosynthetic rate. IR29 cultivar of rice, when exposed to salt stress at booting stage, accumulated soluble starch and showed reduction in sucrose content in flag leaf [25]. In our study too, the leaf blade and leaf sheath showed more reduction in the sucrose than glucose and fructose. This sucrose gradient may be converted to a starch for storage in the plastid or to a hexose for storage in the vacuole. This is because hydrolysis of sucrose is the initial step of sucrose metabolism, catalyzing the breakdown of sucrose by invertase enzyme and is expected to play a major role to support soluble starch synthesis in order to chelate Na^+ entering the cell [26].

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Mutant KDML105 Rice Shows Higher Potential Capability to Retain Total Chlorophyll and Photosynthesis under Low Nitrogen Condition

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Keywords: KDMl105, Nitrogen, Photosynthesis rate, Chlorophyll

Abstract. Nitrogen (N) is an important nutrient for rice production. Growth and development of KDML105 rice and its mutant line MT2 were examined under low nitrogen condition. Both rice samples were grown in a soilless culture system supplemented with modified Yoshida solution at varied concentrations of ammonium nitrate (NH_4NO_3) as 0.18 mM (low N) or 1.43 mM (control N). Samples were cultivated continuously in this condition from seedling until the booting stage (approximately 100 days). Physiological parameters of photosynthetic pigments and photosynthesis rate revealed that low N caused reduced total chlorophyll in KDML105 but not in MT2. Similarly, the result of leaf greenness (SPAD value) also decreased and reflected different net photosynthetic rates (P_n). The MT2 mutant showed higher P_n than KDML105 under both low N and control conditions. Nitrogen is a major component of chlorophyll and amino acids under low N. The MT2 mutant displayed higher efficiency to utilize N sources for physiological mechanisms than its parental KDML105 rice under low N condition. It could be addressed that MT2 mutant displayed higher efficiency to utilize N sources for physiological mechanisms than its parental KDML105 under low N condition, which may reveal for its improvd nitrogen use efficiency. This mutant will be useful to implement as pre-breeding material in rice breeding programs.

1. Introduction

Khao Dawk Mali 105 (KDML105) is a Thai Jasmine rice, recognized as premium aromatic quality in the world market. Average yields for Jasmine rice in Northeast Thailand are about 2.33 Mg ha^{-1}, which is one of the lowest in the world [1]. Soil nitrogen (N) availability is one of the essential macro-elements required for plant growth and development in most agricultural cropping systems [2]. Therefore, nitrogen loss inhibits photosynthesis, chlorophyll synthesis, relevant genes of plasmid protein synthesis and secondary metabolic responses [3]. As a consequence, total crop yields in many intensive farming systems have failed to rise in proportion to the increase of chemical fertilizers over the past 20 years, leading to low nutrient use efficiency and increasing environmental problems [4]. Achieving further increases in yield under well-fertilized conditions requires selecting plants that use fertilizers more efficiently. Understanding how the physiological mechanisms of different rice cultivars respond to diverse concentrations of nitrogen will increase knowledge and promote maximum fertilization usage and efficiency.

2. Materials and methods

Rice cultivars Khao Dawk Mali (*Oryza sativa* L cv. KDML105) and MT2 (derived from a mutant line of KDML105 under radiation and chemical induction and grown until fifth-generation) were used as plant materials. The seeds were sterilized with 70% alcohol for 5 min, with sodium hypochlorite (10% v/v, Clorox®) for 10 min, and then washed three times in distilled water before germination in a tray with distilled water. Seedlings were transplanted and cultivated with full Yoshida solution for a week to ensure relatively uniform nitrogen condition. The working solution was prepared as described by Yoshida et al. [5] with adaptations by Gregorio et al. [6]. The nutrient solution contained 1.43 mM NH_4NO_3, 0.32 mM $NaH_2PO_4{\cdot}H_2O$, 0.51 mM K_2SO_4, 1.00 mM $CaCl_2{\cdot}2H_2O$, 1.67 mM $MgSO_4{\cdot}7H_2O$, 9.09 µM $MnCl_3{\cdot}4H_2O$, 0.08 µM $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$, 0.15 µM $ZnSO_4{\cdot}7H_2O$, 18.2 µM H_3BO_3, 0.16 µM $CuSO_4{\cdot}5H_2O$, 0.04 mM $FeCl_3{\cdot}6H_2O$ and $C_6H_8O_7{\cdot}H_2O$. Then, seedlings were transferred to a soilless system with substrate material (sand: perlite). Low nitrogen treatment was first applied to 35 day-old seedlings. Concentration of NH_4NO_3 was adjusted to 0.72 mM (1/2X) for two week and then reduced to 0.36 mM (1/4X) for a further two weeks and continually reduced to 0.18 mM (1/8X) for the four weeks.

3. Results and discussion

3.1 Change in leaf greenness, chlorophyll content and photosynthesis efficiency

Clearly visible differences in plant morphology were observed between KDML105 and the mutant line MT2 under complete and reduced nitrogen concentration (Fig. 1). Reduction of nitrogen caused a change in leaf color from green to yellow (chlorosis). Greenness was quantified using a chlorophyll meter and expressed in SPAD units. This non-destructive method determines the relative amount of chlorophyll content. Results showed no significant difference between complete and reduced nitrogen condition in MT2, whereas reduction of nitrogen concentration affected the SPAD value of KDML105 at 26.5±3.0 and below critical value, whereas MT2 recorded SPAD values at 33.7±4.2 (Table 1). SPAD values below the critical value indicate that the rice crop suffers from nitrogen deficiency [7]. Our results suggested that KDML105 remains in critical time to suffer nitrogen fertilizer. The first consequence of nitrogen deficiency is leaf chlorosis from production of insufficient chlorophyll [8]. To determine the amount of chlorophyll pigment, chlorophyll extraction was performed. Chlorophyll *a* content in leaves showed no significant difference between KDML105 and MT2 (Table 2). Reduction of nitrogen concentration significantly decreased chlorophyll *b* and total chlorophyll content in KDML105. By contrast, no significant changes were found in chlorophyll *b* and total chlorophyll content under reduced nitrogen treatment for MT2. Photosynthesis measurement of individual leaves was also evaluated to detect the net rate of photosynthetic carbon assimilation. Temporal change in photosynthetic capacity under decreased N concentration is shown in Table 2. Significant differences between complete and reduced nitrogen concentration for net photosynthesis rate (P_n) were noted in both genotypes. KDML105 showed greater reduction in net photosynthesis rate than MT2 at 27.52% and 24.5%, respectively.

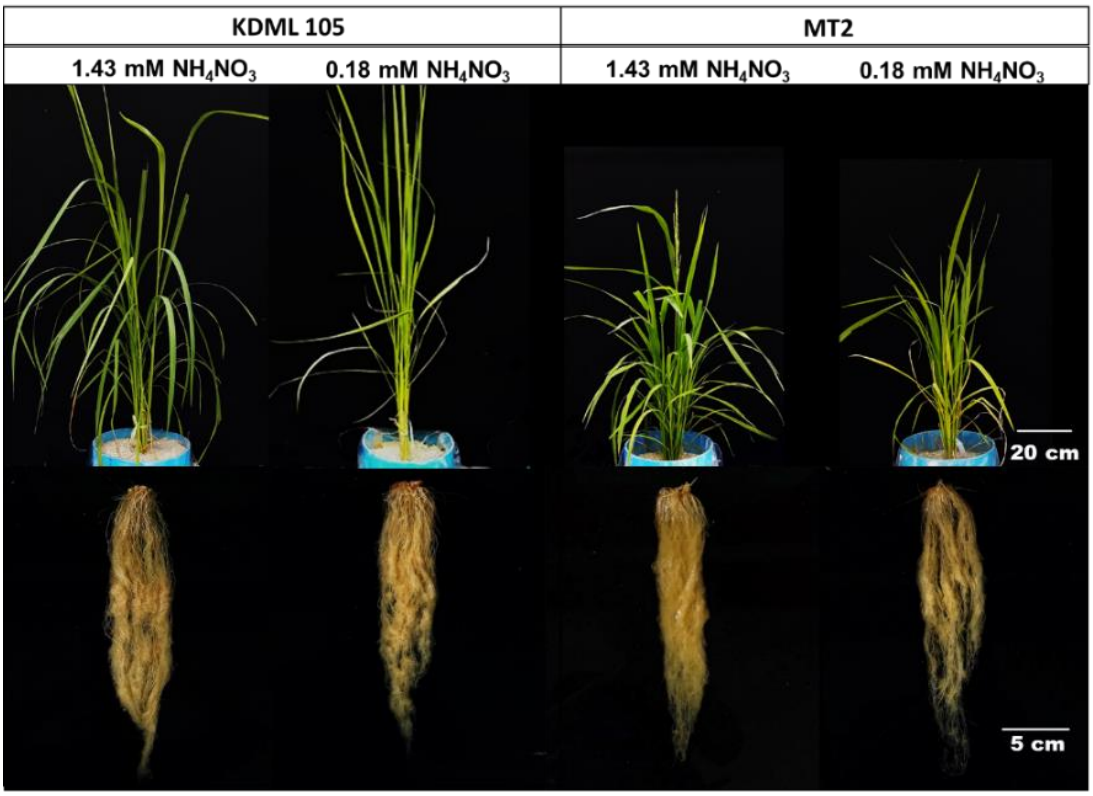

Fig. 1 Morphology of KDML 105 and MT2 treated with 1.43 mM and 0.18 mM NH_4NO_3 at booting stage (100 days after germination)

Table 1 Leaf greenness (SPAD value) of KDML 105 and MT2 treated with 1.43 mM and 0.18 mM ammonium nitrate at booting stage (100 days after germination)

Genotype	Nitrogen concentration (NH_4NO_3)	Leaf greenness (SPAD reading)
KDML105	1.43 mM	35.1±3.3 a
	0.18 mM	26.5±3.0 b
MT2	1.43 mM	39.8±5.1 a
	0.18 mM	33.7±4.2 a
F-test		**

The different letters within the column show significant difference at $p \leq 0.05$ (*) and highly significant difference at $p \leq 0.01$ (**) of means (±SD) analyzed by Duncan's multiple-rang test.

Table 2 Chlorophyll (Chl) content and net photosynthesis rate (P_n) of KDML 105 and MT2 treated with 1.43 mM and 0.18 mM ammonium nitrate at booting stage (100 days after germination)

Genotype	N concentration (NH_4NO_3)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	P_n ($\mu mol^{-1}s^{-1}$)
KDML105	1.43 mM	1.11±0.2	0.86±0.2 a	1.97±0.2 a	14.9±1.4 b
	0.18 mM	1.08±0.2	0.33±0.1 b	1.41±0.1 b	10.8±1.5 c
MT2	1.43 mM	1.24±0.1	1.20±0.7 ab	2.45±0.7 a	20.8±4.4 a
	0.18 mM	1.21±0.1	0.75±0.3 b	1.97±0.3 a	15.7±2.6 b
F-test		ns	**	**	**

The different letters within the column show significant difference at $p \leq 0.05$ (*) and highly significant difference at $p \leq 0.01$ (**) of means (±SD) analyzed by Duncan's multiple-rang test. ns = no significant difference.

3.2 Balance of carbon and nitrogen concentration in leaf shoots and roots of rice plants

Nitrogen is a key constituent of cell molecules and also the pivotal regulator of carbon metabolism [9]. Therefore, both nitrogen and carbon nutrients are essential for plant cellular functions. Ham et al. [10] suggested that increasing nitrogen utilization capacity could be achieved through interaction between nitrogen and carbon metabolism. Therefore, carbon-to-nitrogen ratio (C/N) is a good indicator for synthetically diagnosing the balance of carbon and nitrogen. Here, C/N ratio and total nitrogen (%) were evaluated in both leaf shoots and roots of rice plants. Results showed that C/N ratios were not significant in roots (Table 3). Highest C/N ratio was observed in KDML105 under reduced nitrogen concentration, indicating that KDML105 had poor nitrogen availability and was rich in organic carbon. Furthermore, total nitrogen (%) measurement revealed no significant difference in root parts. Total nitrogen of MT2 showed no change at decreased nitrogen concentration with efficiency to allocate nitrogen limiting resources. Xia et al. [11] suggested that rice had better adaptability to N-free condition due to increased activity of nitrate reductase which absorbed external nitrogen nutrient and maximized utilization of nitrogen resources by increasing chlorophyll pigment synthesis. MT2 showed better adaptability by balancing nitrogen concentration for chlorophyll pigment synthesis with photosynthesis efficiency under reduced nitrogen conditions.

Table 3 Carbon/nitrogen ratio (CN ratio) and total nitrogen (%) of KDML 105 and mutant line MT2 treated with 1.43 mM and 0.18 mM ammonium nitrate at booting stage (100 days after germination)

Genotype	N concentration	CN ratio		Total nitrogen (%)	
	(NH_4NO_3)	leaf	root	leaf	root
KDML105	1.43 mM	16.76±0.8 b	38.38±6.1	2.45±0.2 a	1.11±0.2
	0.18 mM	23.63±2.3 a	40.64±2.6	1.71±0.2 b	0.99±0.0
MT2	1.43 mM	16.33±0.9 b	42.76±2.3	2.44±0.1 a	0.97±0.1
	0.18 mM	19.30±0.2 b	41.46±1.1	2.04±0.0 ab	1.00±0.0
F-test		**	ns	*	ns

The different letters within the column show significant difference at $p \leq 0.05$ (*) and highly significant difference at $p \leq 0.01$ (**) of means (±SD) analyzed by Duncan's multiple-rang test. ns = no significant difference.

4. Summary

KDML105 mutant MT2 showed good physiological adaption to nitrogen, with greater chlorophyll content, and higher net photosynthesis rate and nitrogen balance in leaf tissue under nitrogen reduction treatment than its parent cultivar.

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Evaluation of Salt Tolerant Ability on Selected Thai Rice Cultivars

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Abstract. Salinity is a major abiotic stress affecting rice production. Registered and Traditional Thai rice cultivars obtained from Suphan Buri Rice Research Center were evaluated on salt tolerant ability. Twenty-eight-day old plants were cultured in 0 (control) or 50 mM NaCl solution until evaluation. Pokkali; salt-tolerant cultivar and IR29; salt-sensitive were compared with 9 studied cultivars. Growth parameters and photosynthetic pigment contents were measured at 21 days after stress exposure. The parameters such as shoot fresh and dry weight, and the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were significantly different leading to grouping analysis. However, root performance did not show significant difference among cultivars and were not included in the grouping analysis. Studied cultivars were classified into two groups based on significant changes. Pokkali, Hawm Chonsalit, Ha Ruang, Suphan Buri 60 and Suphan Buri 90 classified into group I showed no significant changes in all studied parameters compared to mock-treated condition, while the group II composed of Mae Lahd, Khao Tah-mon, Sai Yud, Suphan Buri1, Khao Gaw Diaw and, IR29 presented decrease in photosynthetic pigments and shoot fresh weight.

1. Introduction

Salt stress has been a serious problem for rice production. Therefore, the studies of rice cultivars under salt stress condition have been performed in all major rice-producing countries [1, 2]. Salt stress affects rice plants in all developmental stages, i.e. seed germination, vegetative, and reproductive stages [3, 4]. Plant growth, as measured by parameters such as plant height, leaf area, plant fresh weight and plant dry weight is reduced [5, 6]. Photosynthetic pigments and maximal photochemical efficiency of PSII are usually decreased [7]. Salt stress not only affects germination, morphology, and physiology of rice cultivars, but also impacts grain production [8, 9].

Salt tolerance has been evaluated for years on different sets of Thai rice cultivars, along with international rice cultivars in a few studies. For example, 106 rice cultivars including local and improved Thai cultivars from the Thai Rice Department (RD) collection and IRRI improved cultivars (IR) were screened at seedling stage to identify cultivars with salt tolerant ability based on salinity tolerance scores, survival percentage, and Na^+/K^+ ratio, resulting in clustering of cultivars into five groups [10]. In another study, 230 Thai aromatic rice lines were studied and eleven rice cultivars were selected as salinity tolerant cultivars based on chlorophyll index [11]. In addition, eight local black

glutinous rice cultivars and four white rice cultivars were screened for salt tolerant ability based on physiological and biochemical parameters at seedling stage [12]. Moreover, growth and physiological parameters were used to examine salt tolerant ability of 8 upland and 8 lowland Thai rice cultivars, leading to clustering of cultivars into salt tolerant and salt susceptible groups [13, 14]. This type of information has been valuable for improving salt tolerance in Thai rice cultivars.

Despite these efforts, the information about salt tolerance ability among Thai rice cultivars is still incomplete. The majority of low land Thai rice cultivars that might have a potential to tolerate salt stress have never been tested and compared under salt stress condition. This study aimed to evaluate salt tolerant ability of nine lowland recommended and traditional Thai rice cultivars, in comparison with Pokkali and IR 29. These nine cultivars were chosen from a collection due to other distinguished characteristics in our preliminary studies. Growth performance and photosynthetic pigments under salt stress were measured and used for Hierarchical cluster analysis. These data will provide helpful basic information about the selected Thai rice cultivars for further studies.

2. Materials and methods

2.1 Plants materials, growing condition, and salt stress treatment

Seeds of nine Thai rice cultivars including Ha Ruang, Hawm Chonlasit, Khao Gaw Diaw 35, Khao Tah-mon, Mae Lahd, Sai Yud, Suphan Buri 1, Suphan Buri 60, Suphan Buri 90 were obtained from Suphan Buri Rice Research Center to compared with Pokkali (salt tolerant cultivar) and IR 29 (salt sensitive cultivar). Seeds were soaked in water for forty-eight hours before germinating on soil for two weeks. Each seedling was transplanted to individual new pot (2×2 inches) filled with soil. Twenty-eight-day-old seedlings were imposed to 50 mM sodium chloride (NaCl) solution or control (water without NaCl) for twenty-one days. Solutions were renewed every two days.

2.2 Growth performance parameter measurement

Each rice cultivar was harvested at twenty-one days after salt treatment. Shoot length (SL) was measured from the base of the aerial part to the tip of the highest leaf. Plants were separated into two parts to measure shoot fresh weight (SFW) and root fresh weight (RFW). Shoot and root were dried at 70□C in a hot air oven for 48 hours to determine shoot dry weight (SDW) and root dry weight (RDW).

2.3 Determination of photosynthetic pigments

The contents of photosynthetic pigments were determined according to the method of Shabala *et al*. (1998) [15] and Lichtenthaler (1987) [16]. The pigments were extracted from 0.01 grams of the 2rd leaf from the top with 80% acetone. The absorbance was determined at wavelength 470, 644, and 662 nm by UV-Vis spectrophotometer (Jasco v-530). The amounts of chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total carotenoid (C_{x+c}) were calculated from the equation (1), (2), and (3), respectively.

$$Chl_a = 9.784\ A_{662} - 0.99\ A_{644} \quad (1)$$

$$Chl_b = 21.426\ A_{644} - 4.65\ A_{662} \quad (2)$$

$$C_{x+c} = (1000\ A_{470} - 1.90\ Chl_a - 63.14\ Chl_b)/214 \quad (3)$$

2.4 Experimental design and statistical analysis

The experiment was designed as 11 x 2 factorial in completely randomized design (CRD) with 5 replications. The mean values of shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), shoot length (SL), Chlorophyll a, Chlorophyll b, total

chlorophyll, and total carotenoids were analyzed using *t*-test with SPSS software (SPSS Version 22, SPSS Inc., Chicago, IL, USA).

The reduction of shoot fresh weight (SFW), shoot dry weight (SDW) Chlorophyll a, Chlorophyll b, total chlorophyll, and total carotenoids under salt condition compared with control condition were used for Hierarchical cluster analysis in SPSS software.

3. Results

3.1 Growth performance of selected Thai rice cultivars under salt stress condition

Growth characters were measured at 21 days after NaCl treatment. Shoot fresh weight (SFW) of six cultivars: IR29, Khao Gaw Diaw 35, Suphan Buri 1, Mae Lahd, Sai Yud, and Khao Tah-mon was decreased by 13-20%. In contrast, SFW remained unchanged for five cultivars: Pokkali, Hawm Chonlasit, Ha Ruang, Suphan Buri 60, and Suphan Buri 90 (Fig. 1a). Shoot dry weight (SDW) declined by 37% and 28% in cultivars IR29 and Khao Gaw Diaw 35, respectively, while remaining unchanged in other cultivars (Fig. 1b). Root fresh weight (RFW), root dry weight (RDW), shoot length (SL), and root length (RL) were not significantly different between control and salt conditions in all cultivars (Fig. 1c, d, e, f).

3.2 Photosynthetic pigment reduction under salt stress condition

Significant reduction of Chl_a, Chl_b, and total chlorophyll was found in cultivars IR29, Khao Gaw Diaw 35, Suphan Buri 1, Mae Lahd, Sai Yud, and Khao Tah-mon when subjected to salt stress. In these five cultivars, Chl_a, Chl_b, and total chlorophyll content were reduced by 30-42%, 35-48%, and 32-44%, respectively (Fig 2a, b, c). Total carotenoid content under salt stress condition was dropped by 29-39% in cultivars IR29, Khao Gaw Diaw 35, Suphan Buri 1, Mae Lahd, and Sai Yud (Fig. 2d).

3.3 Cluster analysis of cultivars based on salt tolerant ability

Clustering analysis was performed based on growth performance and photosynthetic changes under salt stress. The reduction percentage of each parameter was used to for the hierarchical analysis. The selected rice cultivars were classified into two groups. Group I, which included Pokkali, Hawm Chonlasit, Ha Ruang, Suphan Buri 60, and Suphan Buri 90, was identified as salt tolerant. Group II, which included IR29, Khao Gaw Diaw 35, Suphan Buri 1, Mae Lahd, Sai Yud, and Khao Tah-mon was identified as salt sensitive (Fig. 3).

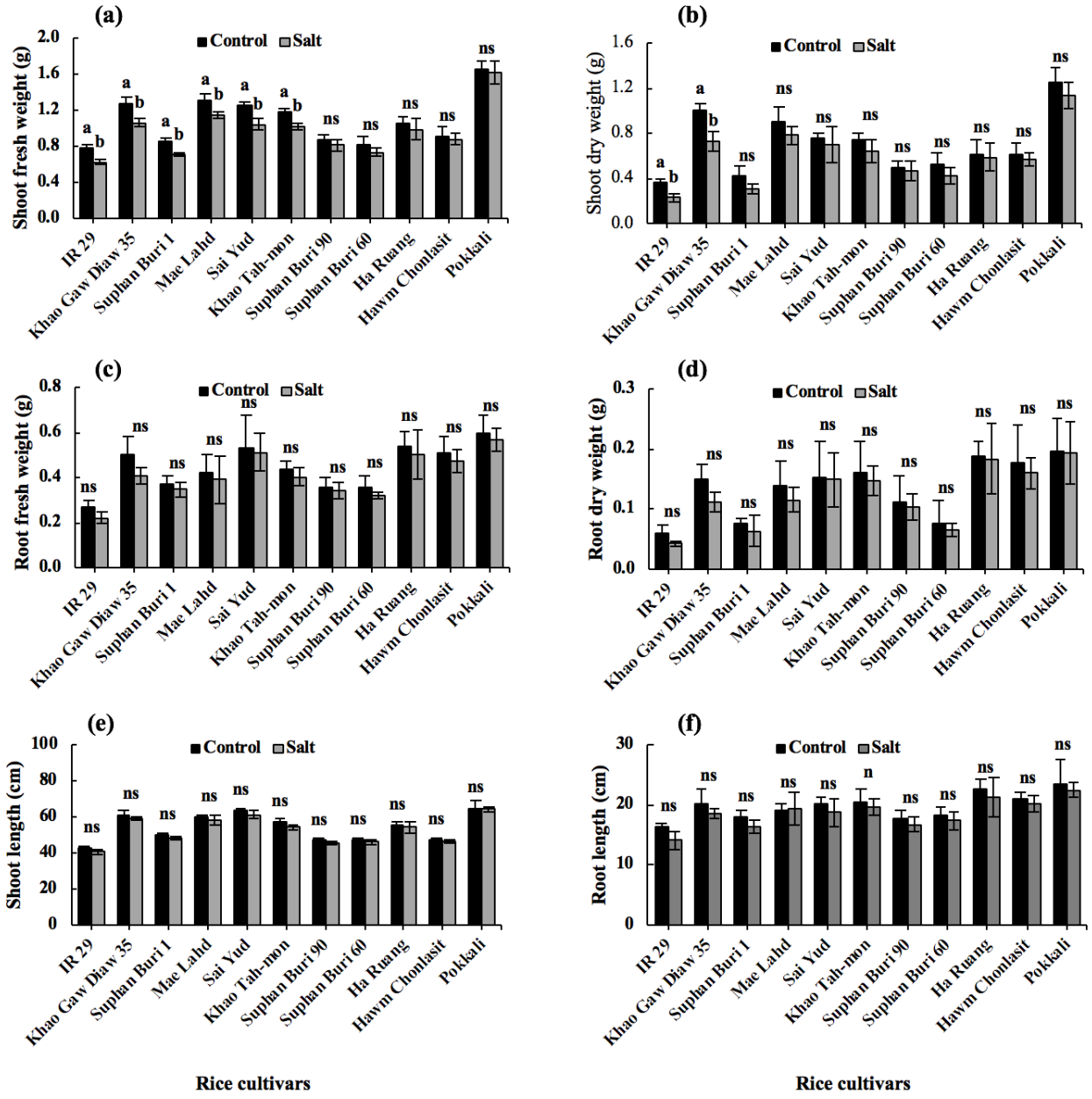

Fig. 1 Shoot fresh weight (a), shoot dry weight (b), root fresh weight (c), root dry weight (d), shoot length (e), and root length (f) of eleven rice cultivars grown under control (no NaCl) and salt stress (50mM NaCl) for 21 days. Data presented as the means of five replicates with standard error (±SE), different letters in each cultivar represented significant difference at $p \leq 0.05$, using *t*-test.

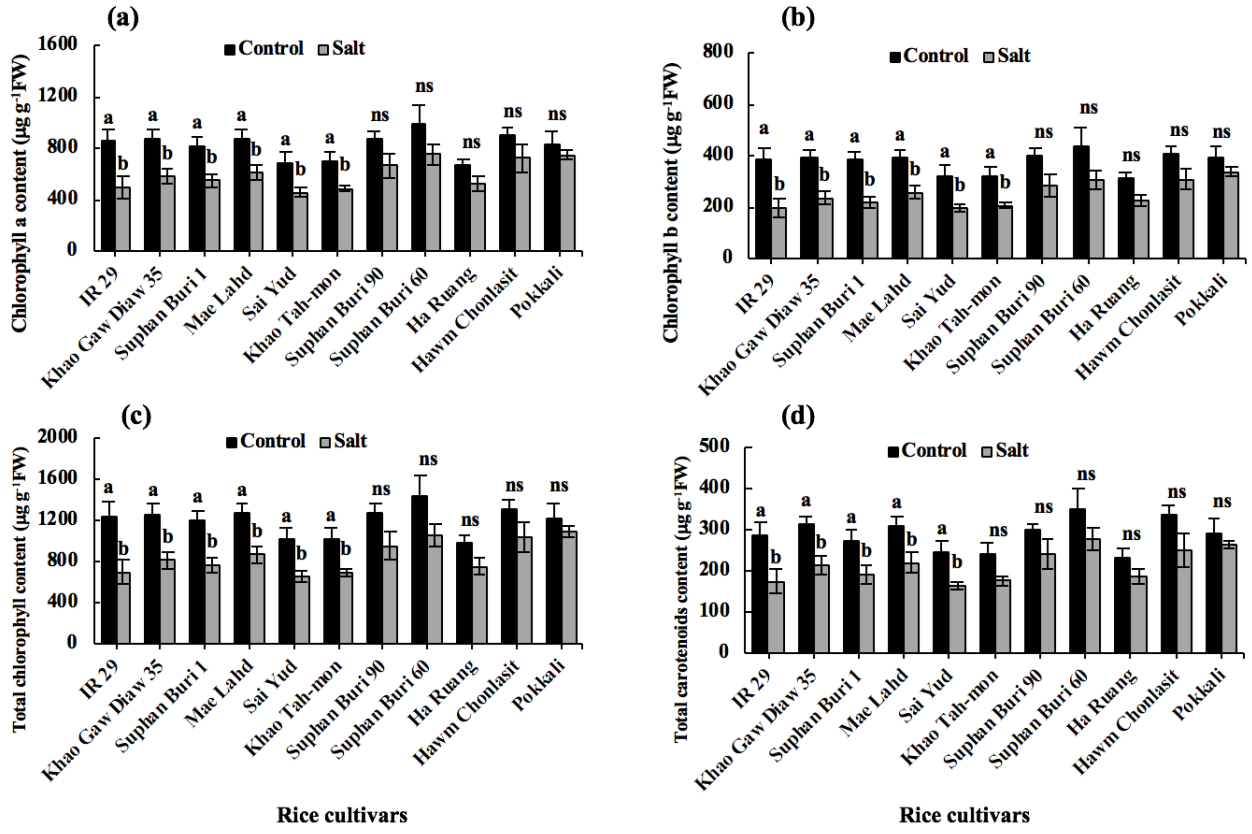

Fig. 2 Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and total carotenoids (d) of eleven rice cultivars grown under control (no NaCl) and salt stress (50mM NaCl) conditions for 21 days. Data presented as the means of five replicates with standard error (□SE), different letters in each cultivar represented significant difference at $p \leq 0.05$, using *t* -test.

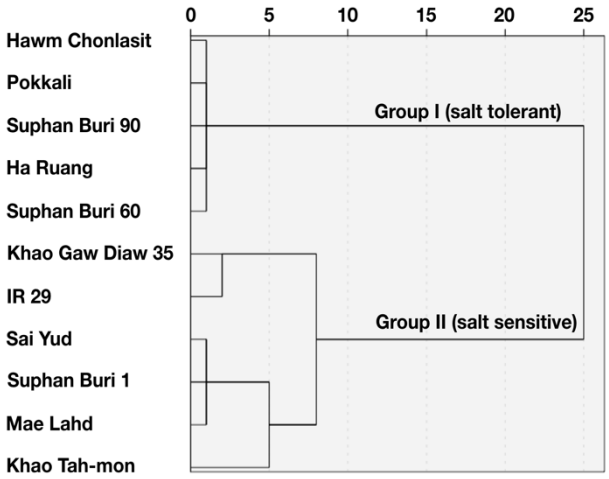

Fig. 3 Cluster analysis of eleven rice cultivars based on reduction of growth and photosynthetic pigments under salt stress (50mM NaCl) for 21 days using Hierarchical analysis.

4. Discussion

In this study, salt tolerant ability was evaluated in eleven registered and traditional Thai rice cultivars. In general, growth performance parameters show obvious changes under salt stress, as reviewed by Hussain *et al.* (2017) [17]. Shoot dry weight, root dry weight, shoot length, and root length were decreased among thirty-three Pakistan rice cultivars treated with 100 mM NaCl at vegetative stage [12]. Pongprayoon *et al.* (2018) showed that shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight were reduced in Thai rice cultivars subjected to 10 dS m^{-1} NaCl treatment for twenty-one days [14]. Our results showed a reduction of shoot fresh weight and shoot dry weight in a few cultivars. However, no change of root performance was found. Our results were in agreement with study of Omisun *et al.* (2018) [18] who reported that SL of all tested eight Indian rice cultivars was affected by 150 mM NaCl, whereas RL of only three cultivars was reduced. Tolerant cultivars may try to decrease water use in the shoot, thereby reducing shoot growth, while still retained root growth under osmotic stress [19].

Another reason for insignificant changes of certain growth parameters, such as shoot length, was the low (50 mM) NaCl concentration used in this study. Similarly, plant height of both Bomba and Bahia rice cultivars did not decrease under both 10 and 20 mM NaCl, but it was reduced under 150 mM NaCl [4]. However, 50 mM NaCl was chosen for this study because it was in the range of moderate saline soil found in Thailand [20]. In addition, it was previously shown to cause different levels of growth reduction in tested rice cultivars with different salt tolerant ability [21, 22].

Beside the growth performance, photosynthetic pigments can be measured as parameter for salt tolerant ability. In this work, photosynthetic pigments did not change in salt tolerant cultivars, while it declined in salt sensitive cultivars. These responses were similar to the results from a previous work by Pongprayoon *et al.* (2017) [14] that chlorophyll a, chlorophyll b, and total carotenoids did not degrade in one tolerant cultivar, Riceberry, whereas the degradation was found in some moderate salt tolerant Thai rice cultivars.

Reduction of growth performance and photosynthetic pigments were used to classify eleven rice cultivars into two groups: salt tolerant and salt sensitive. As expected, Pokkali was classified as salt tolerant and IR29 as salt sensitive cultivars. Suphan Buri 90 was in the tolerant group as Pokkali, similar to a previous study [10]. In contrast, our study categorized Suphan Buri 1 as a salt sensitive cultivar, whereas in the previous study it was classified as a moderate salt tolerant cultivar because more sensitive cultivars were included in the test [14]. Apart from Suphan Buri 90, Hom Chonlasit, Ha Ruang, and Suphan Buri 60 displayed no growth reduction and pigment degradation under salt stress, and thus belong to the salt tolerant group with Pokkali. Hence, they are potential salt tolerant cultivars and should be used for further studies.

5. References

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Electrochromism of G/ITO/NiO_xH_y and G/ITO/WO_3 Prepared by Reactive dc Magnetron Sputtering Technique

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Keywords: Electrochromic Device, Nickel Oxide, Tungsten Oxide, Reactive DC Magnetron Sputtering, Thin Film

Abstract. This work aims to study the electrochromism of NiO_xH_y and WO_3 using cyclic voltammetry technique for electrochromic devices application. All of the samples were deposited on ITO coated glass substrates (G/ITO) by reactive DC magnetron sputtering with the same thickness of 150 nm. NiO_xH_y thin films were prepared at the constant power of 35 W under the mixed gas of Ar:O_2:H_2 at the flow rate ratio of 10:10:20 sccm and WO_3 thin films were prepared at the constant power of 150 W under Ar and O_2 mixed gas at the flow rate ratio of 30:27.5 sccm. The electrochromism properties of G/ITO/NiO_xH_y and G/ITO/WO_3 samples were studied in a 0.1 M of KOH and 0.1 M of H_2SO_4 solution respectively. The results showed that the both of thin film systems had a good electrochromism properties. The highest current density of G/ITO/NiO_xH_y and G/ITO/WO_3 at the 10^{th} cycles repeating scan were 2.07 and 3.89 mA/cm^2 respectively and the optical density changing (ΔOD) of bleached and colored states at the wavelength of 550 nm were 0.59 and 0.35 respectively.

1. Introduction

The electrochromism properties of electrochromic (EC) devices depend on their color and optical transmittance changing of the electrochromic thin films induced by a reversible process. The reversible process occurred when applied the voltage (electric field) to the system. The oxidation state of the transition metal was changed when the chemical reaction (oxidation and reduction) occurred. The conventional electrochromism of EC materials were related to the double injection of positive ions (H^+, Li^+, Na^+ and K^+) and electrons into the multivalent transition metal oxide materials [3,4].

Nowaday, tungsten oxide (WO_3) and nickel oxide hydroxide (NiO_xH_y) are the most widely used for cathodic and anodic electrochromic materials due to a good coloration state, high stability and excellent optical transmittance in visible region. Many researchs have reported that the quality of EC devices did not only depend on EC materials but it was also depend on the quality of ion conductor layer [4,5] which was controlled by the microstructure, composition and ionic conductivity. The electrochromism of all solid-state electrochromic was studied for a long time and it was very complicate. Therefore, the study of electrochromism of EC device in electrolytes solution, it was the one way to understand the mechanism of electrochromic reaction.

In this work, we focused on the study of optical properties and electrochromism of NiO_xH_y and WO_3 thin films deposited on ITO coated glasses (G/ITO) by reactive dc magnetron sputtering technique for all solid electrochromic devices application.

2. Experimental details

The preparation of films: The both of NiO_xH_y and WO_3 thin films were deposited on glass slides (G) and G/ITO substrates by reactive DC magnetron sputtering at room temperature. The NiO_xH_y thin films were prepared in the mixed gas of H_2 (99.99%) and O_2 (99.99%) atmosphere and WO_3 thin films were prepared in O_2 (99.99%). The Ar (99.00%) was used as the sputtering gas for the both films deposition. For NiO_xH_y thin films deposition, a Ni metal (99.99%) disk with the diameter of 3 inch and the thickness of 0.0625 inch was used as a sputtering target. Firstly, the vacuum chamber was pumped down to the base pressure of 3.5 x 10^{-5} mbar. And then, the Ar gas was flew into the chamber at the flow rate of 15 sccm. After that, the 35 W DC power supply was turned on for 5 minutes to remove the contaminant on the target surface. Next, O_2 and H_2 gasses were then flew into the chamber. The flow rate of Ar, O_2 and H_2 gasses were individually adjusted by mass flow controllers. NiO_xH_y thin films were sputtered with the Ar: O_2:H_2 flow rate ratio of 15:10:12.5 sccm. For WO_3 thin films deposition, The sputtering power was set at 150 W and the flow rate of O_2 and Ar gasses were adjusted to be 30 sccm and 27.5 sccm respectively. The films thickness was approximately 150 nm that controlled by quartz crystal thickness monitor.

The characterization of films: The as-deposited and coloration state films were used to characterize the optical transmittance by spectrophotometer (Shimadzu model UV-Vis-NIR 3100). The electrochromism of the films were analyzed by cyclic voltammetry using potentiostat (AMETEK, VersaSTAT 4 Potentiostat Galvanostat). The three electrodes of **(**i**)** G/ITO/NiO_xH_y and G/ITO/WO_3, **(**ii**)** Platinum wire and **(**iii**)** Ag/AgCl, were used as the working electrode, counter electrode and a reference electrode, respectively**.** All electrodes were placed in 0**.**1 M of H_2SO_4 solution for G/ITO/WO_3 and 0**.**1 M KOH solution for G/ITO/NiO_xH_y**.** The voltammogram was measured by scanning potential for 10 cycles which the potential of each cycle was varied between -1**.**0 to 2**.**0 V and -0**.**6 to 0**.**8 V for G/ITO/WO_3 and G/ITO/NiO_xH_y at the same scan rate of 50 mV/s and a step potential of 0**.**01 V**.**

3. Results and discussion

Fig. 1(a), showed the optical transmittance of the as-deposited NiO_xH_y films. The films could be colorless by adding H_2 gas into the sputtering atmosphere of Ar and O_2. Fig.1(b) showed the optical transmittance of as- deposited WO_3 films prepared under the mixed Ar and O_2 atmosphere. The transmittance spectra showed the cut off wavelength edge in uv region but had high transparency in visible region around 80 %.

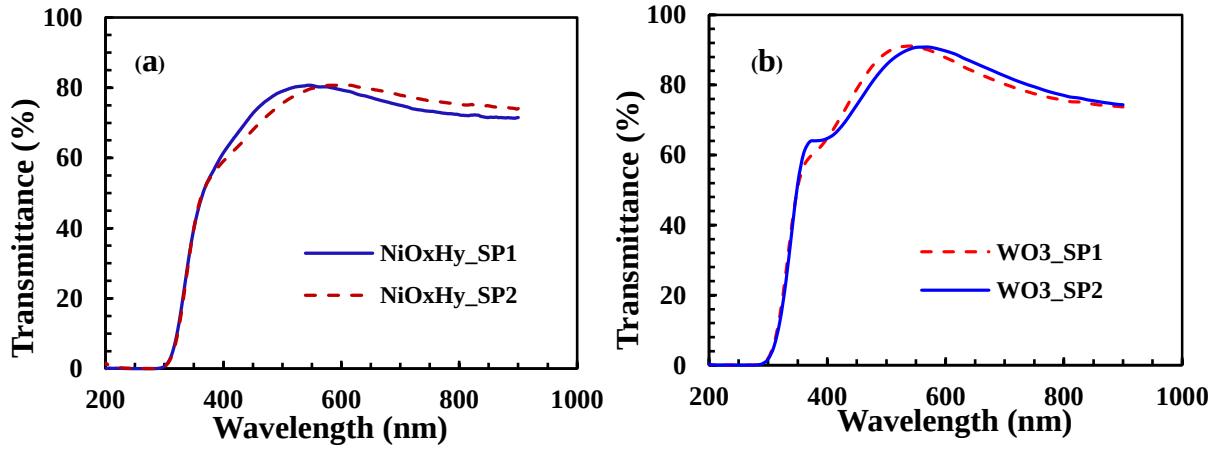

Fig 1. The transmittance spectra of as-deposited (a) G/NiO_xH_y and (b) G/WO_3.

Fig. 2, showed the voltammogram of G/ITO/NiO_xH_y and G/ITO/WO_3 that related to the electrochemical behavior of the samples. A proton (H^+) and electron intercalation was evidenced by redox processes when applied the voltages to the samples. It was found that the exist transient behavior of the films which the redox processes did not reached the steady state condition during the 1st to 5th cycles. However, the electrochromism reaction was stable and reached the saturation state at the 10th cycles. The maximum current density was obtained to be 2.06 mA/cm^2 and 3.89 mA/cm^2 for G/ITO/NiO_xH_y and G/ITO/WO_3 respectively.

To understand the amount of charge that used for electrochemical reaction. The charge that moved through the active surface area of the electrode was proportion to the current density (mA/cm^2). The current density was observed from cyclic voltammograms during the coloration/bleaching process when some ions (H^+, OH^-) and electrons (e^-) transfer in KOH and H_2SO_4 electrolyte solution. The chemical reaction that related with the changing of oxidation state was followed by Eq. 1, 2 and 3, [1, 5].

$$\underset{\text{(Bleach)}}{Ni^{2+}(OH)_2} + OH^- \leftrightarrow \underset{\text{(Color)}}{Ni^{3+}OOH} + H_2O + e^- \quad (1)$$

$$\underset{\text{(Bleach)}}{Ni^{2+}O} + OH^- \leftrightarrow \underset{\text{(Color)}}{Ni^{3+}OOH} + e^- \quad (2)$$

$$\underset{\text{(Bleach)}}{W^{6+}O_3} + xH^+ + xe^- \leftrightarrow \underset{\text{(Color)}}{H_xW^{5+}O_3} \quad (3)$$

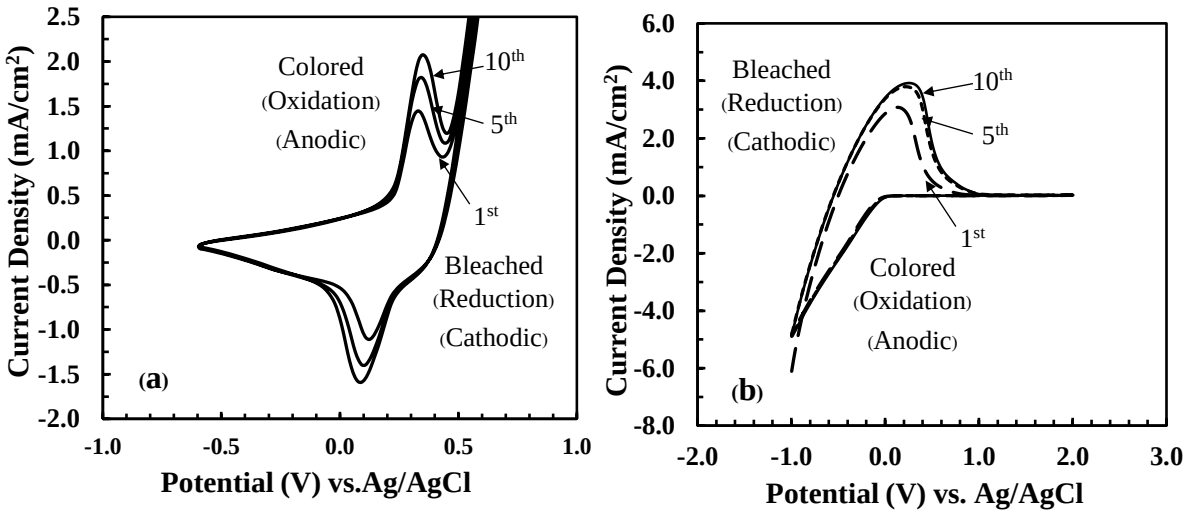

Fig 2. The voltammograms of (a) G/ITO/NiO_xH_y and (b) G/ITO/WO_3. The voltammograms were obtained by scanning the potential for 1, 5 and 10 cycles.

When the DC voltage was applied to the sample, the induced electrons (e^-) were entered into WO_3 film while H^+ ions were entered through the electrolyte solution into WO_3 film too. The oxidation state was changed due to the electrochemical reactions that affected to the transition energy level of electrons in the molecular orbitals. The insertion and withdraw of hydrogen ions through WO_3 films can make the oxidation state changed between W^{6+} and W^{5+} state as shown in Eq. 3. The same mechanism, anodic electrochromism of NiO_xH_y could be explained by electron transition between N^{3+} and N^{2+} as shown in Eq. 1 and 2 of Ni $2p_{3/2}$ orbitals [1, 5, 6]. Figure 3 showed the spectral transmittance of the samples of coloration and bleaching state that were analyzed by spectrophotometer. At the bleaching state of 10th cycles, It still had a high visible transmittance around 70-80 % for G/ITO/NiO_xH_y and 80-90% for G/ITO/WO_3 whereas at the coloration state the

transmittance were decreased to around 15-20% and 35-40% for G/ITO/NiO_xH_y and G/ITO/WO_3 respectively. The anodic peak of G/ITO/NiO_xH_y and cathodic peak of G/ITO/WO_3 were shifted up due to the charge capacity effect. The optical density changing (ΔOD) can be defined by;

$$\Delta OD(\lambda) = \log_{10} [T_{Bl}(\lambda)/T_{Cl}(\lambda)] \quad (4)$$

According to Eq. 4, where T_{Bl} is the percentage of transmittance due to bleached state and T_{Cl} is the percentage of coloration state. The high value of ΔOD represented a good EC property. The optical density changing of G/ITO/NiO_xH_y and G/ITO/WO_3 were calculated from the transmittance spectra of colored and bleached state as shown in Fig. 3. The ΔOD results were shown in Table 1.

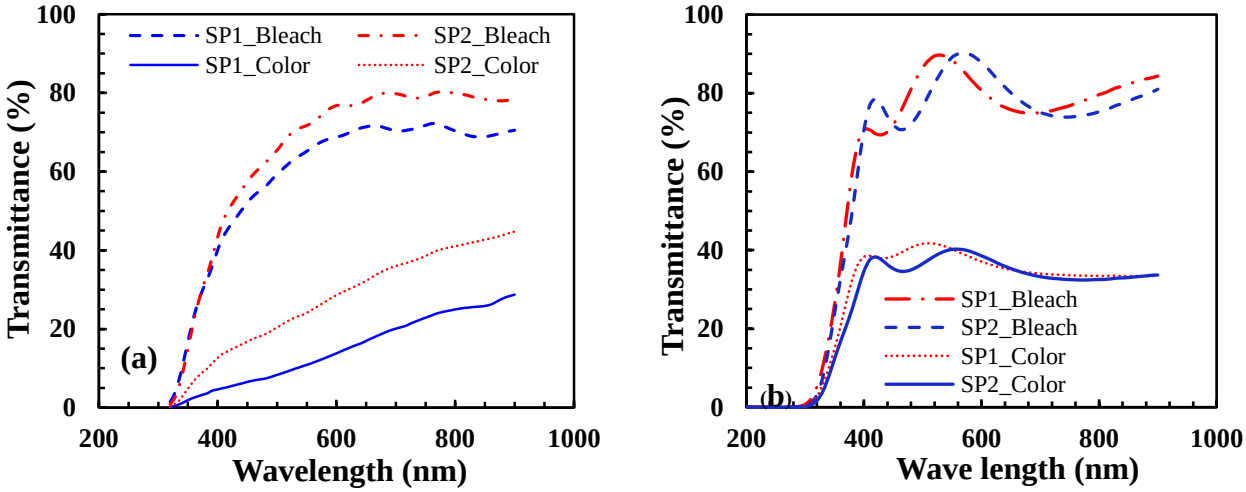

Figure 3. The spectral transparence of bleached and colored states of (a) G/ITO/NiO_xH_y and (b) G/ITO/WO_3.

Table 1. The ΔOD of G/ITO/NiO_xH_y and G/ITO/WO_3

Sample	%T_{Bl} (λ=550nm)			%T_{Cl} (λ=550nm)			ΔOD(λ)		
	SP1		**Av.**	SP2		**Av.**	SP1	SP2	**Av.**
G/ITO/NiO_xH_y	65.16	73.71	**69.44**	15.67	20.11	**17.89**	0.62	0.56	**0.59**
G/ITO/WO_3	88.40	89.14	**88.77**	38.29	40.56	**39.43**	0.36	0.34	**0.35**

4. Summary

In summary, NiO_xH_y and WO_3 thin films have been deposited by reactive dc magnetron sputtering using the metallic Ni and W targets, the high transparency films in visible region of as-deposited films were successfully obtained in this work. The optical transmittance and electrochromism properties of NiO_xH_y and WO_3 films were characterized by spectrophotometry and cyclic voltammetry to observe the coloration and bleaching behavior of the films. The transmittance (λ=550nm) of coloration-bleaching state for G/ITO/NiO_xH_y and G/ITO/WO_3 were around 17.89% - 69.44% and 39.43% - 88.77%, respectively. The average ΔOD of G/ITO/NiO_xH_y and G/ITO/WO_3 samples were obtained to be 0.59 and 0.35 at the maximum current density of 2.07 and 3.89 mA/cm^2,

respectively. The experimental results showed that the G/ITO/NiO_xH_y and G/ITO/WO_3 had a well electrochromism and can be used to fabricate the all solid electrochromic devices.

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Plasma Irradiation Inducing Biochemical Stress in Hairy Root of *Artemisia annua* L.

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Keywords: *Artemisia annua* L. Hairy root, Argon plasma irradiation, Biochemical compounds

Abstract. Biochemical constituent detected in *A. annua* is varied according to genotype and environmental conditions and generally poorly produced in root part. Elicitation can be applied to induce metabolic changes in plant parts using various elicitors. This present study is aimed to investigate the biochemical variations after stress-inducing physical force using argon plasma. Plasma irradiation is a novel electrically ionized radiation technology which was used to apply to hairy root cultures of *A. annua* at dose 0 (control) or 70 watt for 15 or 45 seconds, then the biochemical compounds in root tissue was examined at 3 and 6 hours after plasma irradiation using gas chromatography–mass spectrometry (GC-MS). The results revealed that treatment of 45 seconds showed appropriate time more than 15 seconds. Variation of biochemical compounds was analyzed by GC-MS and it was shown that plasma treatment could elevate or reduce quantitative compounds when compared to non-irradiated roots. Increasing amounts of 1,2-Benzenedicarboxylic acid, a compound against colorectal cancer was detected in harry roots after 6 hours of plasma irradiation (for 45 seconds), which was 3 times higher than untreated hairy root. Moreover, plasma can induce nine compounds that normally disappear in the *A. annua* hairy root. On the other hand, there had seven numbers of biochemical compounds that were reduced after plasma irradiation.

1. Introduction.

Artemisia annua L. (also known as Qinghao) is a medicinal plant and belongs to family Asteraceae, it has been used in Chinese traditional medicine for more than 2,000 years and well-known in modern medicine [1]. The bioactive compounds produced in *A. annua* plant can be used for various pharmaceutical purposes [2]. Most of these active compound was generally detected to accumulate in leave parts but rarely in root. Nevertheless, root culture in bioreactor is one of the industrial production strategies that applied worldwide for various medicinal plant. In our previous report, the hairy root culture induced by *Agrobacterium rhizogenes* showed its potential to vigorously grow and multiply in air-lift type reactor, which potential to produce new pharmaceutical compound. The evidence of a new sesquiterpene, (Z)-7-acetoxy-methyl-11-methyl-3-methylene-dodeca-1,6,10-triene (AMDT) can against the human lung tumor cell lines. This compound was firstly reported to detect in hairy root cultures of *A. annua* [2]. Recently, there has an increasing interest to apply various physical forces to manipulate the variations of plant producing compounds. Physical force such as cold plasma is a novel eco-agricultural technology that has been developed to stimulate plant biochemical compound as

abiotic elicitor. Commercial applications of cold plasma are generally used in microelectronic technology, a lessor for operation in medicine or help keep skin to smooth, bright and anti-aging as beauty and cosmetic, fusion power and ion implantation. Moreover, the cold plasma also used for improving shelf life of fruit as a post-harvest application [3]. It has been reported that irradiation of 100-Watt helium-cold plasma treatment for 15 second significantly increased protein and sugar content in oilseed rape seed and induced seed germination under drought stress [4]. Moreover, the power 80-Watt of helium 15 second for irradiation of cold plasma also increased tomato resistance to *Ralstonia solanacearum* as bacteria that caused of tomato yield reductions [5]. To understand the biochemical responses of plant affected by plasma irradiation, of hairy root culture of *A. annua* L. was used as plant material in this study. Effect of argon plasma on the biochemical changes of *A. annua* hairy roots was demonstrated using Gas Chromatograph-Mass Spectrometer (GC-MS) analysis.

2. Material and Method

2.1 Treatment of Oxygen Plasma Irradiation

The hairy root was irradiated using argon plasma (power 70 W, 13.56 MHz) for 15 or 45 seconds of explosion and 3 or 6 hours for incubation at 25 ^{O}C. The parameters were measured include terpenoid production.

2.2 Biochemical compound analysis

Terpenoid compound was extracted using dichloromethane as solvent because this compounds are non-polar molecule that can be dissolve in dichloromethane. They were then stored in dried crude extract. The biochemical compound in 0.1 g of dried crude extract of root from treatment and leave obtained was examined using Gas Chromatograph-Mass Spectrometer (GC-MS).

3. Results and discussion

Biochemical compounds profile in *A. annua* hairy root which may be affected by the plasma were examined by GC-MS, and these were compared with profiles untreated-hairy root and flesh leaves. Thirty-two compounds were found in the dichloromethane extracts displaying a library database matching quality > 80% (Table 1). The compound variation between leaves, untreated hairy root and plasma treated hairy root were clearly expressed (25 compounds in leaves and 17 compound in normal hairy root). The type grouping was found (Fig. 1), terpenoids (mono-, sesqui-, di- and triterpenes) were the most prevalent compounds found in leaves but lower prevalent in untreated-hairy root. Interestingly, plasma irradiated 45 seconds and 6 hours for incubation can promote the increasing of terpenoids group. Terpenoids accounted for 18, 18, 18, 18 and 50% of total extract compositions in untreated hairy root (b), plasma treated-hairy root 15 second and incubated 3(c) or 6 hours (d) and plasma treated-hairy root 45 second and incubate 3 (e) or 6 hours (f), respectively. Other metabolites (in decreasing order of abundance) were fatty acids and conjugates (47, 46, 46, 46 and 30% in b, c, d, e and f treatment), aliphatic and aromatic hydrocarbons (23, 27, 27, 27 and 15% in b, c, d, e and f treatment), one phenolic was appeared in untreated hairy root and leaves. One other compound was found in every treatment. Interestingly, the quantity of most compound groups remained relative constant (%) between group treatment, some differences were evident in the terpenoid composition of the extracts. Nine terpenoid compounds that disappear in normal hairy root but was appeared in plasma

Table 1 Biochemical compounds of dichloromethane extracts from fresh leaves and untreated or plasma treated-hairy root obtained from GC–MS analysis of *A. annua*

Type	Compound name[a]	Relative content (%) of plant conditions[b]					
		Leave	Untreated hairy root	Plasma treated 15 s/ 3 hr.	Plasma treated 15 s/ 6 hr.	Plasma treated 45 s/ 3 hr.	Plasma treated 45 s/ 6 hr.
Monoterpene	Eucalyptol	-	-	-	-	-	2.8
Sesquiterpenes	Germacrene D	25.5	-	-	-	-	21.5
	α-copaene	2.5	-	-	-	-	1.6
	trans- β -Farnesene	65.8	25.7	9.3	5.9	7.7	38.4
Diterpenes	Neophytadiene	23.44	-	-	-	-	18.9
	Phytol	10.6	-	-	-	-	-
	Trans-phytol	8.6	-	-	-	-	-
	α-tocopherol	6.1	-	-	-	-	22.9
Triterpenes	Squalene	3.5	-	-	-	3.5	2.5
	Campesterol	4.9	-	-	-	-	-
	β-Amyrin	-	-	-	-	-	2.06
	Stigmasta-5,22-dien-3-ol	19.6	4.7	3.7	2.7	3.4	16.1
	b-sitrosterol	10.8	1.3	-	-	-	13.4
Phenolic	2,4-di-tert-Butylphenol	3.0	1.3	-	-	-	-
Aliphatic and aromatic hydrocarbons	Cyclododecane	24.1	8.3	22.5	13.7	20.2	19.6
	Cyclotetracosane	18.6	-	-	-	-	-
	Nonacosane	-	1.6	-	-	-	-
	Docosane	25.1	8.8	10.8	9.6	13.0	14.4
	Heneicosane	26.8	8.4	7.4	10.4	11.2	13.0
	Cyclodocosane, ethyl-	28.7	-	-	-	-	-
Fatty acid and conjugates	Hexadecanoic acid	-	91.1	-	-	-	-
	Decanedioic acid	11.5	-	-	-	-	-
	9,15-Octadecadienoic acid	5.7	2.5	-	-	-	-
	1,2-Benzenedicarboxylic acid	7.8	2.2	4.1	5.7	5.2	5.7
	Tetradecanal	-	-	1.9	1.7	2.8	2.7
	Dibutyl phthalate	8.3	3.5	4.3	4.5	4.3	7.3
	Octadecanal	23.4	7.9	16.7	15.1	17.8	17.9
	9-Octadecenamide	-	1.9	-	-	-	-
	Erucylamide	433.9	214.3	108.7	132.5	197.7	203.1
	Tetradecanamide	20.9	4.6	-	-	-	-
	E-15-Heptadecenal	-	-	-	-	-	27.0
Others	4,5 α -Epoxy-3-methoxy-17-methyl-7 α – (4-phenyl-1,3-butadienyl)-6 β,7 β – (oxymethylene) morphinan	50.4	30.1	16.9	16.7	20.2	14.1

[a] Represent only the compounds with quality higher than 80% quality (% matching) after searched with Wiley 7 no. 1 Library

[b] Relative content (%) were normalized with heptadecanoic acid, methyl ester. Data were average of three replication.

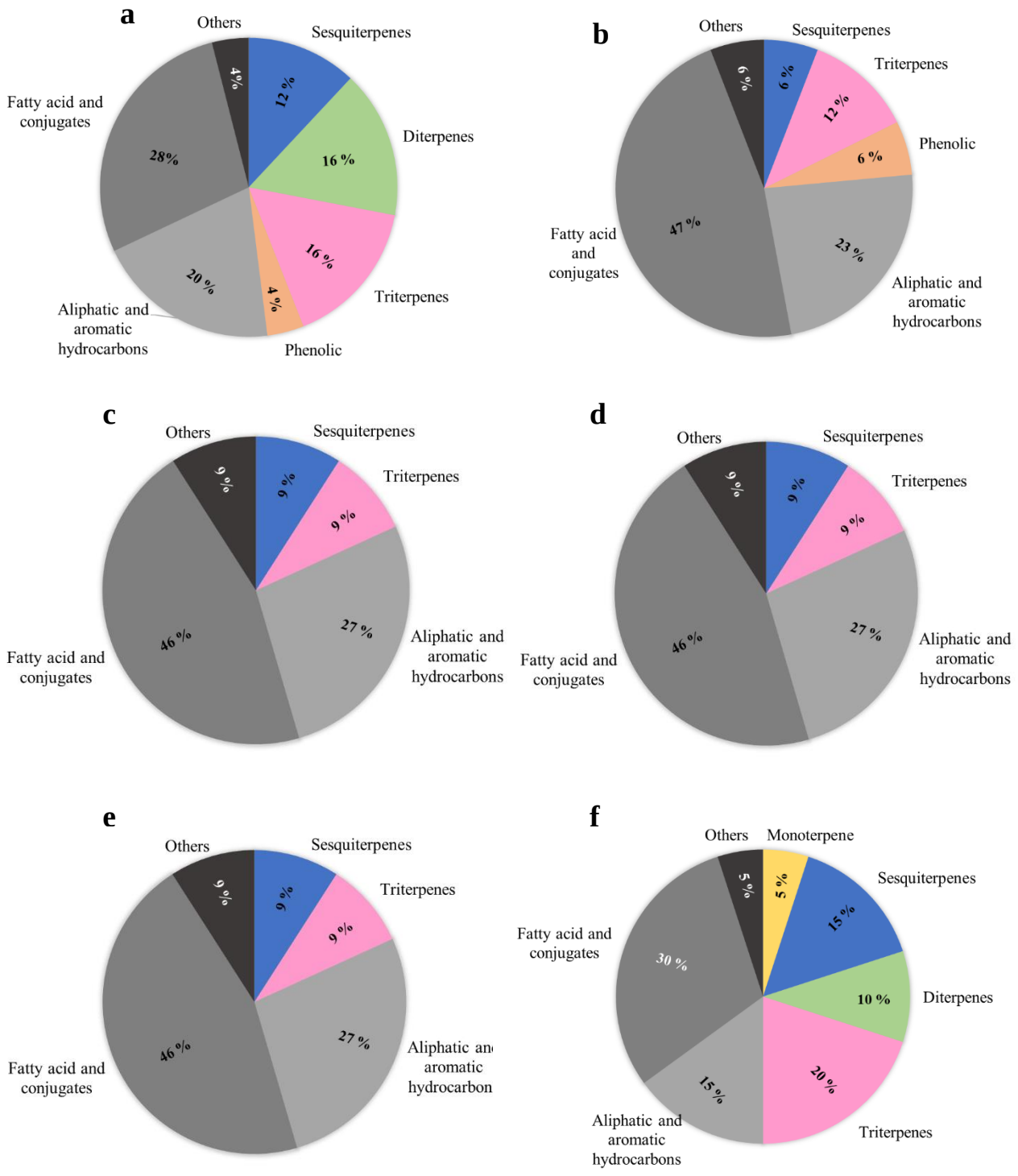

Fig. 1 The quantity proportion of compounds found in dichloromethane extract of *A. annua* leaves)**a**(, Untreated hairy root (**b**), plasma treated-hairy root 15 second and incubate 3 (**c**) or 6 hours (**d**) and plasma treated-hairy root 45 second and incubate 3 (**e**) or 6 hours (**f**). The quantity is the percentage of the total amount.

treated (treatment **e**; eucalyptol, germacrene D, α-copaene, neophytadiene, α-tocopherol, squalene, β-amyrin, tetradecanal and E-15-heptadecenal). Nevertheless, plasma can eliminate a phenolic compound, an aliphatic and aromatic hydrocarbons (nonacosane) and four fatty acid and conjugates (hexadecanoic acid, 9,15-octadecadienoic acid, 9-octadecenamide and tetradecanamide). However, plasma also increase (which was at least 1 times higher than untreated hairy root) the among of two triterpenes (stigmasta-5,22-dien-3-ol and b-sitrosterol), three Aliphatic and aromatic hydrocarbons (cyclododecane, docosane and heneicosane) and three fatty acids and conjugates (1,2-benzenedicarboxylic acid, dibutyl phthalate and octadecanal)

The appearance of nine compounds after plasma treatment, there are five compounds had been reported for pharmacological properties (**eucalyptol**; anti-infective agents and antitussive agents. **A-copaene**; associated autism and Crohn's disease. **A-tocopherol**; dietary supplement for vitamin e intaking**. squalene**; associated cardiovascular disease. and **tetradecanal**; associated celiac disease). The increasing of eight biocompounds, there are four compounds had been reported for pharmacological properties (**stigmasta-5,22-dien-3-ol**; associated cholesterol (LDL-C) reducing in primary hypercholesterolemia. **b-sitrosterol**; hypolipidemic agents. **1,2-benzenedicarboxylic acid**; associated hemodialysis, colorectal cancer and eosinophilic esophagitis. and **dibutyl phthalate**; anti-scrub typhus). The reduction of seven compounds, there are only two compounds had been reported for pharmacological properties (**hexadecanoic acid**; anti-multiple myeloma (MM) cells. and **9-octadecenamide**; hypnotics, sedatives and food additives) [7].

The evidence of biochemical compound appearance and increasing of seventeen compounds may cause by reactive oxygen species (ROS). ROS was produced in plasma system [6]. The effects of plasma (22.1 kV, 12 s) on the biochemical changes and regulation of metabolism-related genes in soybean. There are increasing the concentrations of soluble protein and adenosine triphosphate (ATP) as well as up-regulating ATP a1, ATP a2, ATP b1, ATP b2, ATP b3, target of rapamycin (TOR), growth-regulating factor (GRF) 1-6. Moreover, plasma also increased the demethylation levels of the sequenced region of ATP a1, ATP b1, TOR, GRF 5, and GRF 6 of 6-day-old soybean sprouts [8]. Addition, ROS from plasma are the key factors to activated TET (ten-eleven-translocation) that play important role on DNA demethylation [9]. However, there are report about the repression of *CYP* genes by oxidative stress. The CYPs are a superfamily of ubiquitous enzymes that involved in the metabolism. They observed that, in hepatoma cells, CYP1A1 expression was significantly decreased by oxidative stress (H_2O_2 treatment) [10]. In this plant, CYPs play a role in artemisinin biosynthetic pathway [11]. It is a reason why seven compounds might be reduced or disappeared after plasma treatment.

4. Conclusion

The research revealed that plasma property (external elicitor) in secondary metabolite inducing in plant. The 45 seconds of plasma power 70W and incubated 6 hours can induce nine compounds that normally disappear in the *A. annua* hairy root (five compounds had been reported for pharmacological uses). Increasing of other eight compounds was detected after plasma irradiation (four compounds had been reported for pharmacological uses). However, seven metabolites (in decreasing or disappearance)

were found in treatment of plasma 45 seconds and 6 hours for incubation (two compounds had been reported for pharmacological uses).

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The Effect of Roller's Surface Coating on Sheet Metal Spinning Process

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Keywords: Metal spinning, Surface coating, Wear testing

Abstract. The sheet metal spinning process is a conventional forming process. It is widely used in vary application. Many researches have studied the spinning process parameter, such as, roller design, roller path, feed ratio, spinning depth. The friction coefficient between roller and work piece was studied less than other parameter, because the spinning process is a single point incremental forming. However, in the thin wall spinning process, the friction cause of the failure in spinning process. This research proposes to study the effect of friction on thin wall spinning process. The vary coating was studied for find the minimum friction coefficient. The medium carbon steel (S45C) was used for base material and coating with vary method. TiN (Titanium Nitride), TiCN (Titanium Carbon Nitride), TiAlN (Titanium Aluminum Nitride) and DLC (Diamond Like Carbon) coating was studied. The result shown DLC is the less friction coefficient. After that, the spinning experiment was setup with DLC coating and non-coating roller. By vary spindle speed, feed rate, spinning depth coating and non-coating roller, the result of the experiment will show the effect of friction on spinning force and wear characteristic of the roller.

1. Introduction

The metal spinning process is widely used in many industries. The low forming power, low cost, simple tool are the advantage of it. The spinning process consists of mandrel, blank sheet, roller and tail stock. In the spinning process, roller move to press the blank sheet and move along mandrel profile. The spun work piece is formed into the same shape as the mandrel profile. Recently, the modern spinning processes are developed in vary methods. The mandrel-free spinning is used spinning with asymmetry shape [1, 3]. Some researchers use laser beam to heat the work piece during spinning process [1, 4].

Friction between tool and work piece has a minor effect on spinning process because the contact area between roller and work piece is only single point [2]. In the other hand, the spinning process with thin wall workpiece, where strength is lower, the effect of the friction between roller and workpiece the spinning process is increased [6]. In a forming process, friction between a tool and the workpiece has a significant effect on the material deformation, forming forces, component surface finished, and die wear [5]. The vary friction has an effect on forming process. The causes of difference friction are lubrication, blank sheet material and tool surface coating. Tool surface coating can reduce friction between roller and workpiece. and can improve tool life. The thickness of blank sheet for spinning is ranging 0.4-25 mm [7-9]. In this research, the thickness of blank sheet is 0.4 mm. Variety of reduce friction in forming process such as lubrication, surface coating and cover work piece with

thin film. This research was studied to use surface coating to reduce friction. The 4 types of surface coating are used in this study: TiN (Titanium Nitride), TiCN (Titanium Carbon Nitride), TiAlN (Titanium Aluminum Nitride) and DLC (Diamond Like Carbon).

2. Material and method

2.1 Materials

The substrate material was SC45C steel. The hardness was 88 HRB and surface roughness was Ra = 0.10, Rt = 1.60 and Rz = 0.7 prior to coating. In this study use PVD coating method was used to coat the specimen surface. The coating materials were TiN, TiCN, TiAlN and DLC. The hardness of the surface coating were TiN = 2019 HV0.2, TiCN = 2056 HV0.2, TiAlN = 2217 HV0.2 and DLC = 2509 HV0.2. The thickness of surface coating was 2-4 μm. The substrate surface roughnesses were measured before and after the coating. Table 1 shows that the specimen surface roughnesses were not change after the coating.

Table 1 Surface roughness of specimen.

	Non coating	**TiN coating**	**TiCN coating**	**TiAlN coating**	**DLC**
Hardness	88 HRB	2019 $HV_{0.2}$	2056 $HV_{0.2}$	2217 $HV_{0.2}$	2509 $HV_{0.2}$
Ra (μm)	0.1	0.12	0.1	0.12	0.11
Rz (μm)	0.7	0.74	0.72	0.74	0.7
Rt (μm)	1.6	1.8	1.6	1.8	1.7

(a) (b) (c) (d) (e)

Fig. 1 Wear test specimen (a) non-coating; (b) TiAlN; (c) TiCN; (d) TiN and (e) DLC.

2.2 Methods

The experiment was carried out in two steps. First step was the friction and wear testing. In this step, wear testing machine is used to test the friction coefficient and wear of TiN, TiCN, TiAlN and DLC surface coating. The coating material exhibits the lowest coefficient of friction (COF) and wear will be identified in this step. Second step was the spinning experiment. In this step, the surface of roller was coated with the best coating material. After that, the spinning experiment will set to compare spinning process parameters between coating and non-coating rollers.

2.3 Friction and wear test

In wear testing experiment, the specimens were made from S45C base and coating with TiN, TiCN, TiAlN and DLC material. The experiment followed ASTM G99 standard [10]. Fig. 2 shows the friction and wear test machine. A load cell (6), an amplifier (8) and the LabVIEW program (9) were used to measure friction coefficient. Diameter of stainless 316L ball for testing head was 6 mm. The rotation velocity of spin disk was 120 rpm. The distance of testing was 250 m. Test load were 1 kg, 2 kg and 3 kg. The Optical microscope was used for wear track observation and the surface roughness measurement was used to measure wear profile.

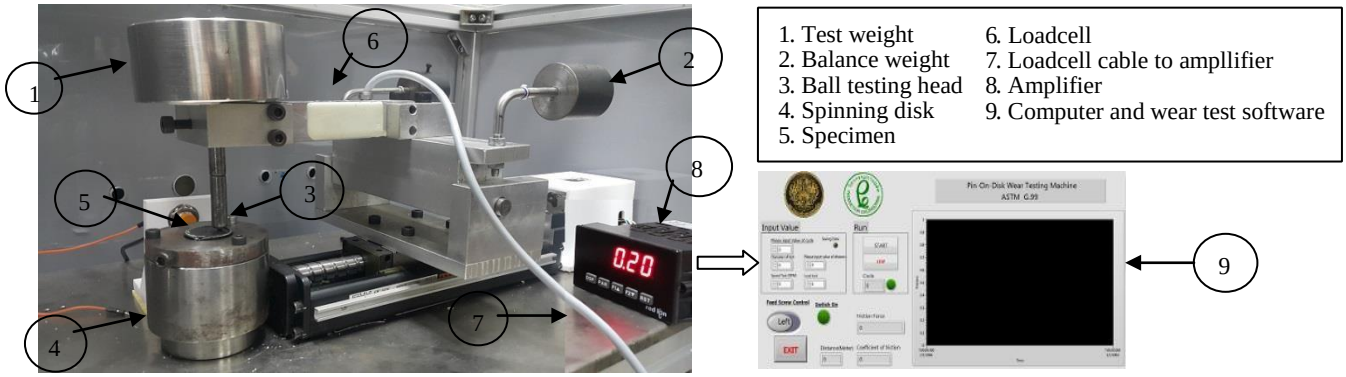

Fig. 2 Wear testing machine.

2.4 Spinning test

This step uses a result from wear testing step. The lowest COF and wear rate of surface coating material was used to coating spinning roller. The spinning experiment proposes to study effect of roller's COF on spinning process. The spinning force, thickness variation and surface roughness of spun were measured as the spinning parameter in this experiment. The experiments compare the spinning parameters between non-coating roller and coating roller. Other spinning parameter such as spindle speed, spinning depth, feed rate, work piece material, work piece diameter and work piece thickness were fixed to all experiment case. The spindle speed = 250 rpm, spinning depth = 0.3 mm, feed rate = 2.4 mm/sec, work piece material = Tin-plate, work piece diameter = 90 mm, work piece thickness = 0.5 mm, roller radius = 2.5 mm, roller diameter = 50 mm and no lubrication.

The spinning experiment is shown in Fig. 3. The roller holder was modified by attach strain gauge for measured spinning force. Strain gauge cables were connected to bridge circuit and amplifier. The computer was used to collect date from amplifier.

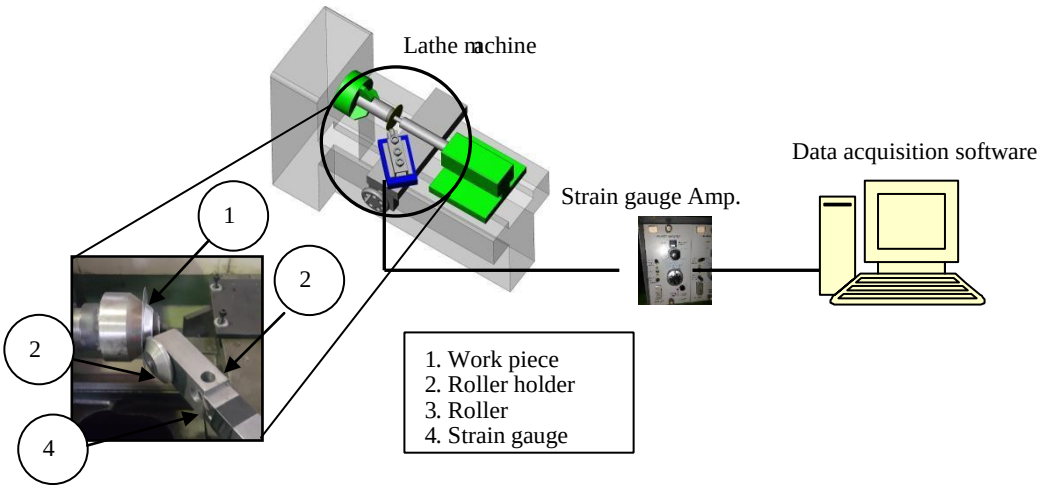

Fig. 3 Spinning test.

3. Result and discussion

3.1 Wear testing result

The results of wear test were shown in Fig. 4. The COF at the start of testing of Non-coating, TiAlN, TiCN, TiN and DLC were 0.55, 0.3, 0.3, 0.22 and 0.23. The average COF of Non-coating, TiAlN, TiCN, TiN and DLC were 0.88, 0.72, 0.72, 0.71 and 0.24. The COF charts of TiAlN, TiCN, TiN and non-coating were start at 0.22-0.55 and the charts were rising rapidly. However, the COF chart of DLC was start at 0.23 and it slightly increase. That mean COF of DLC coating was the lowest COF from other coating. In general, the friction is mainly caused by the dual roles of the mechanical interlock and molecular attraction of friction pairs [11]. The high surface hardness can reduce the initial stage of wear. From table 1, the DLC coating has the most surface hardness. So that the wear rate and COF of DLC coating were lower that other coating.

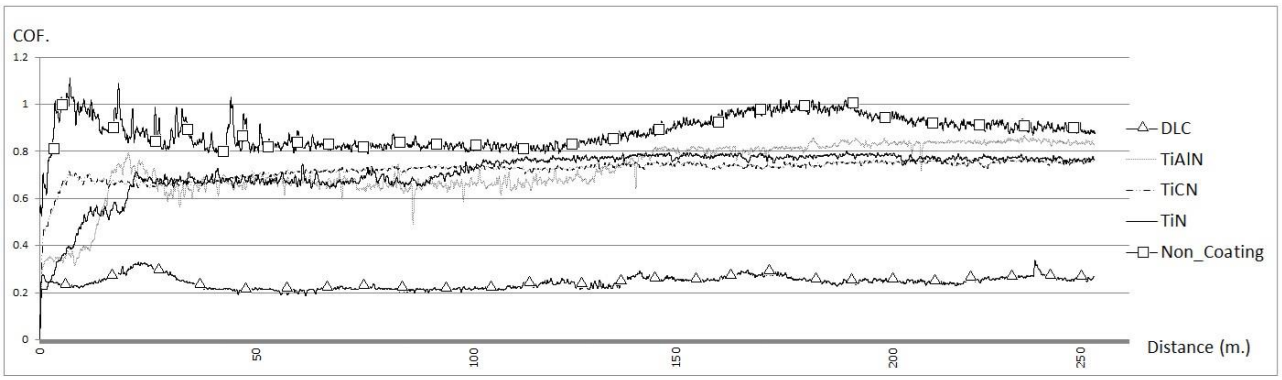

Fig. 4 Result of COF testing.

After finishing the wear test, the wear profiles were measured by surface roughness measurement machine. The results of wear profiles are shown in Fig. 5. Wear rates were calculated from the area under profile of wear track curve multiply by sliding distance. The maximum wear rate is 33.19 (wear volume per sliding distance) that from non-coating specimen. The minimum wear rate is 9.67 (wear volume per sliding distance) that from DLC coating. Moreover, the Fig. 6 shows the wear rates for 1kg, 2 kg and 3 kg test load. The wear rate of non-coating is highest at all test loads.

Meanwhile, the wear rate of DLC is lowest at all test loads. The wear rates of TiAlN, TiCN and TiN were nearly and similar trend. The mainly cause of wear on the coating surface is tensile stress [12]. The tensile stress parallel to the sliding direction were generated the plastic deformation and abrasive wear on the coating surface. The increasing test load cause of coating failure. The coating failure was depending on various factors such as type of coating, surface hardness, surface roughness etc. Fig. 5 show profiles of wear tracks after reaching critical load and distance. In the Fig., the coating failures are occurred. However, the area under the profile of wear track curve and the width of wear failure in Fig. 5 of DLC is lower than other coating. From the COF in Fig. 4 and wear rate in Fig. 5, it is clear the DLC coating is lower COF and wear rate than other coating.

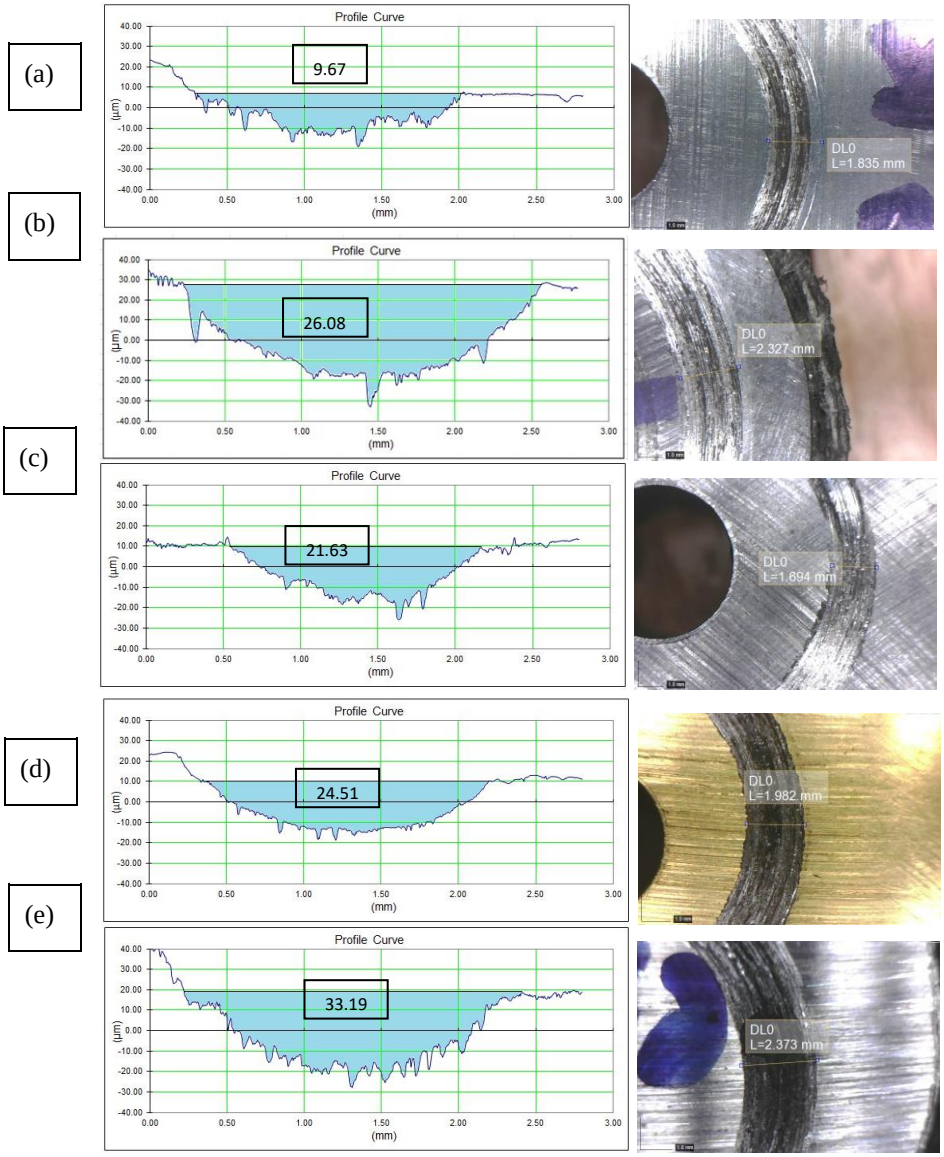

Fig. 5 Sectional profiles of wear tracks on (a) DLC; (b) TiAl; (c) TiCN; (d) TiN and (e) noncoating.

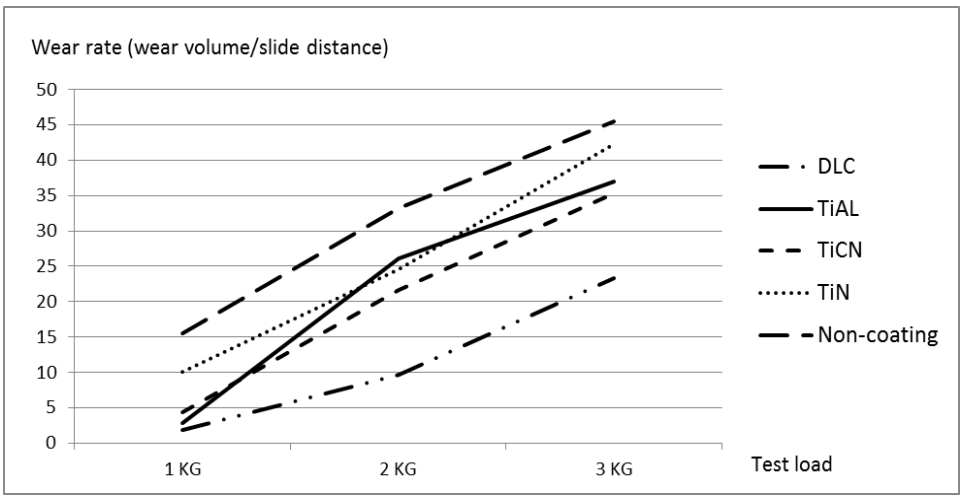

Fig. 6 Wear rate.

3.2. Spinning experiment result.

The result of spinning force in axis direction is shown in Fig. 7. The result compares spinning force between non-coating roller and DLC coating roller. Spinning force of Non-coating roller is 9.97 Kg at start of experiment and increase when the roller moves along the mandrel. Spinning force of DLC coating roller is 9.96 Kg at start of experiment. The DLC roller's spinning force is slightly increased throughout the experiment. The average spinning force of noncoating roller is 9.973 Kg and DLC coating is 9.964 Kg. Summary, DLC coating can slightly decrease axis spinning force from 9.973 Kg to 9.964 Kg or 0.09%.

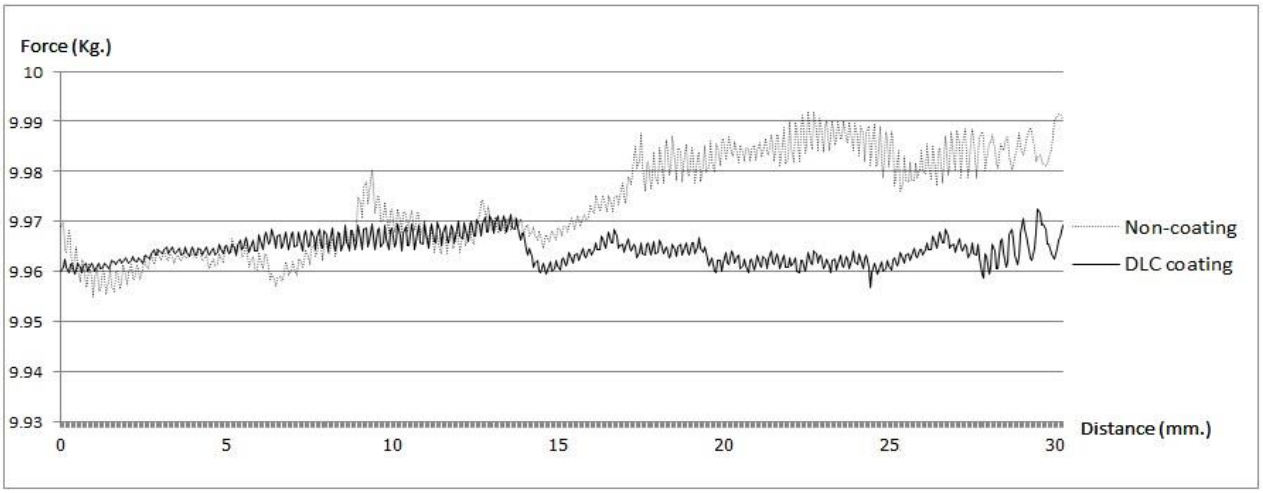

Fig. 7 Spinning force in axis direction.

The thickness variation of spun was measured by cut the spun to 4 section and then each section was measured the wall thickness at 3 points. The result of thickness variation is show in Fig. 8. The thickness of spun from coating roller is ranging 0.28 to 0.3 mm. The thickness of spun from non-coating roller is ranging 0.24 to 0.34 mm. That mean, the thickness variation of spun from coating roller is less than non-coating roller.

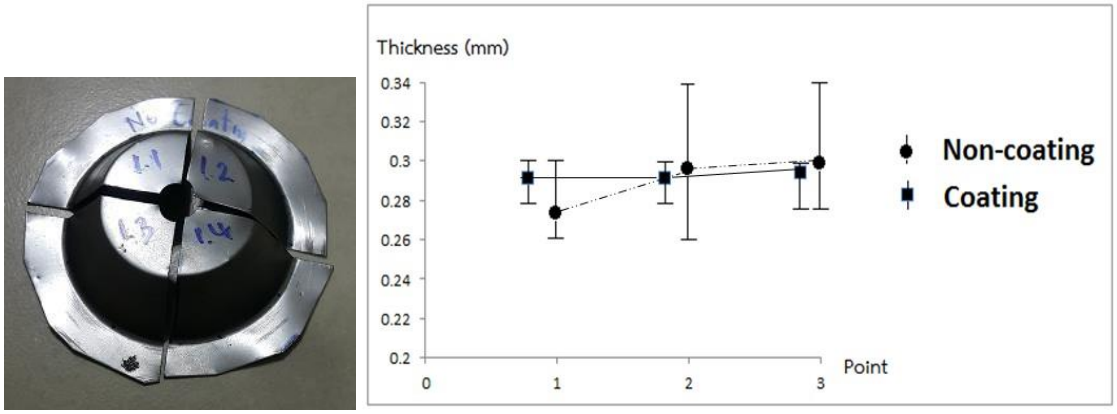

Fig. 8 The thickness variable of spun.

The surface roughness of spun were measured by surface roughness measurement machine. The result is show in table 2. The surface roughness of spun from coating roller is better than noncoating roller. For example, Ra of spun from non-coating roller is 0.30 meanwhile Ra of spun from coating roller is 0.2. Rz of spun from non-coating roller is 1.50 meanwhile Ra of spun from coating roller is 1.20. Rt of spun from non-coating roller is 3.30 meanwhile Ra of spun from coating roller is 2.30. The surface roughness equations are:

$$Ra = \frac{1}{l}\int_0^l |Z(x)|dx$$
$$Rz = Rp + Rv$$
$$Rt = \max(Zpi) + \max(Zvi)$$
$$Rq = \sqrt{\frac{1}{l}\int_0^l Z^2(x)dx}$$

When $Z(x)$ = roughness profile
l = sampling length
Rp = maximum value of profile peak high
Rv = maximum value of profile valley depth
Zpi = maximum value of profile peak high in small phase
Zvi = maximum value of profile valley depth in small phase

Table 2 Result of surface roughness, spinning force and thickness range.

	Non-coating	Coating	%Improve
Average Ra (μm)	0.3	0.2	33.33
Average Rz (μm)	1.5	1.2	20
Average Rq (μm)	0.4	0.3	25
Average Rt (μm)	3.3	2.3	30.3
Maximum spinning force (N)	98	95.15	2.91
Average range of thickness maxmin (mm)	0.06	0.02	33.33

4. Conclusion

The wear testing was found DLC coating has the lowest COF and wear rate from 4 types of coating. In the spinning experiment, the DLC coating can slightly decrease axis spinning force from 9.973 Kg to 9.964 Kg or 0.09%.

The surface roughness of spun from coating roller is better than non-coating roller. For example, Ra of spun from non-coating roller is 0.30 meanwhile Ra of spun from coating roller is 0.2. Rz of spun from non-coating roller is 1.50 meanwhile Ra of spun from coating roller is 1.20. Rt of spun from non-coating roller is 3.30 meanwhile Ra of spun from coating roller is 2.30. That means the COF of roller has an effect on spun surface roughness. The less friction between roller and work piece can improve the spun surface roughness.

The thickness variation of spun from coating roller is less than non-coating roller. The thickness of spun from coating roller is ranging 0.28 to 0.3 mm. The thickness of spun from non-coating roller is ranging 0.24 to 0.34 mm.

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Biological Activity of Partial Purified Polysaccharides from Thai Macroalgae

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Abstract. Macroalgae are a rich source of natural polysaccharides with several important biological activities. This research is the first reported instance on biological activities of partial purified polysaccharides and rare sugar of some macroalgae from Thailand. Polysaccharides were extracted from local macroalgae i.e. *Ulva* spp., *Sargassum* spp., *Spirogyra* spp., and a mixture of *Cladophora* spp. and *Rhizoclonium* spp. which are locally known as Sarai Sai Kai, Sarai Toon, Sarai Tao, and Sarai Kai, respectively. Total sugar content in each sample increased 1.52, 1.26, 1.38 and 1.16 times after partial purification. Markedly, a variation of sugar composition was discovered in algal polysaccharide samples; a rare sugar tagatose was found in polysaccharides from Sarai Sai Kai, Sarai Kai and Sarai Tao, meanwhile psicose was found in Sarai Kai and Sarai Tao, whereas allose was found only in Sarai Kai. No rare sugar was reported in Sarai Toon. Subsequently, polysaccharides of Sarai Toon showed the highest biological activity of antioxidant properties by scavenging DPPH and ABTS with IC_{50} equalling 3.59±1.00 and 0.86±0.32 mg/ml. The results indicated a polysaccharide from Sarai Toon was more effective than vitamin C. Moreover, IC_{50} of anticancer activity against Caco-2 cells was 0.66±0.09 mg/ml. Macroalgal polysacharide are subjected for further intensive experiments for their natural antioxidant and anticancer properties.

1. Introduction

Macroalgae are plant-like organisms since they lack true leaves, roots and stems of plants [1]. Their growth can be found in natural water sources including freshwater and marine water sources. Usage of macroalgae are various in terms of food, livestock feed, fertilizer, wastewater treatment and medicine [2]. Macroalgae, therefore, are considered as a valuable natural resource. Macroalgae contain large amounts of polysaccharides [3], which are long chains of carbohydrate molecules, consisting of monomer units linked glycosidic bonds in either straight or branching forms. Polysaccharides found in macroalgae are cellulose, hemicelluloses, agar, alginate, carrageenan, beta-glucan, laminarin and fucoidan. They pose bioactive activities such as antioxidants, antitumor, anticoagulant, anticancer, anti-inflammatory, antiviral and antityrosinase [4]. Some types of rarely present sugar can be found in polysaccharides extracted from macroalgae. These particular types of sugar are scarce in natural sources and contain few calories [5]; they are also rare and expensive. This research aimed to study rarely present sugar in macroalgal polysaccharides and their biological activities after a partial purification.The macroalgae samples are cosmopolitant and widely spread in both freshwater and marine water sources in Thailand. The freshwater samples were identified as *Spirogyra* spp. and a mixture of *Cladophora*/*Rhizoclonium*, while those found in marine water sources were *Ulva* spp. and *Sargassum* spp.

2. Materials and Methods

2.1 Polysaccharides extraction from macroalgae

Fifty grams of dried macroalgae including *Ulva* spp., *Sargassum* spp., *Cladophora*/ *Rhizoclonium*. and *Spirogyra* spp., which are locally known as Sarai Sai Kai, Sarai Toon, Sarai Kai, and Sarai Tao, were extracted with 1500 mL distilled water at 98ºC for 1 h. The supernatant was separated from algae residues by successive filtration through cloth, with the remaining residues filtered with water for two more occassions. The supernatant was evaporated at 60ºC for concentration and precipitated with 95% ethanol . The extracts were subsequently concentrated and submitted to graded precipitation with ethanol and the mixture was kept overnight at 4°C to precipitate the polysaccharides [6].

2.2 Monosaccharides composition analysis

Monosaccharides composition analysis was carried out by the methods described by Yuan *et al* [4]. High-performance liquid chromatography (HPLC) was used for the identification of monosaccharide composition. 1 g of the obtained crude polysaccharide was hydrolyzed with HCl (pH=2), and kept at 98°C for 3.5 h. The content was neutralized to pH 7.0 with 1 M NaOH and filtrated. It was then applied to a gel-filtration chromatographic column of Shodex VG-50 4E, maintained at a temperature of 50°C, eluted with CH_3CN:CH_3OH:H_2O (85:10:5) at a flow rate of 0.6 mL/min and detected by a refractive index detector (RID).

2.3 Partial purification of polysaccharides from macroalgae

Partial purification of polysaccharides from macroalgae with re-extraction and re-precipitation were modified from the methods of Phinyo [6] and Khanavi *et al* [7]. Six grams of dried crude polysaccharide was extracted with 300 mL of distilled water at 98°C for 1h. The extracts were then precipitated with ethanol and the mixture of each were kept overnight at 4°C to precipitate the polysaccharides. Total sugar concentration was measured by phenol-sulfuric acid method [11]. The total sugar yield was calculated as detailed below [8].

$$\text{The total sugar yield (\%, w/w)} = \frac{\text{The content of glucose in polysaccharides}}{\text{Dry weight of polysaccharides}} \times 100$$

2.4 Antioxidant activity of partial purified polysaccharides from macroalgae

2.4.1. DPPH radical scavenging assay

This assay was performed based on the microplate-adapted method as previously described by Terres *et al*. [9]. 20 µl of partial purified polysaccharides aliquots from macroalgae, standard solution of gallic acid, negative controls, and 280 µl of a methanolic solution of DPPH (0.142 mM; absorbance of 1.0 ± 0.09) were added to each microplate well. The microplates were incubated at 25 °C for 20 minutes under constant shaking, followed by the immediate measurement of absorption at 515 nm. using a microplate reader.

2.4.2. ABTS radical cation decolourization assay

This assay was performed based on the microplate-adapted method previously described by Terres *et al*. [9]. The solution of the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) was prepared by mixing 1 mL of 7 mM ABTS dissolved in methanol with 17.6 µl of 140 mM potassium persulfate. This solution was incubated in dark conditions at room temperature for 16 hours.

The absorbance of the ABTS solution was adjusted to the range of 1.0 ± 0.09 by dilution within methanol (1/40; v/v). Aliquots of 20 µl of partial purified polysaccharides from macroalgae, standard solution of trolox, negative controls and 280 µl of ABTS solution (pH 6.7) were added to each well of the microplate. The microplates were incubated at 25 °C for 20 minutes under constant shaking, with the absorbance immediately measured after at 734nm. using a microplate reader.

Three technical replicates of each experiment were performed and the half maximal inhibitory concentration (IC_{50}) of each polysaccharide from macroalgae was calculated as detailed below.

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of treated cells})}{(\text{Absorbance of control})} \times 100$$

2.4.3. Anticancer of partial purified polysaccharides from macroalgae

Caco-2 cells and normal fibroblast cells (Vero) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% (v/v) fetal calfserum (complete medium). $1x10^4$ Cultured cells in 100 µl complete media were transferred into each well of a 96 well plate and incubated at 37°C in an enriched humidified air atmosphere with 5% (v/v) CO_2 for 24 h to allow the attachment of each cell to the bottom of each well. Subsequently, the cells were treated with partial purified polysaccharides from macroalgae, incubated for 48 h before the media was removed. In addition, 20 µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution was added. After four hours of further incubation, the MTT solution was removed. Finally, a 200 µl mixture of DMSO was added to each well and mixed to ensure cell lysis and the dissolving of the formasan crystals, before the absorbance at 540 nm and 630 nm were measured. Three replications of each experiment were performed and the percentage survival of the treated cancer and normal cultured cells was calculated according to the formula below [10].

$$\text{Percentage survival} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control}} \times 100$$

3. Results and Discussion

3.1 Quantity of polysaccharides from macroalgae

The polysaccharide extract from Sarai Sai Kai showed the highest amount of polysaccharides (28.7%), followed by Sarai Kai (25.10%), Sarai Tao (11.80%) and Sarai Toon (10.56%). Because polysaccharide is a polar compound, water can be used for extraction, similar to other highly polar solvents [11]. However, water extraction can only extract water soluble polysaccharides [6]. Other types of polysaccharides that are water insoluble should be extracted by other methods to collate the polysaccharides.

3.2 Monosaccharide Composition Analysis

The residue obtained after extraction of algal biomass with hot water contained mainly polysaccharides [6]. Hydrolysis by HCl (pH=2) produced the monosaccharide constituents of readily hydrolyzable polysaccharides, detected by HPLC analysis (Table 1). The retention time of standard monosaccharide samples highlighted that Sarai Sai Kai contained rhamnose, xylose and tagatose. Sarai Toon contained rhamnose and glucose. Sarai Tao contained rhamnose, psicose, xylose and tagatose, and Sarai Kai contained psicose, xylose, tagatose, allose and galactose. This result is the first report

on rare sugar content in polysaccharides from macroalgae in Thailand. It should be noted that glucose content of hydrolysates may depend on the physiological status of the alga at the moment of harvesting during different seasons. Using water for extraction can only extract water soluble polysaccharides. This could be due to the complex mucilage composed of rhamnose, arabinose, xylose, galactose and uronic acid [12].

Table 1 Monosaccharide composition of polysaccharides from macroalgae

Macroalgae / Sugar profile (mg/L)	Sarai Sai Kai (*Ulva* spp.)	Sarai Toon (*Sargassum* spp.)	Sarai Kai (*Cladophora* /*Rhizoclonium*)	Sarai Tao (*Spirogyra* spp.)
Rhamnose	111.702	152.654	-	99.094
Xylose	20.194	-	160.994	24.406
Galactose	-	-	139.548	-
Glucose	-	31.324	-	-
Tagatose	54.604	-	48.644	72.95
Psicose	-	-	54.04	127.678
Allose	-	-	8.826	-

3.3 Total sugar content in crude polysaccharides and partial purified polysaccharides

From the partial purified polysaccharides by re-extraction and re-precipitation, the total sugar of purified polysaccharides from Sarai Sai Kai, Sarai Toon, Sarai Kai and Sarai Tao, shown in Table 2, increased from 13.60±0.69, 4.03±0.06, 8.20±0.91 and 5.67±0.58%, respectively, indicating that the concentration of polysaccharides increased.

Table 2 Total sugar content of crude polysaccharides and partial purified polysaccharides from macroalgae

Macroalgae	Total sugar %(w/w)	
	Crude polysaccharides	Partial purified polysaccharides
Sarai Sai Kai (*Ulva* spp.)	27.60 ± 1.06^{d}	41.87 ± 0.46^{g}
Sarai Toon (*Sargassum* spp.)	15.60 ± 0.10^{a}	19.63 ± 0.06^{b}
Sarai Kai (*Cladophora*/*Rhizoclonium*)	22.00 ± 0.90^{c}	30.27 ± 0.23^{c}
Sarai Tao (*Spirogyra* spp.)	35.73 ± 0.42^{f}	$41.40\ 0.20^{g}$

3.4 Antioxidant activity of polysaccharides from Macroalgae

DPPH radical scavenging assay is widely used for evaluating the effects of free radicals in various samples because of the high stability. The antioxidant activities of gallic acid and polysaccharides against the radicals of DPPH are shown in Fig. 1 which show that the antioxidant activities of polysaccharides increased as the concentration increased. The polysaccharide from Sarai Toon had the highest antioxidant activity, IC_{50} equal to 3.59±1.00 mg/mL, followed by Sarai Tao, Sarai Kai and Sarai Sai Kai at 6.10±1.02, 18.90±1.82 and 54.84±2.83 mg/mL respectively. The ABTS radical cation decolourization assay uses the same principle as the reduction of DPPH free radicals,

but with ABTS, they are positively charged. Polysaccharides from Sarai Toon were most effective in removing ABTS radicals,with an IC_{50} equal to 0.86±0.32 mg/mL, followed by polysaccharides from Sarai Tao, Sarai Sai Kai and Sarai Kai at 2.62±0.28, 8.84±2.00 and 8.91±1.90 mg/mL respectively. From this experiment, it can be seen that the polysaccharides from Sarai Toon have the ability to inhibit free radicals more than polysaccharides from other macroalgae. Sarai Toon contain elements of phenolic compounds in the phlorotannins group found only in brown algae and classified as phenolic compounds that have a high oxidative activity [13]. In addition, different types of polysaccharides in each type of macroalgae [14], as well as the amount and location of sulfate groups on different sugar structures of polysaccharides extracted from different macroalgae also affects the effectiveness of antioxidants [15, 16, 17]. The major difference of polysaccharides from Sarai Sai Kai, Sarai Toon, Sarai Kai and Sarai Tao is the content of rhamnose, which leads to different antioxidant properties [18]. Polysaccharides from Sarai Toon were the most effective antioxidant with a rhamnose content of 152.654 mg/L, followed by polysaccharides from Sarai Sai Kai (111.702 mg/L), Sarai Tao (99.094 mg/L) and Sarai Kai (no rhamnose).

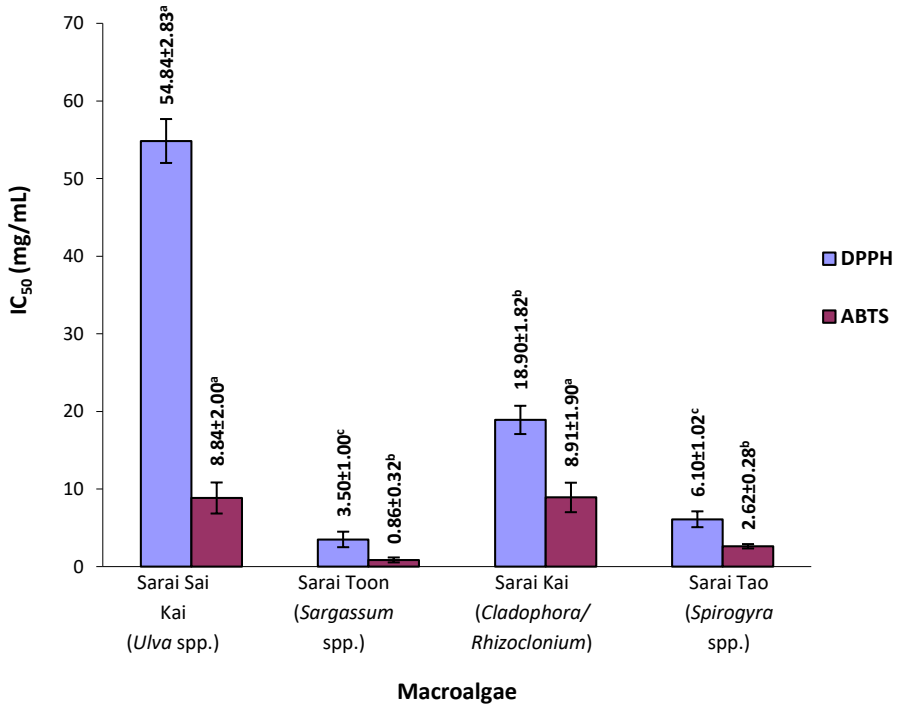

Fig. 1 The concentration of polysaccharides from Sarai Sai Kai, Sarai Toon, Sarai Kai and Sarai Tao that can inhibit 50% of free radicals (IC_{50}) from DPPH radical scavenging and ABTS radical cation methods.

3.5 Caco-2 cell cancer toxicity of polysaccharides from macroalgae

The cell toxicity induced by algal polysaccharides examined in human colon cancer cells using MTT assay analysis is shown in Fig. 2. It was found that all four macroalgal polysaccharides can inhibit Caco-2 cells. Sarai Toon polysaccharides can inhibit cancer cells the most, with the percentage of survival cells, IC_{50} equal to 0.66±0.09 mg/mL, followed by polysaccharides from Sarai Tao, Sarai Sai Kai and Sarai Kai at 1.37±0.15, 3.29±0.61 and 12.20±0.72 mg/mL respectively. The cytotoxicity test against normal cells indicated that IC_{50} equalled 2.12±0.25, 0.07±0.04, 9.69±0.17 and 0.65±0.04 mg/mL respectively (Fig. 3). Previous reports showed that many natural polysaccharides possess anti-

cancer activities. Although the antimicrobial activity of natural polysaccharides was less than the synthetic anticancer agents such as Cis-platin, natural polysaccharides were less toxic to cells than synthetic anti-cancer substances [19]. Sarai Toon had the highest toxicity to normal cells. Potential factors affecting cell toxicity were the sulfate group and the replacement position of the sulfate group. Polysaccharides of Sarai Toon contained carrageenan, which had a replacement sulfate group in carbon at position 6, the highest toxicity position [20]. Therefore, natural polysaccharides have been developed to be used as anti-cancer agents.

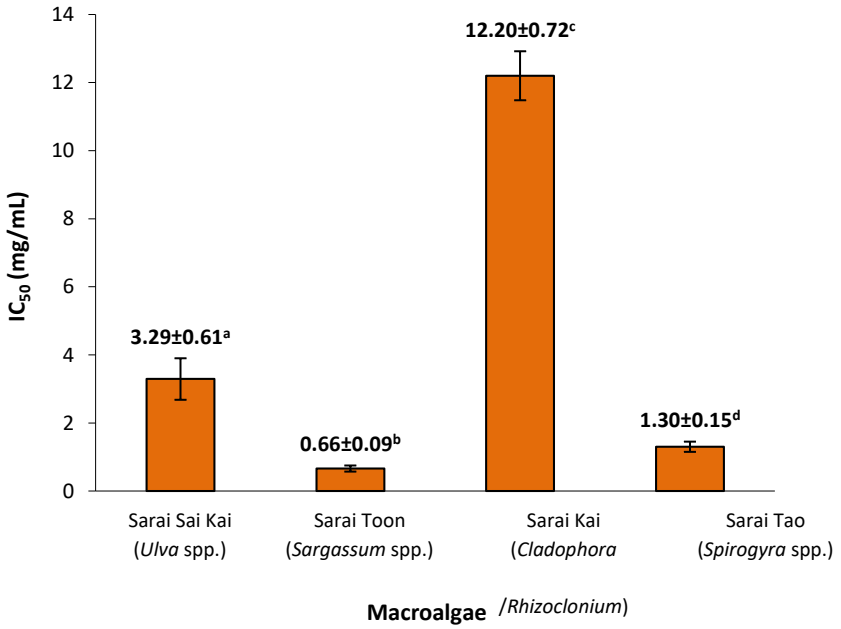

Fig. 2 Cytotoxicity of macroalgal polysaccharides against Caco-2 cells.

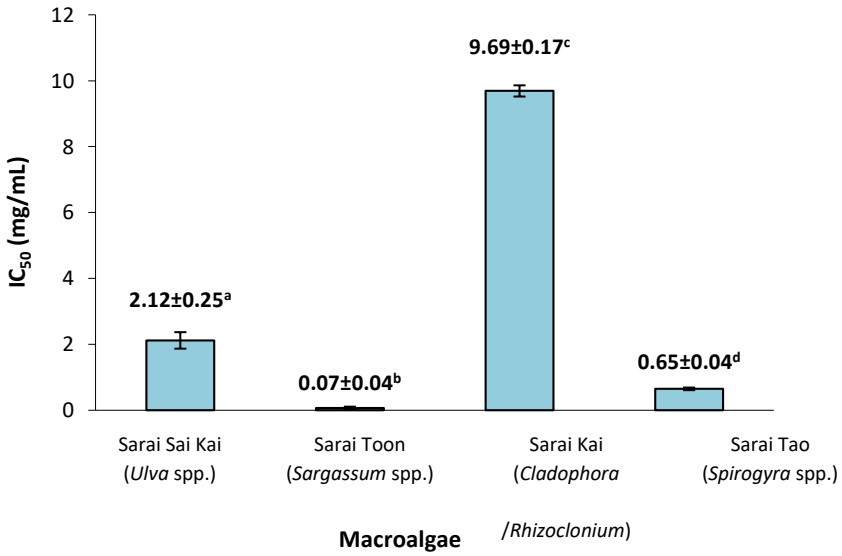

Fig. 3 Cytotoxicity of macroalgal polysaccharides against Vero cells.

4. Summary

Rare sugar can be found in polysaccharides from Sarai Sai Kai, Sarai Toon, Sarai Sai Kai. and Sarai Tao comprised of tagatose, psicose and allose. In addition, polysaccharides from partial purification are effective against free radicals and inhibit colon cancer cells (Caco-2). Polysaccharides from macroalgae may be an alternative for applications in the food and drug industry in the future.

5. Acknowledgement

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Improved Ethanol Tolerance in *Spathaspora passalidarum* CMUWF1-2 through Adaptive Evolution

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Keywords: *Spathaspora passalidarum*, Bioethanol, Adaptive evolution, Ethanol tolerance, Yeast

Abstract. *Spathaspora passalidarum* CMUWF1-2 is a yeast that has highly efficient produce ethanol from xylose, which is the major sugar present in lignocellulosic biomass at high temperature. However, this yeast is sensitive to the inhibitors such as furfural present in the pretreated lignocellulosic biomass. Previous study, CMUWF1-2 was improved the furfural tolerance through evolutionary adaptation. During ~17 generations of adaptive evolution, a furfural tolerant mutant AF2.5 was obtained. After characterizing this strain in this study, it was found that AF2.5 not only had furfural tolerance but also had ethanol tolerance, higher than wild type. To focus on its ethanol tolerance, AF2.5 was examined growth and cell morphology in YPD broth medium containing with and without 4% (v/v) ethanol comparing with wild type. In the presence of 4% (v/v) ethanol, AF2.5 showed better growth than wild type, while cell morphology of AF2.5 was not different with wild type. Their cell walls were thin, when grown in the presence of ethanol. Moreover, the surviving capacity of AF2.5 that was exposed to 4% (v/v) ethanol was evaluated comparing with wild type. The result showed that a percent of viability in AF2.5 had two times higher than wild type.

1. Introduction

Bioethanol is a renewable fuel that play an important role in using as alternative energy instead of fossil fuels because it is a clean energy and less environmental concerns. The mostly production of bioethanol often uses the yeast fermentation process from various biomasses [1]. In the beginning, the first-generation biomass that was sugar-based raw materials or food crops such as corn or sugarcane was used to produce ethanol, but it led to the food prices increasing and the food crops lacking. Therefore, the second-generation biomass that was lignocellulosic biomass such as agricultural waste was interested because it has the abundance of sugars and it can reduce the biomass cost [2]. Lignocellulose components are 40% cellulose, 30% hemicellulose, and 15% lignin [3]. In the part of hemicellulose, it has both of pentose (C5) sugars (e.g. xylose and arabinose) and hexose (C6) sugars (e.g. mannose, galactose, and glucose) [4].

To obtain ethanol from lignocellulose, normally, there are at least three steps: pretreatment, hydrolysis and fermentation. After completing the pretreatment and hydrolysis steps, sugars can be released from lignocellulose [5]. In addition, inhibitors are also present in pretreatment lignocellulose hydrolysate, which can inhibit the growth of microbe and cause the reduction of ethanol yield. These inhibitors can be put into three major groups; furans, weak acids, and phenolics [6,7]. In addition, not only inhibitors but also ethanol, which is the end product of this process, is able to disturb growth and metabolism of yeast cells. When the ethanol concentration increasing, cell membrane of yeast cell is led to the integrity and fluidity decrease and the permeability damage. Furthermore, the high ethanol concentration is the cause of acidify intracellular and vacuolar condition in yeast cell by perturbing the transmembrane electrochemical potential and it affects the protein conformation of key glycolytic

enzyme, protein denaturation and dysfunction. In addition, it has the effect to the inferior uptake of glucose, maltose, ammonium, and amino acid [8, 9].

The industrial yeast *Saccharomyces cerevisiae* is a popular yeast strain for biofuel industries from corn-based and sugar-based. However, it is unable to utilize xylose, the most common pentose sugar in the hemicellulosic hydrolysate, as a sole carbon source for growth and fermentation [10, 11]. *Spathaspora passalidarum* is one of a few yeasts known to efficiently ferment and metabolize xylose and exhibits minimal glucose repression [12, 13]. This rare ability makes this species of great interest to bioenergy research as effective xylose fermentation and utilization is a major step in the production of biofuel from lignocellulose materials. Nevertheless, this yeast is not tolerated to several stresses such as hydrolysate inhibitors and high concentration of ethanol [2, 14]. In order to improve their stress tolerance abilities, adaptive evolution method, which can cause a random mutagenesis is noticeable. Since, the mechanism of inhibitor tolerant remain unclear, random mutagenesis may be useful for obtaining desired strain. The mutation will be induced through environmental stress condition [15, 16, 17]. In this study, we are interested in *S. passalidarum* CMUWF1-2 that is effective in ethanol production from various sugars with no glucose repression and it is a thermotolerance yeast [13]. However, it is less tolerant to inhibitors such as furfural. Therefore, in previous study, *S. passalidarum* CMUWF1-2 was improved the furfural tolerance by adaptive evolution. The adapted mutant, AF2.5 was obtained. Fortunately, we found that AF2.5 was not only had furfural tolerance but also had ethanol tolerance. Thus, AF2.5 was characterized in growth, viability and morphology under both ethanol-stressed and non-stressed conditions.

2. Material and methods

2.1 Yeast strain and culture conditions

S. passalidarum CMUWF1-2 and furfural-adapted mutant, AF2.5 were used in this study. AF2.5 was obtained from adaptive evolution method by gradually increasing the furfural concentration of the repeated cultivations from 1.0 g/l to 2.5 g/l. The cultivation was repeated around 17 times (data not shown). These strains were maintained on YPD agar plate (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, and 15 g/l agar) and the pure culture was stored at -20ºC by adding with 25%(w/v) glycerol for long-term storage.

2.2 Ethanol tolerance in AF2.5

To test an ability of ethanol tolerance in AF2.5 comparing with CMUWF1-2 (wild type), spot test analysis was used. The yeast cells were cultured in YPD broth medium at 30ºC, 150 rpm for 18-24 h. Cells were harvested and measured OD_{600} using a spectrophotometer. Adjusted an initial OD_{600} as 1.0 with sterile distilled water and then ten-fold sequentially diluted from 10^0 to 10^{-5}. Five µl of each dilution was spotted onto YPD agar plate with and without various concentrations of ethanol from 6.0-12.0% (v/v). These plates were incubated at 30ºC for 72 h. Each experiment was examined at least three replicates.

2.3 Effects of ethanol on cell growth, cell viability and cell morphology in AF2.5

For investigating cell growth, cell viability and cell morphology of AF2.5 under ethanol-stressed condition comparing with wild type, AF2.5 and CMUWF1-2 were pre-cultivated at 150 rpm for 18 h in 5 ml of YPD medium. The pre-culture was inoculated with an initial OD_{600}=1 into a 125-ml Erlenmeyer flask containing 50 ml of a fresh YPD medium with and without (w/wo) 4.0 % (v/v) ethanol. Cells were grown under a shaking condition (150 rpm) at 30 °C. Cell cultures were sampled every 12 h and immediately subjected to centrifugation at 8000 g for 5 min. Cell growth was determined turbidimetrically at 600 nm. Cell morphology was observed under microscope (Nikon

ECLIPSE Ts2R, Japan). Cell viability was determined by counting the number of colony-forming units on YPD plate, after cultivating cells in YPD broth w/wo 4.0% ethanol at regular intervals during incubation. Survival curves are represented as the percentage of viable cells using the following formula. Data were reproduced by three independent experiments.

$$\text{Viability (\%)} = \frac{\text{Number of colonies grown in YPD broth with 4.0\% EtOH}}{\text{Number of colonies grown in YPD broth}} \text{ X } 100$$

3. Results

3.1 Ethanol tolerance in AF2.5

From previous study, we found that AF2.5 was able to grow on YPD agar plate supplemented with 2.5 g/l furfural higher than CMUWF1-2 (wild type) (unpublished data). In this study, AF2.5, a furfural-adapted mutant was further examined the ethanol tolerance comparing CMUWF1-2. It was found that both CMUWF1-2 and AF2.5 were able to grow at 6% (v/v) ethanol with the same level. However, at 7% (v/v) ethanol, AF2.5 had an ability of ethanol tolerance higher than CMUWF1-2. At 8% (v/v) ethanol, AF2.5 was slightly grown better than CMUWF1-2. However, at 10% and 12% (v/v) ethanol, no growth was observed in both stains (Fig. 1).

From this result, AF2.5 not only was improved furfural tolerance but also ethanol tolerance. Moreover, AF2.5 was already confirmed that was not due to contamination but its phenotype was changed by adaptive evolution.

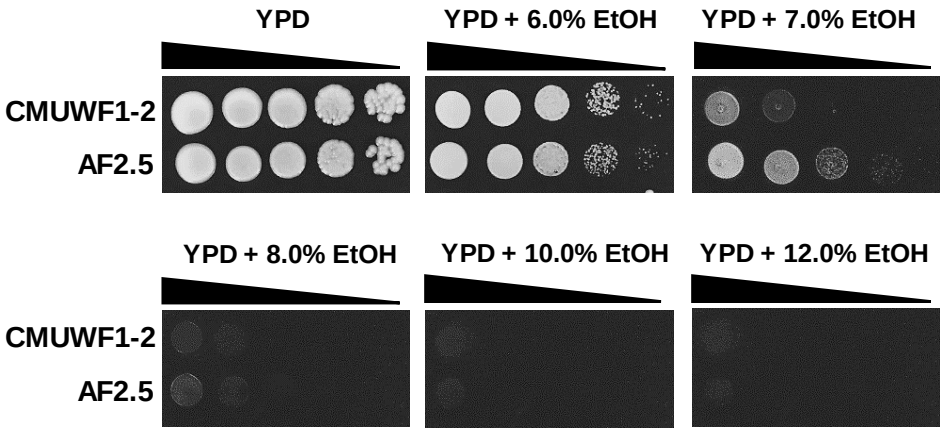

Fig. 1 Ethanol tolerance in adapted mutant AF2.5 comparing with CMUWF1-2. Cells were tested on YPD medium containing 0%, 6%, 7%, 8%, 10% and 12% (v/v) ethanol by spot test analysis. The experiment was examined at least three replicates.

3.2 Effects of ethanol on cell growth, cell viability and cell morphology in AF2.5

The effects of ethanol on cell growth, cell viability and cell morphology of AF2.5 comparing with CMUWF1-2 were investigated by culturing in YPD medium supplement with and without 4%(v/v) ethanol. The result showed that cells growth of AF2.5 and CMUWF1-2 were not different in YPD (Fig. 2a), but AF2.5 was able to grow in the presence of 4%(v/v) ethanol better than wild type (Fig. 2b). In addition, cells viability of both strains was evaluated during cultivation in YPD medium

with 4.0% (v/v) ethanol. The highest percentage of viability found in AF2.5 and CMUWF1-2 after inoculation at 0 h were 37.92% at 48 h and 22.82% at 84 h, respectively. Percentage of viability at 48 h in AF2.5 was 2.3 times higher than CMUWF1-2 (Fig. 2c). Furthermore, cell morphology of both strains grown in YPD medium w/wo 4.0% (v/v) ethanol was observed under microscope. It was found that cell morphology of both stains was not different when grown in YPD medium (Fig. 3a), but their cell walls were thin when grown under ethanol-stressed condition (Fig. 3b).

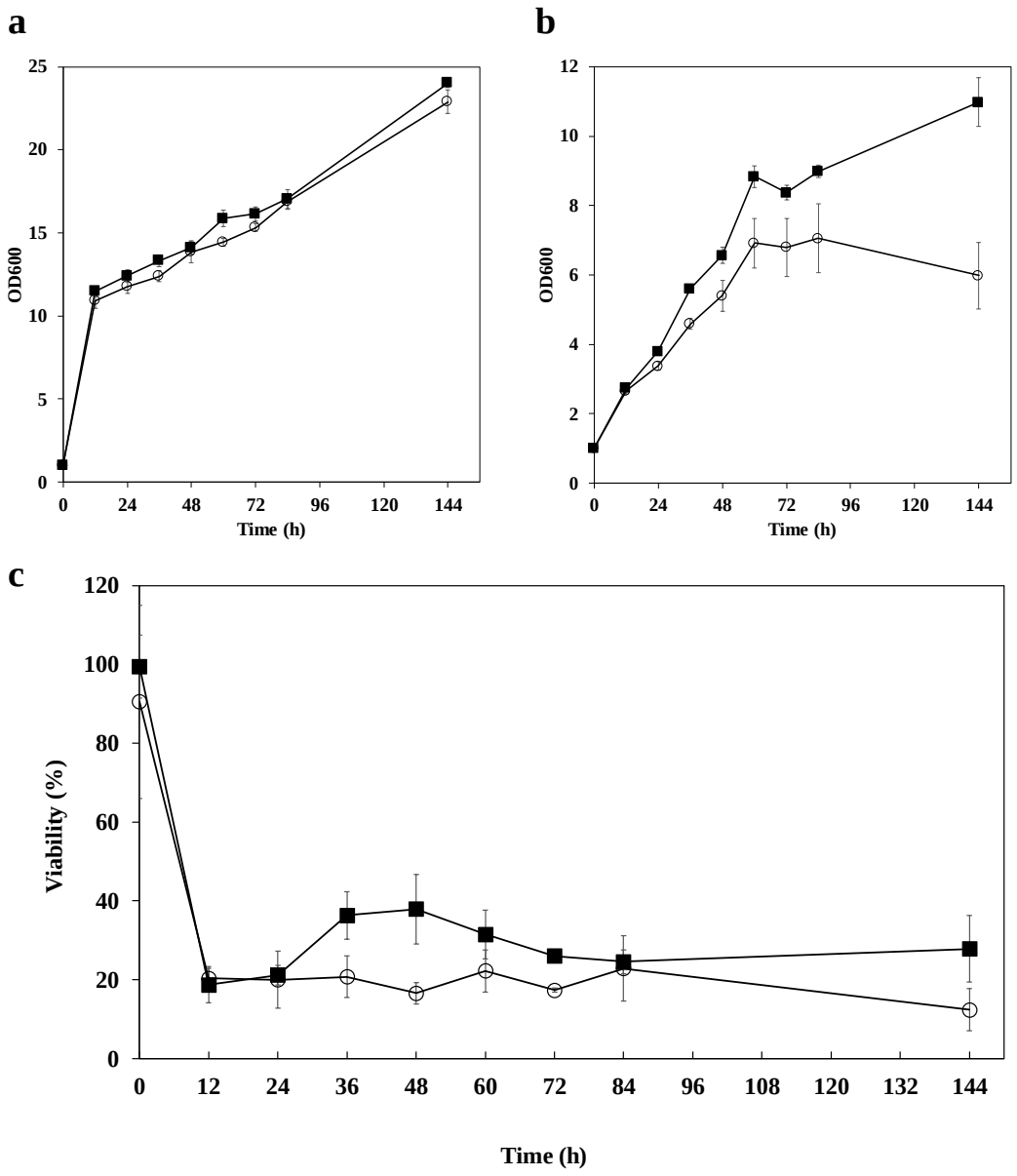

Fig. 2 Effects of ethanol on cell growth and cell viability in AF2.5 comparing with CMUWF1-2. Cells of both strains were grown in YPD medium containing without ethanol (**a**) and with 4%(v/v) ethanol (**b**). Cell viability of both strains grown in YPD medium containing with 4%(v/v) ethanol (**c**). Data were reproduced by three independent experiments.
Symbols: ⊖, CMUWF1-2; ■, AF2.5

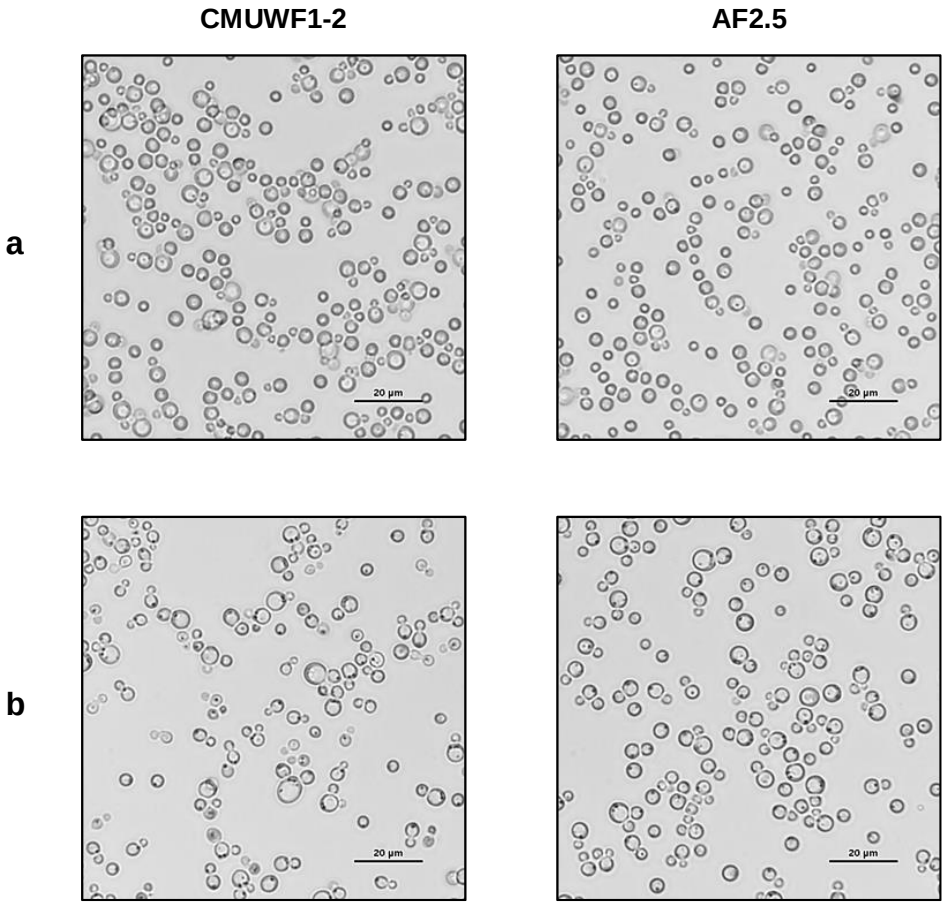

Fig. 3 Cell morphology of *S. passalidarum* CMUWF1-2 and AF2.5 grown in YPD medium containing without ethanol (**a**) and with 4%(v/v) ethanol (**b**). Cells of both strains grown at 30ºC, 150 rpm for 84 h were observed under microscope. The experiments were examined at least three replicates.

4. Discussion

The xylose-fermenting yeast *S. passalidarum* CMUWF1-2 was isolated from soil in Mae Taeng District, Chiang Mai Province. It was shown highly efficient produce ethanol from various sugars, especially xylose, presenting in lignocellulosic biomass at high temperature [13]. However, it has less toleration to inhibitors that present in hydrolysate of lignocellulosic biomass such as furfural, which is a major inhibitor classified in the furan group. Furfural has been shown to cause DNA damage and could induce DNA mutations in many microorganisms such as *Salmonella typhimurium*, *Escherichia coli*, lambda phage, and *Drosophila melanogaster*. Moreover, the furfural had the effects to yeast cell by reducing fermentation rate and/or stop growing [18].

Our previous study, *S. passalidarum* CMUWF1-2 was improved furfural tolerance by using adaptive evolution. This approach can arouse a random mutation in the genome of microorganisms and make them to present a desired phenotype via provide a selection pressure favoring the growth of mutants in the evolving population [16, 17]. Adaptive evolution was a successful method to improve *S. cerevisiae* strain Ethanol Red, because the evolved stains, *S. cerevisiae* ISO12 was capable to grow and ferment in the presence of inhibitors and high temperature better than parental strain [19]. Likewise, in the experiment of Shui and co-workers (2015) [20], *Zymomonas mobilis* ZM4 was improved the furfural and acetic acid tolerances by adaptive evolution. Four evolved mutants that were

ZMA7-2, ZMA7-3, ZMF3-2, and ZMF3-3 exhibited higher furfural and acetic acid tolerances than parental strain. From our study, AF2.5 obtained after 17 generations of adaptive evolution in furfural stress medium showed furfural tolerance better than CMUWF1-2 (unpublished data). Moreover, it showed higher ethanol tolerance than CMUWF1-2 in this study.

In this study, we focused on its ethanol tolerance. The evolved mutant, AF2.5 and CMUWF1-2 were characterized the cell growth, cell viability and morphology. AF2.5 showed that cell growth and cell viability under ethanol-stressed condition were better than CMUWF1-2. This change might be due to furfural that we used in adaptive evolution approach. Furfural might induce the mutations of genes that related to the ethanol tolerance such as the vacuolar H^+-ATPase and the plasma membrane H^{+}-ATPase genes, which involved in reducing cytosolic acidification in yeast cells [21, 22], the heat shock proteins (HSPs) genes (e.g. *HSP12*, *HSP26*, *HSP30*, *HSP78*, *HSP104*, *SSA3*, and *SSA4*) and trehalose metabolic enzyme genes (e.g. *TPS1*, *TPS2*, and *NTH1*), which played an important role in protecting protein structure denaturation from high concentration of ethanol [23, 24, 25], and *PUT4* genes encoding a high-affinity proline transporter, which increased proline uptake due to proline was able to protective effect against ethanol stress by reducing the ROS levels and increasing the survival rate of yeast cells [26, 27].

In addition, cell walls of AF2.5 and CMUWF1-2 grown in YPD medium with 4% (v/v) ethanol were thinner than that of grown in YPD medium without ethanol. These might be both strains modified plasma membrane compositions to be an unsaturated fatty acid (UFAs) and ergosterol for increasing membrane integrity and fluidity when exposed to ethanol stress for protecting themselves and increasing survival rate from environmental stress [28, 29].

5. Summary

The furfural-adapted mutant, AF2.5 was successfully improved ethanol tolerance from *S. passalidarum* CMUWF1-2 by adaptive evolution. It had the abilities of furfural and ethanol tolerances higher than wild type. Its phenotype changing might be occurred from the genetics improving and/or the cell membrane modifying that were induced mutations by furfural for preventing themselves and increasing viability of their cells under environmental stress conditions.

6. Acknowledgement

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7. References

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Activity Evaluation of Bioactive Compounds from Edible Freshwater Green Macroalgae Extracted by Ultrasound Assisted Extraction

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Keywords: Ultrasound-assisted extraction (UAE), Algae, Bioactive compounds, Antimicrobial activity, Antioxidant activity

Abstract. Freshwater macroalgae are important sources of natural bioactive substances. They are considered high value products that have been applied in food, cosmetic and pharmaceutical industries. This research aimed to evaluate ultrasound-assisted extraction (UAE) method on bioactive compounds (antimicrobial, antioxidant and anti-tyrosinase activities) derived from *Spirogyra* spp. (namely "Tao") and *Cladophora* spp./*Rhizoclonium* spp. (namely "Kai"). Ultrasonic device with an input frequency of 45 kHz and sonication time of 30 and 60 min were employed in this process. The results revealed that Tao showed higher antibacterial activity and antioxidant activity than Kai. The minimum bactericidal concentrations (MBC) against *Staphylococcus aureus* and *Propionibacterium acnes* were 15.6 mg/ml and 62.5 mg/ml, respectively. In addition, Tao extracted by UAE for 30 min presented the highest yield of total phenolic compounds (TPC) followed by 2, 2-Diphynyl-1-picryhydrazyl (DPPH), with 15.5±0.6 and 10.4±1.6 mg gallic/g extract, respectively. On the other hand, the highest yield of anti-tyrosinase was 20.1±1.1 mg kojic/g extract when sonicated for 60 min. In summary, the appropriate method and extraction time depended on purpose, bioactive compound and kind of algae. These results could potentially be useful for macroalgal natural compound extraction in cosmetic and pharmaceutical production in the future.

1. Introduction

Freshwater macroalgae are a rich source of structurally novel and biologically active metabolites. Primary or secondary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Recently, much attention has been focused on macroalgae as sources of novel biologically active compounds such as phycobilin, phenols, terpenoids, steroids and polysaccharides [1]. Algal phenolic compounds were reported to be a potential candidate to destroy free radicals, which are harmful to the human body. Nowadays, many chemically unique compounds with various biological activities have been isolated. Some of these are under investigation and being used to develop new pharmaceuticals. [2]

Spirogyra is a genus of filamentous green macroalga of the order Zygnematales. It is commonly found in freshwater areas and naturally grows in freshwater habitats such as small stagnant water bodies, streams, shallow water, ponds and rivers. There are more than 400 species of *Spirogyra* in the world. In Thailand, an edible freshwater alga, *Spirogyra* (known locally as Tao) [3], has been considered as a source of food for Northern and Northeastern Thailand. It consists of proteins, carbohydrates, lipids, dietary fibers, multivitamins, mineral substances and phenolic content [2]

Cladophora spp. and *Rhizoclonium* spp. naturally grow in Northern Thailand. It is commonly known as "Kai" [2]. Local people have traditionally used this algae as an ingredient in several Northern Thai dishes [4]. Previous *in vivo* studies have suggested that *Cladophora glomerata* extract exhibited antigastric ulcer, anti-inflammatory, hypotensive and antioxidant activities [5].

Conventional extraction techniques are time-consuming and not ecofriendly due to the use of organic solvents. Therefore, ultrasound-assisted extraction (UAE) has been proposed as an alternative to conventional extraction, providing higher recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactive properties. The spectrum of waves involved in ultrasound are called ultrasonic waves and the frequencies of these waves are those above the audible range (>20 kHz).

UAE is an inexpensive, simple and efficient alternative to conventional extraction techniques. The apparatus is cheaper and easier to operate compared to other novel extraction techniques such as microwave-assisted extraction and supercritical fluid extraction [6]. It has acoustic cavitation force as the main driving force capable of inducing a series of compressions and rarefactions in the molecules of the present solvent. This causes the formation of bubbles as a result of changes in temperature and pressure (Fig. 1)

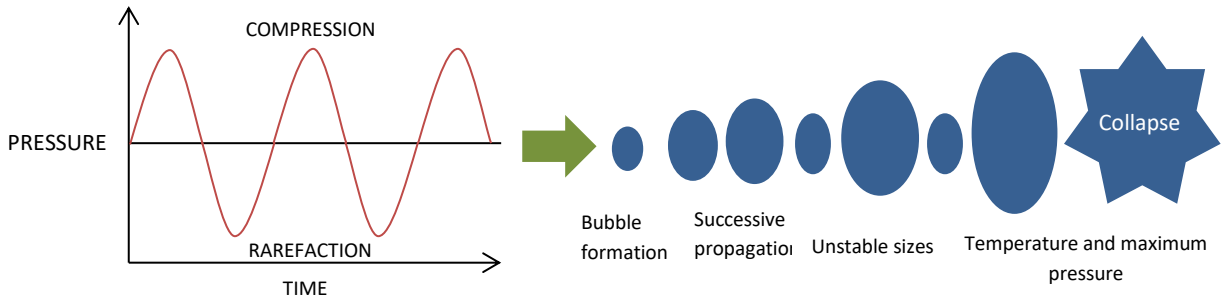

Fig. 1 The principle of acoustic cavitation

When the ultrasound intensity is sufficient, bubbles will absorb the energy from the sound waves, grow during the expansion cycles and recompress during the compression cycle. Furthermore, bubbles may start another rarefaction cycle or collapse leading shock waves of extreme conditions of pressure and temperature [7]. Thus, the implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate cells causing the release of the desired compounds. Currently, UAE has been used for the extraction of proteins, sugars, polysaccharides-protein complex, oil, etc. Nevertheless, recent studies have revealed that UAE of phenolic compounds were less degraded than others and even no degradation has been observed under optimized conditions. Each of these parameters affected the extraction efficacy of bioactive compounds. By increasing the extraction temperature, the extraction yields increase due to a higher mass transfer and solvent diffusion rate. Also, the extraction time has to be optimized for the prevention of degradation of bioactive compounds and for the increase of extraction efficacy. Thus, this research aimed to study the effect of ultrasound-assisted extraction on some bioactive compounds in two freshwater macroalgae; Kai and Tao.

2. Materials and methods

2.1 Sample Collection

Tao was collected from a cultivation pond in Ban Na Tao, Saun Kean sub district, Muang district, Phrae province. Kai was purchased from Ban Had Krai, Wiang sub district, Chiang Khong district, Chiang Rai province. Both sampling sites are located in Northern Thailand. Primarily, the algal samples were cleaned with distilled water and dried, then pulverized into powder and stored in a vacuum desiccator at room temperature for further study.

Prior to the experiment, algal samples were identified using the morphological features of their macroscopic structures [8, 9].

2.2 Extraction

A 50 g sample of each dried powder was prepared in 500 ml of 95% (v/v) ethanol, then processed with UAE 45 kHz at 50°C for 30 and 60 mins. The solutions were separated from the algal residues by filtration using No.1 Whatman filter paper, then dried to obtain ethanolic crude extracts. These were done in duplicate. In the drying process, the ethanolic extracts were evaporated under vacuum and freeze-dried by lyophilizer. The percentage yield of the dry weight of each extract was calculated.

2.3 Determination of antimicrobial activity

2.3.1 Agar well diffusion method

The antimicrobial activities of the algae extracts were examined by agar well diffusion method [10, 11]. Brain heart infusion (BHI) broth was inoculated with *Staphylococcus aureus* ATCC 25923 and *Propionibacterium acnes* DMST 14916 (obtained from the Applied Microbiology Laboratory, Faculty of Science, Chiang Mai University) and adjusted to 0.5 McFarland standards. DMSO and gentamicin were used as negative and positive control, respectively. 35 µl of the samples was dropped into the wells. *S. aureus* plate was incubated at 37°C for 24 hours and *P. acne* plate was incubated at 37°C for 72 hours under anaerobic conditions. The antimicrobial activity was determined by observation, measuring the inhibition zone diameter. Subsequently, the conditions that provided the highest inhibition zone were selected for further study on minimum inhibitory concentration (MIC) to define the lowest concentration of antimicrobial extract that prevents visible growth of a bacterium. It was determined using the modified method by Sameeh *et al*. [12]. Meanwhile, the lowest concentration of samples showing no visible growth of bacteria was taken as MBC was assessed using the method by Natrah *et al*. [13]

2.4 Determination of Antioxidant Activity

2.4.1 Determination of antioxidant activity by free radical scavenging activity on 2, 2'-diphenyl-1-picrylhydrazyl (DPPH•)

Gallic acid, 0.001-0.01 mg/ml, was used as a standard. Amount of 0.5 ml of gallic acid, crude ethanolic extracts of algal were added into 1.5 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) ,0.1 mM, and mixed well. The mixtures were left in darkness at room temperature for 20 min. Afterwards, the absorbance of the sample (A_{sample}) was measured using a spectrophotometer at 517 nm against ethanol blank. Methanol was used as a negative control ($A_{control}$). The half maximal inhibitory concentration

(IC_{50}) and the antioxidant activity were calculated by Bhadoriya *et al*. [4] according to the equations below:

$$\text{DPPH inhibition (\%)} = (((A_{control} - A_{sample}) \times 100) / A_{control})$$

$$\text{Antioxidant activity} = IC_{50}control / IC_{50}sample$$

2.4.2 Determination of total phenolic content (TPC)

Total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent method [14]. Each sample was mixed with 10% Folin-Ciocalteu reagent for 5 min, followed by the addition of 5% (w/v) sodium carbonate. The solutions were mixed using a vortex mixer. The reaction mixture was incubated for 1 hour in dark conditions. The absorption of the mixture was measured at 765 nm using a UV-2450 Spectrophotometer (Shimadzu Corporation). The concentration of total phenolic compounds in all samples was expressed as micrograms of gallic acid equivalents (GAE) per gram of the extract.

2.4.3 Determination of tyrosinase inhibition assay

Tyrosinase inhibition assay was modified from the method previously [15], using L-DOPA as the substrate. Algal extracts (20 µL, varying from 10 to 500 µg/mL), mushroom tyrosinase aqueous solution (20 µL, 50 units/mL), and phosphate buffer (pH 6.8, 80 µL) were mixed and pre-incubated at 37 °C for 5 min. Then L-DOPA (90 µL, 1 mg/mL) was added. The mixture was then incubated for 10 min at 37°C. The amount of dopachrome was measured at the wavelength of 475 nm. Kojic acid was used as positive control. Phosphate buffer was used as the blank. The percent inhibition of tyrosinase enzyme was calculated using the equation:

$$\text{\% Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where, A(sample) is the absorbance of the sample extracts and A (control) is the absorbance of the assay using the buffer instead of inhibitor (sample).

2.5 Statistical analysis

Experiments were performed in triplicates. The data were statistically evaluated using analysis of variance (ANOVA) with SPSS. Duncan's multiple range tests were carried out in order to test any significant difference between the species of algae and the extraction time. Significance levels were defined using $p < 0.05$.

3. Results and discussion

3.1 Sample collection

Tao was obtained from Phrae province and its morphological investigation found that Tao possessed unbranched filamentous and spirally coiled chloroplasts. Fig. 2 shows the morphologies of Tao observed under a light microscope**.** On the other hand, Kai was received from Chiang Rai province and its morphology shows typical branched thalli (Fig. 3).

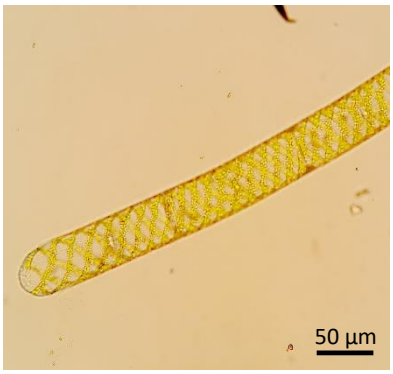

Fig. 2 Microscopic picture of Tao (*Spirogyra* spp.)

Fig. 3 Microscopic pictures of Kai (A: *Rhizoclonium* spp. B: *Cladophora* spp.)

3.2 Extraction

Tao and Kai were extracted by UAE at 45 kHz with two durations. It was found that Kai at 30 min showed the highest extraction yield (17.02±0.14%). Fig. 4 presents the percentage yield of extraction. Mungmai *et al.* [16] reported that the extraction yield of *Rhizoclonium hieroglyphicum* (C.Agardh) Kützing by conventional extraction was 12.58±0.44%. This indicates that yields obtained with conventional extraction (extracted by distilled water and 95% (v/v) ethanol, at 50°C for 1 hour) are lower than UAE.

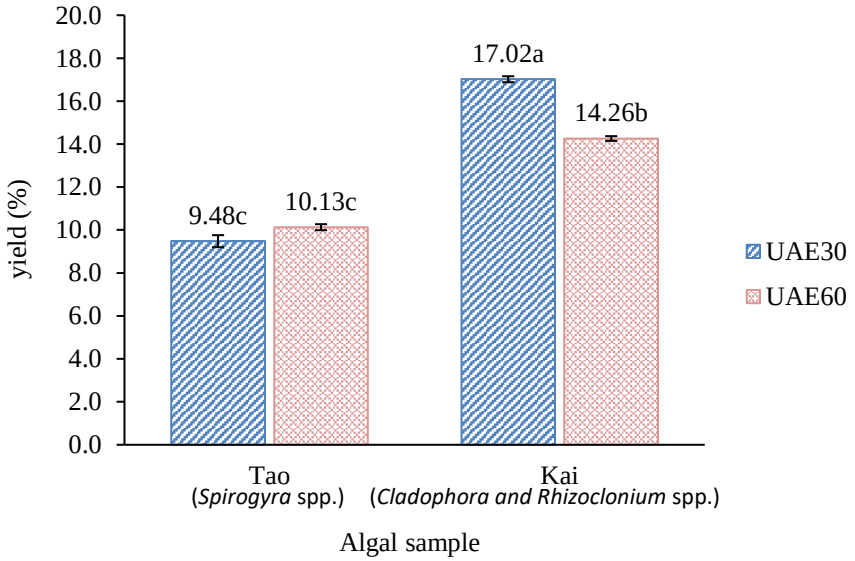

Fig. 4 The percentage yield of algal extraction extracted by ultrasound assisted extraction at 30 and 60 minutes. Data are expressed as mean ± standard deviation (n=3). The alphabet on the error bar was significantly different ($p < 0.05$) according to Duncan's multiple range tests.

3.3 Determination of antimicrobial activity

Tao and Kai crude extracts obtained from both 30 and 60 extraction times could inhibit the growth of *S. aureus* and *P. acnes* with an inhibition zone ranging between 8.3 and 16.7 mm (Fig. 5). Soltani *et al*. [17] studied about the antimicrobial activities of *Cladophora glomerata* extract and found that it could inhibit the growth of *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus mirabilis*. Kamble *et al*. [18] reported that *Eschericha coli* and *Staphylococus albus* was

inhibited by *Cladophora callicoma* and *Spirogyra plena.* Also, our results indicate that Tao and Kai crude extracts contained active antimicrobial compounds. Soria *et al*. [7] suggested that UAE extraction provided bioactive compounds higher than the conventional extraction method. However, extraction at 60 min which had 79°C resulted in a zone of inhibition less than 30 min which had 58°C of extraction may cause of high temperature during extraction process

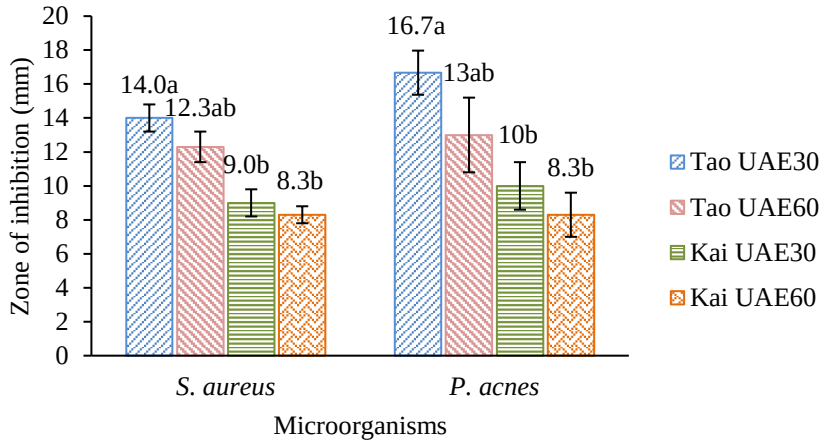

Fig. 5 Antimicrobial activity of algal extraction was evaluated on *S. aureus* and *P. acnes* by an agar well diffusion method. Data are expressed as mean ± standard deviation (n=3). The alphabet on the error bar was significantly different ($p < 0.05$) according to Duncan's multiple range tests.

Minimum inhibitory concentration determination found that crude extract of Tao at 30 min of extraction time had an MIC value of *S. aureus* and *P. acnes* as 15.6 and 62.5 mg/ml, respectively while Kai had an MIC value as 62.5 mg/ml. For minimum bactericidal concentration evaluation, crude extracts of Tao and Kai at 30 min of extraction time had MBC values of S. aureus as 15.6 and 31.2 mg/ml, respectively.

Table 1 Minimum inhibitory concentration and Minimum bactericidal concentration

Extracts (500mg/ml)	**Time (min)**	***Staphylococcus aureus***		***Propionibacterium acnes***	
		MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Tao (*Spirogyra* spp.)	30	15.6 ± 0.0c	15.6 ± 0.0c	62.5 ± 0.0b	62.5 ± 0.0b
	60	31.3 ± 0.0b	62.5 ± 0.0a	125 ± 0.0a	62.5 ± 0.0b
Kai (*Cladophora* spp. & *Rhizoclonium* spp.)	30	62.5 ± 0.0a	31.2 ± 0.0b	62.5 ± 0.0b	125.0 ± 0.0a
	60	62.5 ± 0.0a	62.5 ± 0.0a	62.5 ± 0.0b	125.0 ± 0.0a

Data are expressed as mean ± standard deviation (n=3). The alphabet was significantly different ($p < 0.05$).

3.4 Determination of antioxidant activities

Using DPPH assay found that crude extract of Tao at 30 min of extraction time had the highest antioxidant activities with 10.4 mg gallic acid/g extract. Hossain *et al*. [19] reported that the recovery, antioxidant capacity and profile are strongly influenced by extraction variables where extraction time, temperature and frequency are the most important. Short extraction period gave antioxidant activities higher than long extraction time. The results indicated increasing the temperature during extraction may lead to a loss of bioactive compounds. Nevertheless, in total phenolic content evaluation, Tao crude extract at 60 min of extraction had the highest total phenolic content (28.7 mg gallic acid/g extract). Chimsook *et al*. [20] studied the effect of UAE on total phenolic content and found that high temperature leads to increased cavitation of ultrasound assisted extraction by assisting cell wall breaking in order to release the polyphenols. Fig 6 demonstrates the antioxidant activity of algal extraction. Fig. 6 The antioxidant activity of algal extraction. Data are expressed as mean ± standard deviation (n=3). The alphabet was significantly different ($p < 0.05$).

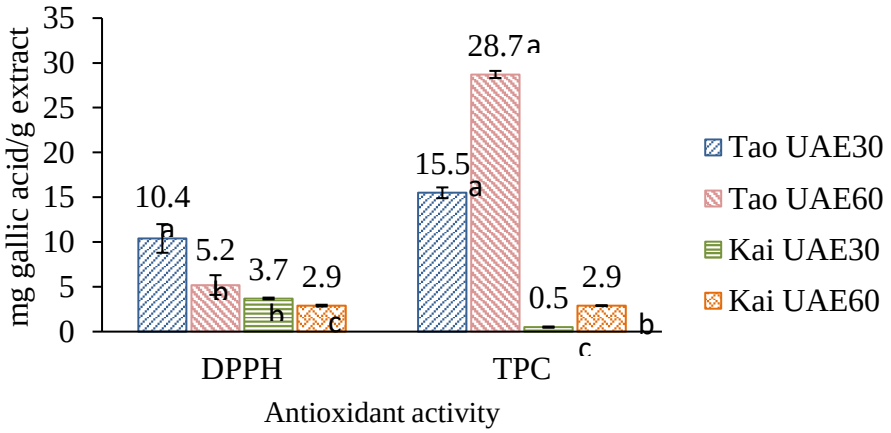

Fig. 6 The antioxidant activity of algal extraction. Data were express as mean ± standard deviation (n=3). The difference alphabet was significantly different ($p < 0.05$).

3.5 Determination of anti-tyrosinase activity

The crude extracts of Tao and Kai could inhibit tyrosinase enzyme, 11.0-20.1 mg kojic acid/g extract (Fig. 7). The previous study of Stankovic´ [21] found that polyphenols, fatty acid, carotenoids, vitamins and chlorophyll inhibited the tyrosinase activity. Moreover, the extract from ultrasonic extraction are also used in some pharmaceutical formulas to obtain anti-tyrosinase and antioxidant activities. However, the results show that Tao UAE60 has a higher anti-tyrosinase activity than UAE30, while Kai UAE60 has a lower activity than UAE30. Therefore, inhibition ability depends on type of algae as well as their cell walls that play a vital role in substance secretion. Wurdack [22] reported that the cell walls of Kai compose three layers (chitin, pectose and cellulose) while Tao composes two layers (pectose and cellulose).

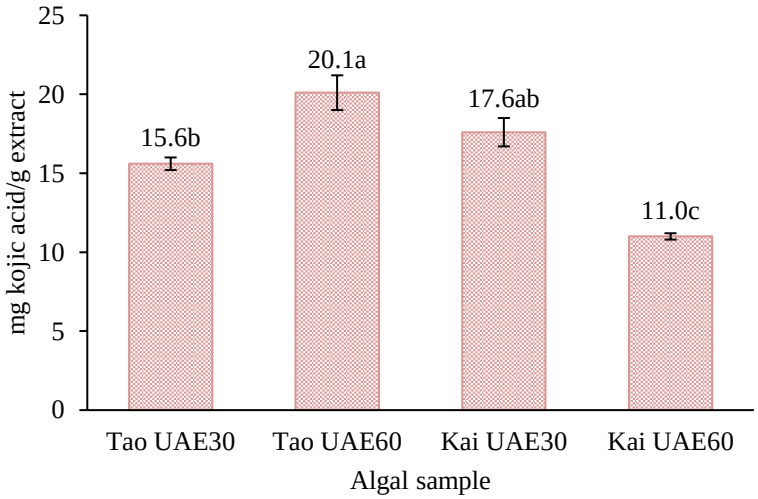

Fig. 7 Anti-tyrosinase activity of algal extraction. Data are expressed as mean ± standard deviation (n=3). The alphabet was significantly different ($p < 0.05$).

4. Conclusions

The bioactive compounds of Tao and Kai with the application of ultrasound at various times were compared in this study. The results clearly indicate that the two macroalgae can inhibit growth of *S. aureus* and *P. acnes*. Moreover, crude extracts of Tao and Kai contain antioxidant and anti-tyrosinase activities which will be useful for algal based cosmetics and pharmaceuticals.

5. Acknowledgement

Grateful and sincere thanks are given to the Microbiology Section, Department of Biology, Faculty of Science for facilities and Center of Excellence on Biodiversity (BDC-PG3-160018) for the research grant.

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Determination of Humic Acids as a Precursor of THMs in Raw Water from the Chao Phraya River, Thailand Using Feem Technique

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Keywords: FEEM, Humic acids, WTP, D, THMs, DOC, Water supply

Abstract. The concentration of humic acids in raw water canal (the Prapa canal) of Metropolitan Waterworks Authority (MWA) Bangkok, Thailand were measured by fluorescence excitationemission matrix (FEEM) spectroscopy. This technique is capable to evaluate insights and classify the types of organic matters. Water samples were collected from Chaopraya river and raw water from water treatment plant (WTP) for 4 months period. The analytical technique was selected to examine the humic acids. Results displayed that humic acid-like organic group occurring at the excitation/ emission wavelengths of Ex / Em 250-250 nm /410-450 nm and Ex / Em 300-320 nm / 410 nm. The dissolve organic carbon (DOC) in the Prapa canal was less than 2.00 mg/l. In addition, there was a correlation between the seasonal changes and the concentration of DOC. Moreover, as a result of the humic acids are very important because It had ability react with disinfectant an increasing the THMs concentration in tap water. Therefore, the concentration of THMs should be considered for the control of the humic acids at the raw water canal before operating to the water treatment plant.

1. Introduction

Chao Phraya River is an important source of drinking water in Thailand. Similarly, most countries in the world use their major rivers as their main source, processed through Water Treatment System (WTP) to assure the drinking water quality for consumers.

Natural organic matter (NOM) is a broad range of suspended, dissolved and aromatic organic [1], which is commonly found in raw water that plays major roles in aquatic environments and water quality and treatment. More importantly, it creates disinfection by products (DBPs), which are harmful to health such as trihalomethanes (THMs), consisting of chloroform, bromodichloromethane, dibromochloromethane, and bromoform [2,3] at the time of reacting with chlorine during the Pre-Chlorination and Post-Chlorination process.

However, the current water treatment system cannot remove all NOM, as a consequence, the organic matter is likely to react with chlorine, and generate THMs formation. The determination of the concentration of humic acid and Fulvic by measuring the DOC method was applied, but the fractionation of humic substance is still required to determine its composition. Only a few works in

literature demonstrate the elements of NOM in Chao Phraya River, plus the existing research has many problems in representing analytical techniques.

Fluorescence excitation-emission matrix (FEEM) has been widely used to characterize dissolved organic matter (DOM) in water and also applied to track DOM in a WTP [4]. As has been previously reported in the literature, there is a link between the DBP formation potential and DOM fluorescence [5]. EEMs were characterized into five excitation–emission regions including aromatic protein I, aromatic protein II, fulvicacid-like, humic acid-like, and soluble microbial by-product-like Chen et al. (2003). The purpose of this research is to efficiently isolate the humic substance component. The results can benefit water supply operation system as well as preventing THMs formation.

2. Material and Methods

Samples were collected from 4 sampling station, along the 18 kilometers long Prapa canal, and the last sampling station was at the entrance of the Bangkhen Water Treatment of Metropolitan Waterworks Authority (MWA) in Thailand as shown in (Fig.1).

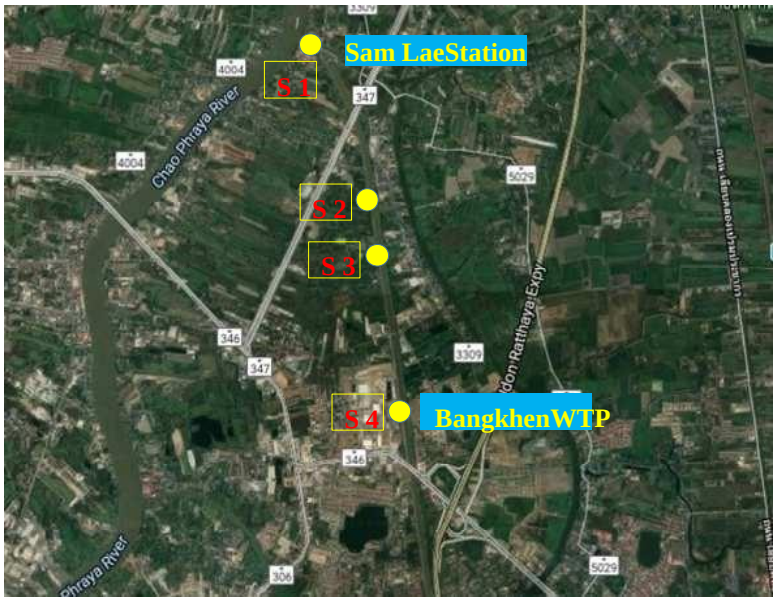


Fig. 1 Water sampling stations

The water sample was separated in a set of 3, each sample was filled into a 120 ml bottle. All samples were respectively collected in one day from 4 sampling stations, beginning at the Sam Lae water intake station directed toward the Bangkhen Water Treatment every month. After the collection within 24 hours, each sample was filtered through 0.45 µm cellulose acetate membrane filter. Duration of data collection was 4 months starting from March-June 2018.

The concentration of humic substance was measured by diluting a volume of 1 cm quartz cell water sample to be equal to a DOC concentration of no more than 10 mg/L, and adjusted it to pH 7, then inserted into the Fluorescense Excitation Emission Matrix (FEEM).Model a Jasco FP-8200 Spectrofluorometer. The EEM spectra was set at excitation (Ex) wavelengths from 220 to 600 nm and emission (Em) wavelengths from 230 to 650 nm, to be interval 5 nm. The results were analyzed to determine the types of humic acid based on Chen et al., 2003's chart as shown in (Fig.2)

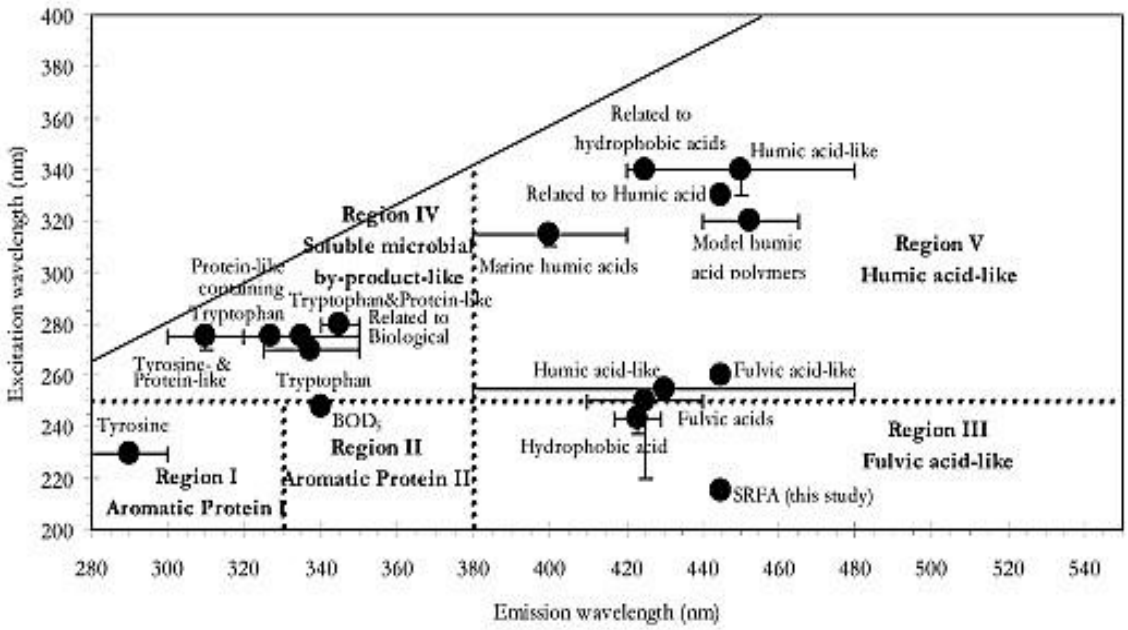
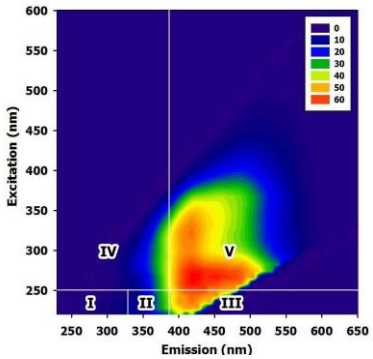


Fig. 2 Location of EEM peaks (symbols) according to literature reports and operationally defined excitation and emission wavelength boundaries (dashed lines) for five EEM regions [6].

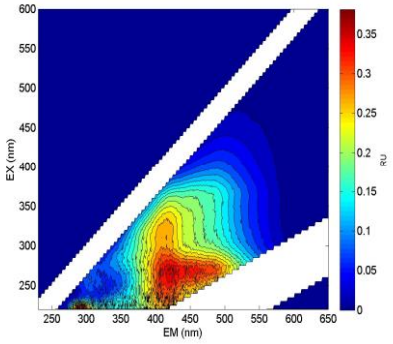
3. Result and Discussion

The result revealed that the concentration of humic substances of Chaopraya river (Fig.3) from the beginning to half way through the raw water canal and the point before entering the water treatment plant remained constant. It was found that THMs formation occurred due to the reaction between these precursors and chlorine

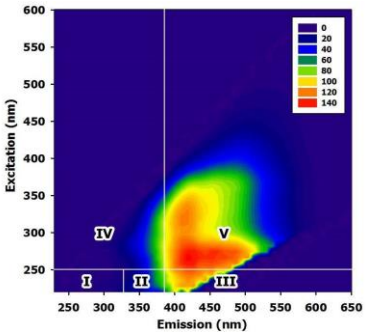
In the present study, the concentration of humic acid was determined by using the FEEM technique. Samples were firstly collected at the S1-S4 water sampling station from the Chao Phraya River. The second sampling point was at the Si Phon Bang Luang, then, Si Phon Rangsit and lastly, the sampling station located at the location before the stream flowing into WTP. Results showed that the maximum fluorescent light intensity (Peak) was located at Ex / Em 250-250 nm / 410-450 nm and Ex / Em 300-320 nm / 410 nm. The findings were compared to the wavelengths of Chen et al., (2003) and it shows that the positions were grouped in Region V, which represented the humic acid-like organic group.



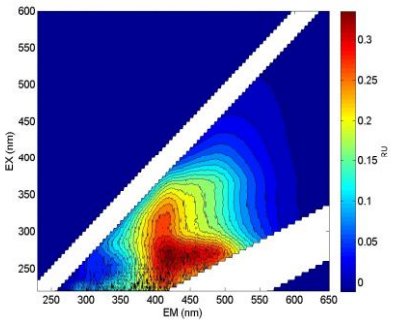
S1: Sam Lae water intake station (QSU)



S1: Sam Lae water intake station (RU)



S2: Si Phon Bang Luang (QSU)



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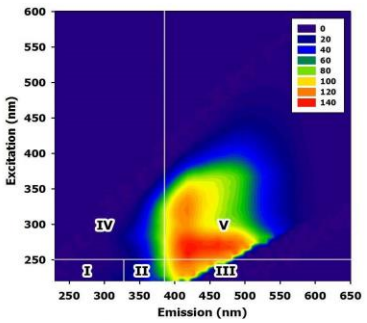

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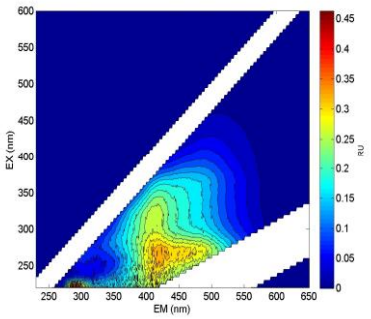
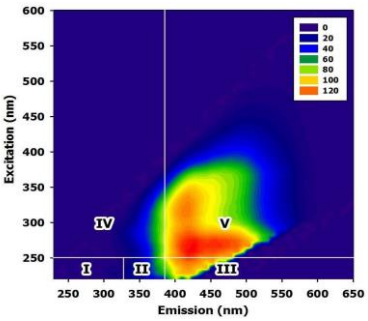
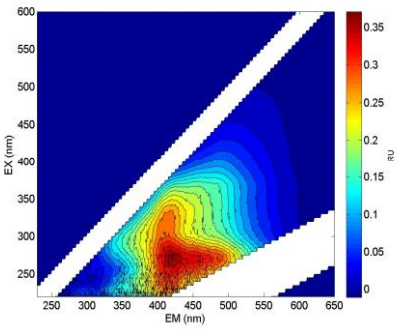


S3: Si Phon Rangsit (RU)



S4: Entrance of WTP (QSU)



S4: Entrance of WTP (RU)

Fig. 3 Results of humic acid measurement using the FEEM technique (S1-S4)

4. Conclusion

Fractionation of humic substance by using FEEM is capable of effectively tracking humic, which is the principal THMs precursor source. More importantly, FEEM procedure is not time consuming and does not require a sample pretreatment.

FEEM is well suited for monitoring and preventing the occurrence of THMs not to exceed THM standard in accordance with WHO. The data results obtained from the FEEM can used as a tool in optimizing and improving the current water treatment process to create confidence among water users.

5. Acknowledgements

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Inhibition Efficiency of Extracts from Leaves of Piper betle Linn, Piper sarmentosum Roxb and Andrographis paniculata (*Burm.f.*) nees on the Growth of Colletotrichum gloeosporioides in Nam Dok Mai Mango (*Mongifera indica Linn.*)

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Keywords: Betal leaf, Wildbetal lefbush, Kariyat, Extraction, Nam Dok Mai Mango

Abstract. The efficiency of betal leaf, wildbetal leafbush and kariyat extract for fungus inhibition of Colletotrichum gloeosporioides on Nam Dok Mai Mango was studied. The objectives were to find the herbs that are suitable for inhibiting Colletotrichum gloeosproiodes that cause anthracnose disease on Nam Dok Mai Mango and the appropriate concentration to extend the shelf life of Nam Dok Mai Mango. Three concentrations of three herb extracts were used at 10, 15 and 20 ppm. The lowest concentration was tested for anthracnose disease inhibition and shelf life of Nam Dok Mai Mango by using Dilution Susceptibility Test using concentration at 10,15 and 20 ppm. The growth of hyphol on was measure day 7 in PDA. Auti fungus efficacy Showed that betal leaf ertract was 0.00±0.00 mm in all 3 concentrations. The wildbetal lefbush and kariyat extract were 3.23±0.25, 0.67±0.29, 0.40±0.17, 6.17±0.29, 6.00±0.50 and 5.00±0.50 mm respectively. Betal leaf can inhibit fungus 100 percent at all concentration specify which value corresponds to what. The lowest oncentration of 10 ppm. can inhibit fungus on Nam Dok Mai Mango and one soaked with distilled water and the other not soaked 100 and 96 percent respectively.

1. Introduction

Thailand is a land with abundant natural resources, is a land that has various kinds of natural plants suitable for cultivation and agriculture. There are different climates resulting in a variety of distribution of tropical wood products to the market continuously throughout the year. Fruit planting areas according to different regions. Thailand has more than 9.68 Million rai. Thai fruits are considered economic crops that can make income to Thailand several million baht per year and is commonly consumed. Both domestic and international Fruit trees that are of economic importance and have high export value are widely consumed in various countries such as longan, durian, mangosteen, lychee, mango, pomelo, rambutan, pineapple, coconut, perfume and tamarind etc [1]. Mango is an economic fruit that is popular with both fresh fruit and processed fruits. In addition to domestic consumption, can be exported to foreign countries. The major foreign markets of Thailand are Malaysia, Singapore, Japan and other countries [2]. In the current competition in the export market of mangoes, there are 2 main issues: price and quality of products. One important quality problem Anthracnose disease After harvest Which causes damage to the product while transporting and distributing Resulting in lower quality of output Affect the price to be lower as well or may not be able to sell that product. The production situation of Nam Dok Mai Mango Export 2016/60, faced with the problem of inclement weather over the previous year. Which was damaged by the rain of the season which lasted for several

days Causing mangoes to be damaged by fungi Both in the production process Post-harvest storage, transportation and distribution [3]. Which anthracnose disease Caused by fungi Collctotrichum gloeosporioides Which causes the disease to cause post-harvest rot. Control of post-harvest diseases of mango is commonly used in chemicals. benzimidazole, such as benomyl, dip the results before filling into containers Which chemicals can penetrate into the product Causing insecurity to consumers and workers ,but currently there are reports that chemical groups benzimidazole The effective control of the disease has decreased in both Thailand and Florida, the United States, due to the fungus species that are resistant to fungicides such as Colletotrichum gloeosporioides and Lasiodiplodia theobromae [4]. And in the United States has announced the abolition of benomyl for all types of post-harvest agricultural products and many countries that are aware of the problem of chemical residues in agricultural products that affects the health of consumers Ecological and environmental changes and microbial resistance. At present, people are interested in new ways to control fungal diseases by using natural products such as plant extracts. To replace the use of chemicals

The crude extract from betel leaf was studied. Chaplo leaves and buffalo leaves It was found that the extracts from betel leaf and plu leaf Can inhibit mold Colletotrichum gloeosporioides and the research Andrographis paniculata and Moringa leaves It was found that the extracts from the paniculata can inhibit the fungus causing Anthracnose in chili Therefore, if there is a study of the utilization of medicinal plants, betel leaves, plu leaf and Fah Talai bandit for inhibiting fungi Colletotrichum gloeosporioides That occurred in Nam Dok Mai Mango It will increase the value and can also help reduce the use of chemicals. It can also help extend the shelf life of Nam Dok Mai mango in another way [5].

1.1 Objective

1.1.1 Study the herbs that are suitable for inhibiting fungi *Colletotrichum loeosporioides* that cause Anthracnose disease in Mango Nam Dok Mai

1.1.2 Study the optimal amount of extract to extend the shelf life of Mango Nam Dok Mai

2. Research methodology

2.1 Preparation of herbal extracts

Select herbs Baked at 50 ^{o}C for 10 hours and then blended herbs Weighing herbal powder 250 grams per 95 % ethanol 1,000 ml soaked in room temperature 24 hours after maturity and then filtered to separate waste with filter paper No. 4 Repeat 2 times and take the filtered part.To evaporate with Rotary Evaporator at room temperature Keep the extract that has been put in a sealed tea jar. At temperature 4 ^{o}C to wait for further analysis Save the extract weight to calculate Find the percentage of extracts

$$\text{Percentage Extract} = \text{Extract Weight} / \text{Plant Specimen Weight} \times 100 \quad (1)$$

2.2 Preparation of *Andrographis Paniculata* Extract

Andrographis paniculata powder 250 grams

↓

Add 95 % ethanol, volume 1,000 ml. Stir and leave for 24 hours.

↓

Bring a lot of secondary separation with filter paper No.4 Collect the extract in a bottle

↓

Bring the Talisman To add 95 % ethanol, 500 ml volumeStir and leave for 24 hours.

↓

Bring a lot of secondary separation with filter paper No. 4 Collect the extract in a bottle

Extract the extract with vacuum evaporator distillation at 35 ^{o} C

↓

Until no solvent evaporated

↓

Keep the extract that has been put in a sealed tea jar at 4 ^{o} C for further analysis.

↓

Remove the dried Talay Farmer, then weigh to calculate the percent extract

2.3 Fungus preparation *Colletotrichum gloeosporioides*

Fungus C. Gloeosporioides Is an anthracnose fungal strain of Nam Dok Mai mango (Which is taken from the plant disease clinic Kasetsart University Kamphaeng Saen Campus.

Remove mold C. gloeosporioides Seed germination

↓

By using a loop to fire, sterilize and then cut the jelly surface of the mold

↓

By measuring 3 mm in diameter

↓

Place the jelly pieces in the center of the culture medium. In the petri dish

↓

Cured in a temperature of 30 ^{o} C for 7 days

↓

Observe the result

2.4 Preparation of herbal extracts at various concentrations.

Remove herbal extracts to reduce the concentration level. By using diluted distilled water every 2 times Of the extracted concentration is 30, 20 and 10 ppm, which distilled water is used as a control sample By adjusting the volume concentration of the extract in Table 1.

Table 1 To adjust the volume level of concentration of the herbal extract.

Desired concentration (ppm)	**The desiredvolume** (ml)	**Concentration used (ppm)**	**Volume, intensityused (ml)**	**Adjust volume with solution (ml)**
10	50	100	5	
15	50	100	7.5	distilled water
20	50	100	10	

2.5 To test the effectiveness of antifungal C. Gloeosporioides.

By weighing the finished PDA medium put in a 50 ml beaker using 500 ml Graduated Cylinder, measuring 500 ml distilled water, pouring into Duran bottles, 1000 ml. Stir well, bring to Autociave at 121 ° C for 15 minutes, then put in a test tube. 9 ml 3 Leave the tube until the temperature drops to 45-50 0 C. Suck the solution but concentration (Table1). Volume 1 ml. Mix into the prepared PDA food. Mix together using the Vortex mixture and pour into the dish. infection repeat 3 repeat for each concentration tested, it is a herbal extract for experiments 10 ml of the culture medium used as a comparison to food PDA 10 ml after you empty and then put aside one day to complete. City Pages food and then move the tested fungus

The fungus C olletotrichum gloeosporioides Fed on PDA food in the culture medium, place it at a temperature of about 30 °C for 5 to 7 days. Then use a loop of a diameter of 3 mm . Flush, disinfect the light, cool and then penetrate the fibers in the colonies to make new fibers That is growing Then move the jelly pieces onto the PDA food that contains herbal extracts. At concentrations 10 ,15 and 20 ppm Place the jelly pieces on the center of the petri dish. By overturning the side with the fiber of the fungus to contact the food surface Then incubated at 30 0 C for 7 days by experimental design was completely random (Complete Randomized Design, CRD) record the results of experiments by the growth of the fiber. Fungi that are growing on the surface of the food, mixed with herbal extracts. At various concentrations by measuring the diameter of the colonies that grow in food Comparison of fungal growth in control units Therefore stopped recording results and then calculate the percentage Inhibition of growth from the following formula [5].

$$\text{Percentage of growth inhibition} = [(A - B) / A] \times 100 \quad (2)$$

A = The average of the colony diameter on the culture medium
B = average of the diameter of the colony on the food plate that contains the extract

3. Summary

From the extraction of 3 types of herbs, including betel leaf, plu leaf, and paniculata with Absolute Ethanol solution with a ratio of 1 : 2 (herb powder 250 grams per Absolute Ethanol 1,00 0 ml) at room temperature. By immersing in the Absolute Ethanol solution for 24 hours. Once due, filter the residue with filter paper No. 4 Repeat 2 times and then the filtered part can be evaporated with a Rotary Evaporator. Can be a dark green viscous liquid. Results according to Table 2

Table 2 Percentage of extracts after evaporation of all 3 herbs

Herb type	The amount of extract after evaporation (ml)	Percent extract (%)
betel	32	12.8
Chaplo	22.5	9
Andrographis paniculata	20	8

In the petri dish with fungal cultivation alone found that the growth of the fungus was spread all over the plate. The fungus will grow from the center of the plate to the surrounding area. As shown in Fig. 1

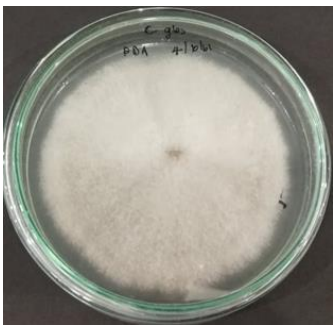

Fig . 1 *C. Gloeosporioides* fungus Fed on PDA food for 7 days at 30 ^{o} C

From the use of extracts from 3 types of herbs, including betel leaf, plu leaf and Fah Talai bandit The extraction solvent Absolute Ethanol Dilution Susceptibility Test by observer mold growth *C. Gloeosporioides* for 7 days. The experiment by measuring the growth of mycelial *C. Gloeosporioides.*That grows on PDA media mixed with herbal extracts By measuring the diameter of the colonies that grow horizontally compared with the bacteria in the control plate Table 3 and Fig. 1

Table 3 Compares the growth of the fungus *C. Gloeosporioides.* On PDA media containing herbal extracts at various concentrations

Herb type	The average colony diameter (mm) [1]		
	10 ppm	15 ppm	20 ppm
Betel leaf [ns]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Chaplo	3.23 ± 0. 25 [a]	0. 67 ± 0. 29 [b]	0. 40 ± 0. 17 [bc]
Andrographis paniculata	6. 17 ± 0. 29 [a]	6. 00 ± 0. 50 [a]	5. 00 ± 0. 50 [b]

Note: 1 = average of 3 repeated samples, letters horizontally different mean values are significantly different statistically significant ($p \leq 0.05$).

Table 3 shows the type of herb and concentrations as low as possible, which can inhibit the growth of mold *C. Gloeosporioides.* By experimenting on PDA from testing the efficacy of herbal extracts, all 3 types of betel leaves PLU and Creat at concentrations of 10, 15 and 20 ppm in inhibiting mycelial growth of the fungus. *C. gloeosporioides* Causes of Anthracnose in Nam Dok Mai Mango compare with the control unitIt was found that the extract from betel leaf at concentration level

10 ,15 and 20 ppm Not significantly different at the level of 0.05 (P> 0.05), but plu leaf extract at concentrations of 10 ppm with different plu leaf extract at concentrations of 15 and 20 ppm are statistically significant at the 0.05 level.(P ≤ 0.05) and an extract of Andrographis paniculata at concentrations 10. and 15 ppm, different from the extracts from the paniculata at concentration of 20 ppm with statistical significance at the level 0.05 (P ≤ 0. 05) from the betel leaf extract at concentrations of 10, 15. and 20 ppm can inhibit fungal growth *C. Gloeosporioides* The best is not 100 percent difference, followed by extracts. Chaplo at concentration level 10, 15 and 20 ppm, is 95, 92.5 and 60 percent, respectively, and an extract of Andrographis paniculata at concentrations of 10, 15 and 20 ppm was 37.5, 25 and 22.5percent respectively extract. Betel leaves at a concentration of 10 ppm were tested with Nam Dok Mai mango.

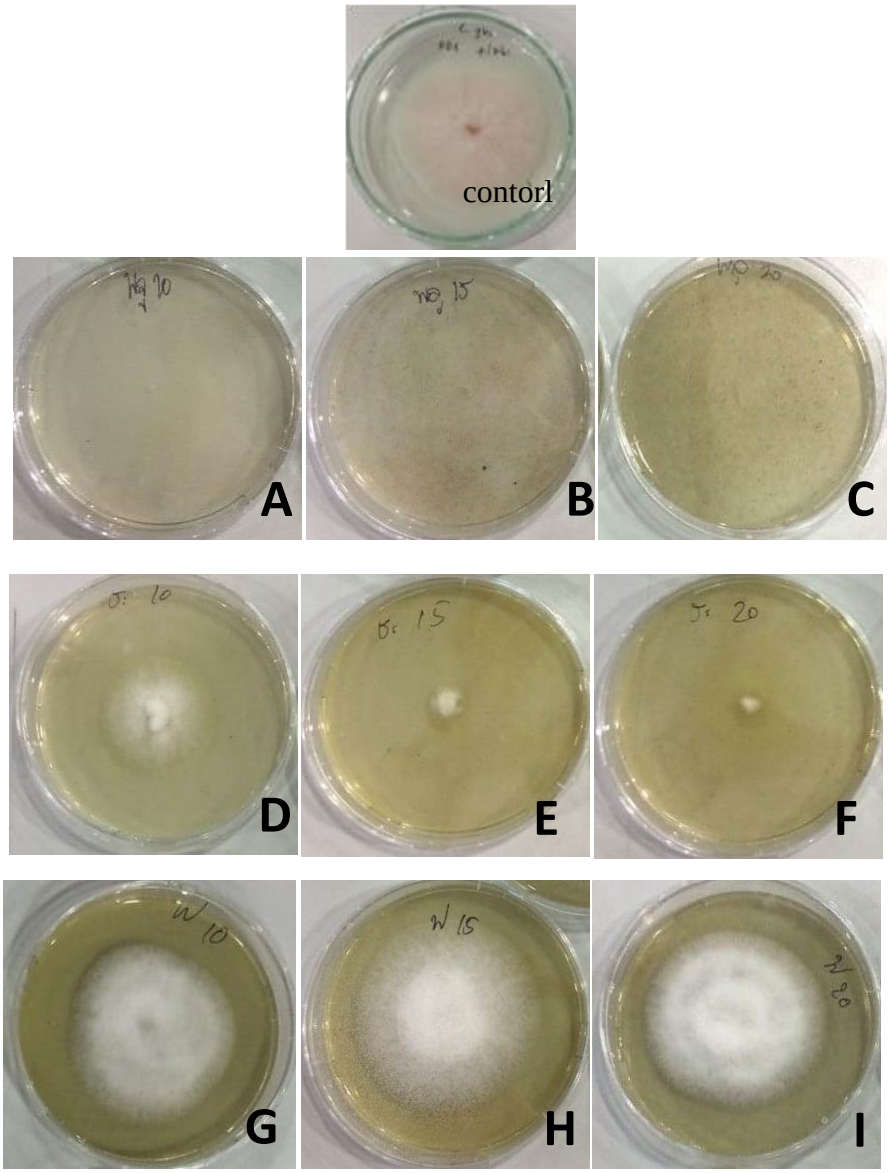

Fig. 2 The growth of the fungus *C. Gloeosporioides.* On PDA food that contains herbal extracts for 7 days

A : 10 ppm betel leaf extract
B : Mixed betel leaf extract 15 ppm
C : Mixed betel leaf extract 20 ppm
D : Mixed with Chaplo extract 10 ppm
E : mixed with leaf extracts, Chaplo 15 ppm
F : Mixed leaf extract of Chaplo 20 ppm
G : Mixing Andrographis Paniculata Extract 10 ppm
H : Mixing Andrographis Paniculata Extract 15 ppm
I : Mix Andrographis Paniculata Extract 20 ppm

Table 4 The test of the efficiency of extracts from Nam Dok Mai mango at the concentration of 10 ppm with Nam Dok Mai mango fruit for 7 days

Day	Not soaked	Soaked in distilled water	Soak the extract
0			
1			
2			
3			
4			
5			
6			

Table 4 Shows the change of Nam Dok Mai mango fruit From the test of the efficiency of betel leaf extract at the concentration level of 10 ppm for inhibiting fungal growth *C. Gloeosporioides* On mango fruits Compared with the Nam Dok Mai mango fruit that is not soaked in distilled water and

soaking distilled water and save the image as Table 4.2, the betel leaf extract at concentrations of 10 ppm is effective in inhibiting fungal *C.Gloeosporioides*. that causes the anthracnose disease. In addition, the level of 0.05 (p> 0.05) compared to the compound. Purple Nam Dok, which is extracted with the mango fruit is not soaked extract can inhibit the growth of fungus *C.* .. *Gloeosporioides* and 100 percent compared to frozen mango with mango extract, the distilled water can inhibit the growth of fungus *C. Gloeosporioides* was 96 percent.

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INNOVATIVE TECHNOLOGY AND SUSTAINABILITY ENGINEERING

Effects of Membrane Pretreatment and Operating Temperature on Performance of Alkaline-Acid Direct Glycerol Fuel Cell

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Keywords: Alkaline-acid, Direct glycerol fuel cell, Nafion 115 membrane, Temperature, Membrane treatment, Kinetic of chemical reaction.

Abstract. Alkaline-acid direct glycerol fuel cell was fueled by glycerol to replace ethanol due to its interesting economic aspect when compared to ethanol. However, glycerol provides lower performance of fuel cells than ethanol. The effect of membrane treatment condition consisting of alkaline and acid on the cell's performance was investigated. Another observation was on the effect of operating temperature for the benefits of daily use as a portable device operating at low temperature. It was found that the membrane treated under acid condition by sulfuric acid solution yielded higher performance of fuel cell than did the alkaline condition treatment. The immersion of Nafion 115 membrane in the acid solution resulted in the addition of H^+ ions to the inner structure of the membrane which assists in the transfer of H^+ ion to the cathode side. The reduction of cell's performance as a result of decreasing operating temperature from 80˚C to 70, 60, 50, 40 and 30˚C is because the cell exhibited higher activation loss at low temperature according to the Butler-Volmer Equation.

1. Introduction

In the last decade, alternative power sources are important for solving an increasing of energy demand and having an impact on environmental problem from fossil fuel. Any fossil fuel used in such activities possess low fuel conversion efficiencies since there are many conversion steps concerned in the process consuming more fossil fuel. There are many forms of alternative energy sources such as solar cells, nuclear power, biofuels, geothermal power and fuel cell. Among them, fuel cells can directly convert chemical reaction into electrical power which have given interests to many researchers since the past decades. In fact, fuel cells have advantages more than other energy sources by providing higher performance and no CO_2 emission [1]. Proton exchange membrane fuel cell (PEMFC) is a promising alternative power source because of its ability to operate at low temperatures and give high power density. However, it was challenged with its disadvantages in terms of hydrogen storage that requires high pressure always with transportation and handlings which are the main concerns in PEMFC operation [2]. Hence, liquid chemicals have been received many interests as possible fuels for fuel cells due to their ease of transportation, safety to handling as well as high energy densities stored. Some alcohols e.g. methanol and ethanol have been widely tested in fuel cells [4,5,6]. It is because of the simplicity of their molecules that can be easily oxidized and the availability as they

were produced in great quantities worldwide, leading to their lower price and more advantages in mobile application than hydrogen fuel cells.

Direct methanol fuel cells (DMFCs) generally have two limitations; methanol crossover from the anode through the membrane into the cathode and slow electro-oxidation kinetics [7]. For direct ethanol fuel cells (DEFCs), the problems are similar to DMFCs with high volatility, but it is non-toxic [8].

Glycerol, a non-toxic, non-flammable and non-volatile liquid, is one of the possible chemicals that can be used as a fuel in fuel cells. In spite of its relatively high viscosity compared to other liquid fuel candidates, glycerol is considered very interesting from an economic aspect since it is a by-product from biodiesel production processes. It can be electrochemically oxidized via 14 electrons in complete oxidation of glycerol case. Normally the oxidant of direct glycerol fuel cells (DGFCs) is O_2. There are two main conventional types of DGFCs, namely acid and alkaline DGFCs.

The electrochemical reactions governing the acid DGFCs are as follows:

Anode: $C_3H_8O_3 + 3H_2O \rightarrow 14H^+ + 14e^- + 3CO_2$ (1)

Cathode: $7/2O_2 + 14H^+ + 14e^- \rightarrow 7H_2O$ (2)

However, acid DGFC is not widely implemented because oxidation reaction of alcohols under acidic condition is very sluggish.

The electrochemical reactions governing alkaline DGFCs are as follows:

Anode: $C_3H_8O_3 + 14OH^- \rightarrow 3CO_2 + 11H_2O + 14e^-$ (3)

Cathode: $7/2O_2 + 11H_2O + 14e^- \rightarrow 14\ OH^-$ (4)

According to previous studies, anion exchange membrane (AEM) has lower ion conductivity and thermal stability when compared to conventional proton exchange membrane such as Nafion®. It was discovered that mixed condition of alkaline-anode and acidic-cathode DGFCs have higher performance than acid DGFCs and alkaline DGFCs [9].

The electrochemical reaction governing the alkaline-acid DGFCs are as follows:

Anode: $14NaOH \rightarrow 14Na^+ + 14OH^-$ (5)

$C_3H_8O_3 + 14OH^- \rightarrow 14e^- + 3CO_2 + 11H_2O$ (6)

Cathode: $7H_2SO_4 \rightarrow 14H^+ + 7SO_4^{2-}$ (7)

$7H_2O_2 + 14H^+ + 14e^- \rightarrow 14H_2O$ (8)

The overall reaction:

$$C_3H_8O_3 + 14NaOH + 7H_2O_2 + 7H_2SO_4 \rightarrow 3CO_2 + 25H_2O + 7Na_2SO_4 \quad (9)$$

Peak power density mixed condition of alkaline-acid DGFCs are 91.6% higher than only alkaline DGFCs [9].

An et al. suggested that Nafion membrane should be pre-treated by NaOH solution because the functional group of Nafion can receive Na^+ from NaOH solution [10]. Nafion membrane have sulfonic acid (SO_3H) in its functional group. Na^+ will replace H^+ in the sulfonic acid (SO_3H) group to form sodium sulfonate ($NaSO_3$). It will push Na^+ which dissociates from fuel at the anode side to cathode side, but since Na^+ has larger atomic radius than H^+ which makes it difficult for Na^+ to enter and replace Protons (H^+) in sulfonic acid (SO_3H). From alkaline-acid DGFCs electrochemical reaction, NaOH is part of the anode fuel where Na^+ is released. Normally, nafion membrane should be pre-treated by sulfuric acid to received SO_4^{2-} , not Na^+ and to remove any metallic/ionic impurities [11]. Neeva et al. discovered that when increasing temperature, the performance of ion transfer can be increased same in DGFCs under alkaline condition. The maximum power density of this research was 129.9 mW/cm^2 found while operate at 80˚C [12].

Therefore, the effects of membrane treatment under alkaline & acid conditions on the performance of an alkaline-acid DGFC were experimentally investigated in this work. In addition, the effect of operating temperature on the cell performance was studied.

2. Materials and Methods

2.1. Preparation of membrane electrode assembly (MEA)

The MEA having an active area of 5 cm^2 was fabricated employing a Nafion 115 membrane and two electrodes of anode and cathode. Nafion 115, was pretreated before being fabricated into an MEA. First, the membrane was washed and submerged in deionized water for 1 h. After that the membrane was immersed in a 3wt% H_2O_2 solution to remove organic contaminants deposited on the membrane surface. After the membrane was cleaned, it was submerged again for 1 h. into deionized water to remove chemicals from previous treatment. Two types of membrane treating conditions under alkaline and acid media are 10wt% NaOH solution and 0.5 M H_2SO_4 solution which was used to protonate and activate the side chain of the membrane, by immersing the membrane in the solution for 1 h. After that, both membranes were submerged again in deionized water for 1 h to remove left at external surface of the membrane alkaline and acid. For both anode and cathode, non-wetproof carbon cloth (ETEK, Type A) was used as the backing layer. Microporous layer was coated onto the backing layer of only the cathode. Microporous layer ink was a mixture of carbon powder (Vulcan XC72) at 1 mg/cm^2 with 20wt% Nafion and isopropyl alcohol. The mixture was submerged in an ultrasonic bath for 30 minutes to form a homogenous ink. The ink was then coated onto the backing layer using the SONO-TEK ultrasonic-coating machine. The catalyst ink of both anode and cathode was a mixture of 20wt% Pt/C (ETEK), Nafion solution (30% of overall catalyst layer weight) and isopropyl alcohol. The mixture was submerged in the ultrasonic bath for 30 min. The catalyst ink was ultrasonically coated at 1mg/cm^2 onto 5 cm^2 of the backing layer with microporous layer (for cathode)

2.2. Electrochemical and cell performance measurements

The MEA was installed in a stack and connected to a fuel cell testing station (Asia Pacific Fuel Cell Technologies, Model: FCED-PD50) for potentiostat and cell polarization test. Two Peristaltic Pump (Masterflex L/S Digital Drive) were used for feeding liquid solution fuels to the cell. Potentiostat test was done at a constant cell potential of 0.4V and maintained for 30 min followed by a quick scan of current using cell polarization test. The cell polarization technique was performed at a waiting time between potentials of 3 s.

3. Results and Discussion

The operating condition of the fuel cell was fixed for both anode and cathode side which is.

Anode Fuel : Solution of 1M Glycerol + 5M NaOH at 1 mL/min

Cathode Fuel : Solution of 1M H_2O_2 + 1M H_2SO_4 at 3 mL/min

For both sides of the cell, there was no preheating of fuel or oxidant prior to entering the cell. Preparation of membrane electrode assembly (MEA)

3.1 Effect of membrane treatment under alkaline-acid conditions

Fig. 1 shows that the cell assembled with NaOH-treated membrane provides higher stability of current density that the cell with H_2SO_4-treated. The average current density of the cell with NaOH-treated membrane was 382.61mA/cm^2 which is less than that of the cell with H_2SO_4 treated membrane with average current density of 448.65 mA/cm^2. The standard deviations of NaOH and H_2SO_4 treated membrane were 12.82 and 36.29 mA/cm^2, respectively as summarized in Table1.

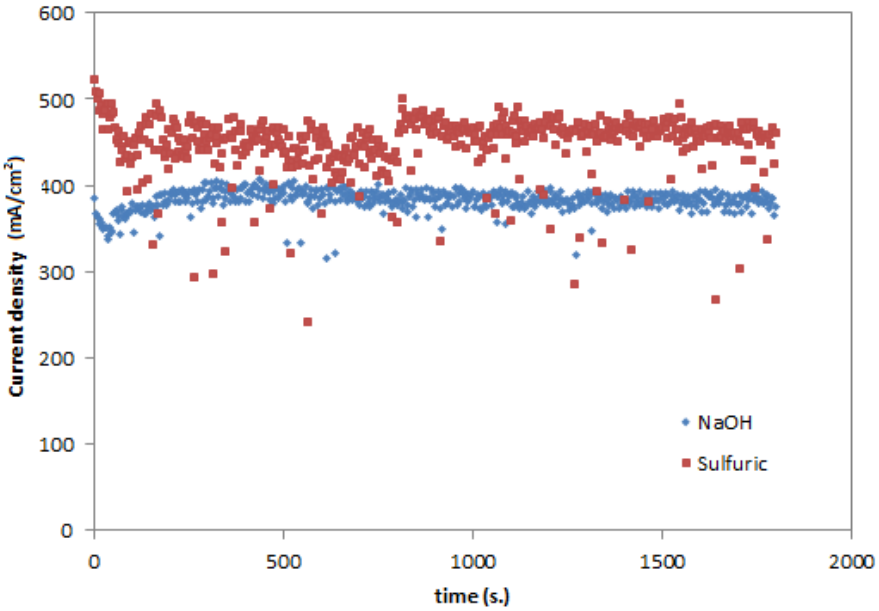

Fig. 1 Potentiostat results of the cells having membrane treated with NaOH and H_2SO_4

Table 1 Average current density and its standard deviation of the cells having membrane treated with NaOH and H_2SO_4

Solution	Average current denity (mA/cm^2)	SD
NaOH	382.61	12.82
H_2SO_4	448.65	36.29

As shown in Fig. 2, the polarization curves of the cell with H_2SO_4 treated membrane had higher maximum power density than that of the cell with NaOH treated. The maximum power density of the cell with H_2SO_4 treated membrane was 292.2 mW/cm^2 while that of the cell with NaOH treated mambrane was 243 mW/cm^2 as shown in Table 2. It shows that sulfuric acid solution was more suitable for treating Nafion membrane than NaOH solution. This Nafion membrane containing sulfonic acid (SO_3H) functional groups will transfer Na^+ from NaOH from anode to cathode through its redox reaction. The goal of immersing membrane in NaOH solution is to add Na^+ into the sidechain of the membrane to replace H^+. The purpose is for Na^+ in the sidechain of the membrane to push away Na^+ form anode fuel to cathode. However, Na^+ ion size is large compared to H^+ , hence it is difficult for Na^+ to replace H^+ in the functional groups of the membrane. However, immersing of membrane in H_2SO_4 solution will help to add SO_4^{2-} from H_2SO_4 which is useful in the improvement of ion transfer in the membrane. Therefore, membranes treated with H_2SO_4 solution have more ion transfer ability than the ones treated with NaOH solution.

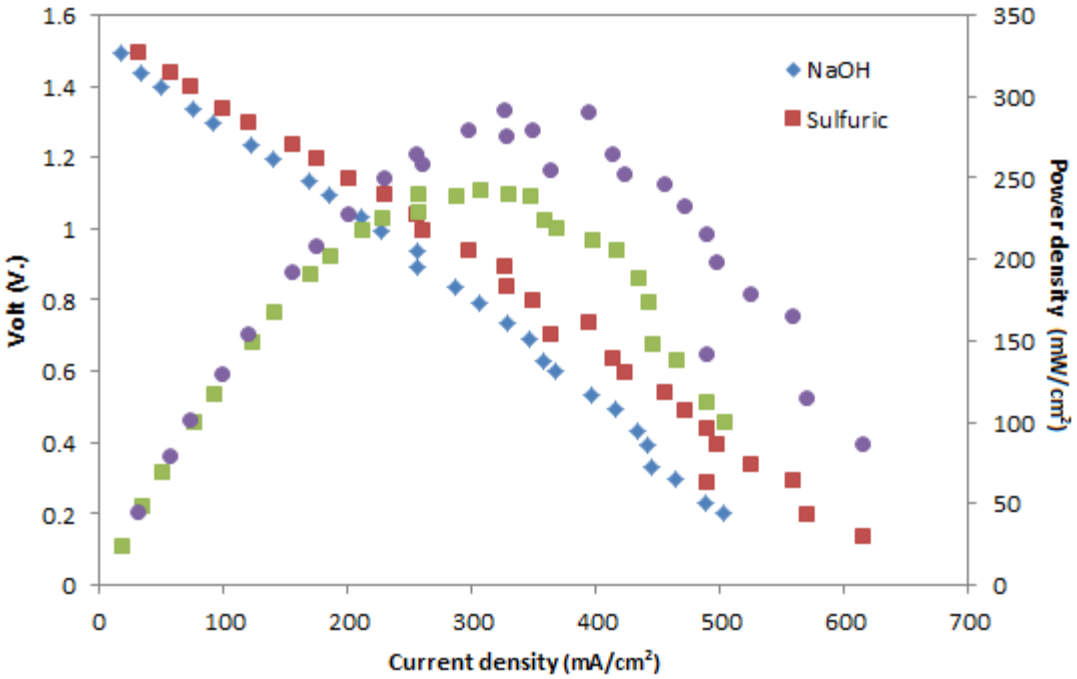

Fig. 2 Polarization curves of the cells having membranes treated with NaOH and H_2SO_4

Table 2 Maximum current density and maximum power density of the cells having membranes treated with NaOH and H_2SO_4

Solution	Maximum current density (mA/cm^2)	Maximum power density (mW/cm^2)
NaOH	502.8	243.0
H_2SO_4	614.0	292.2

3.2 Effect of operating temperature on the performance of an alkaline-acid DGFCs.

After obtaining the appropriate treating solution for membrane, it is also important to study the behavior of the cell when the operating temperature was changed. The nafion 115 membrane treated with H_2SO_4 solution was used to obtain potentiostat and cell polarization curves when the operating temperature was varied at 30˚C, 40˚C, 50˚C, 60˚C, 70˚C and 80˚C. The current discharge at 0.4V displayed in Fig. 4 and 5 show that the performance in terms of average current densities increased when operating temperature increased. The best average current density was 501.67 mA/cm^2 at 80˚C. But, when operating temperature was decreased to 70˚C, the average current density decreased to 367.33 mA/cm^2. Therefore, when operating temperature was extremely decreased the average current density was also extremely decreased.

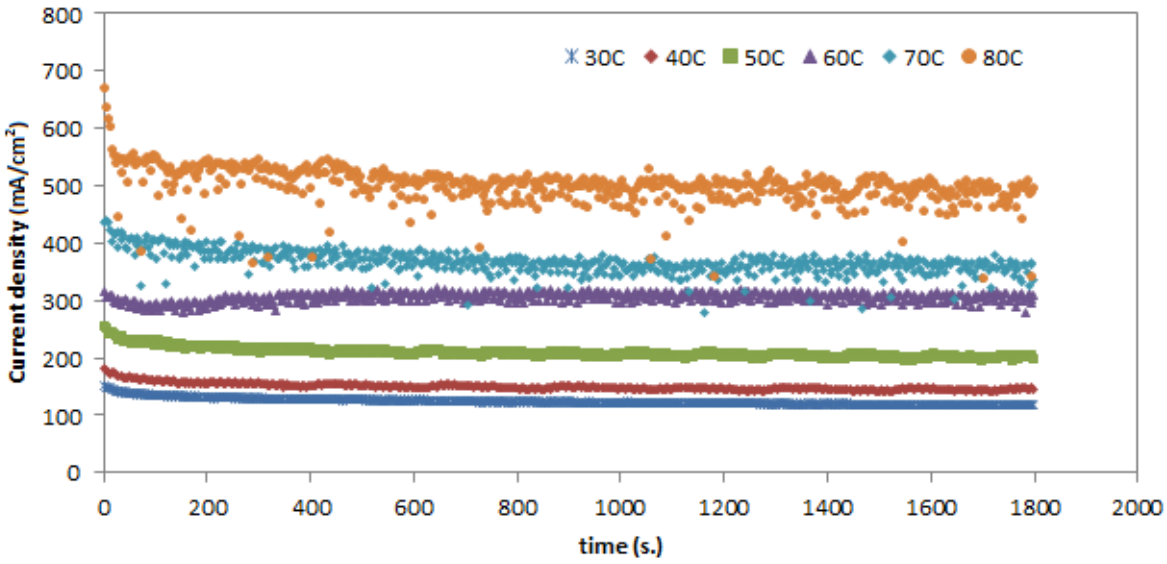

Fig. 3 Potentiostat of the cells operated at different temperatures.

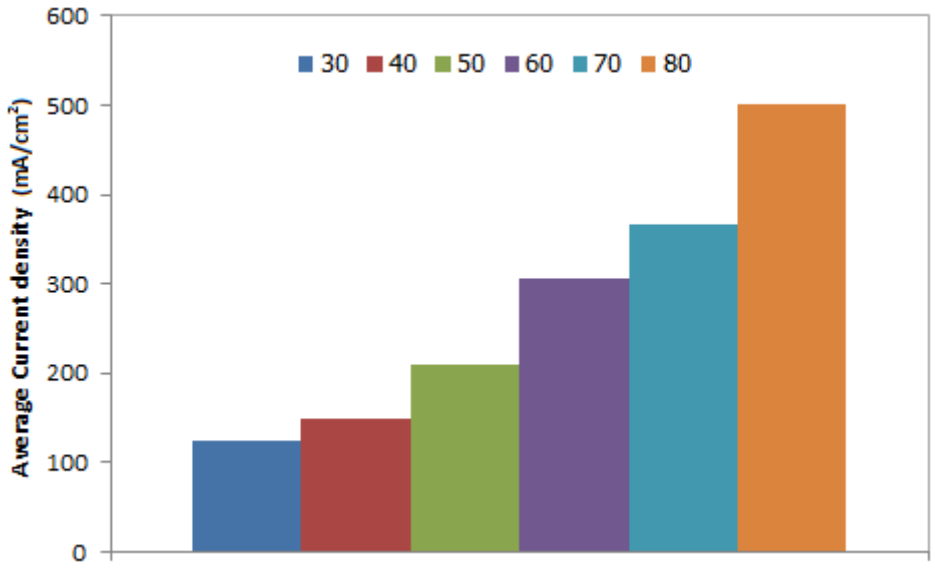

Fig. 4 Average current density at different operating temperature.

The same trend of the cell performance was also observed in the cell polarization test as shown the current density and power density curves in Fig. 5 and Table 3 respectively. The lowest operating temperature of 30˚C gave the worst cell performance with peak power density of only 54.2 mW/cm^2. The highest maximum power density of 334.4 mW/cm^2 was obtained from the case of 80˚C operating temperature. These results conform to Butler-Volmer Equation which, kinetic of chemical reaction can be improved by increasing the temperature.

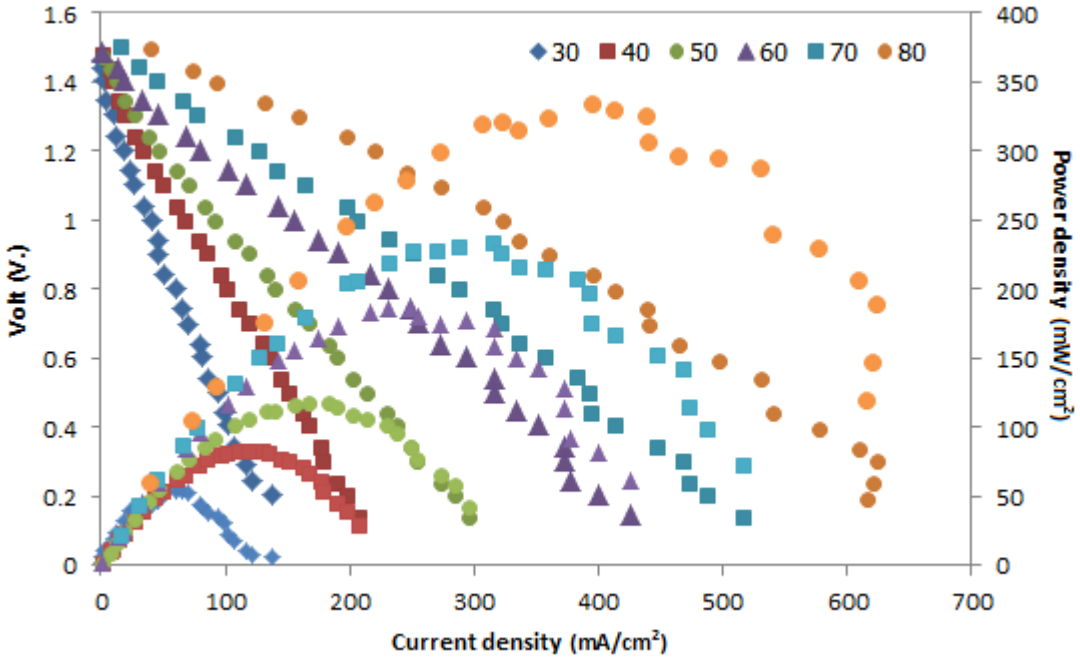

Fig. 5 Polarization curve of the cells operated at different temperatures.

Table 3 Maximum current density and maximum power density at different temperatures

Temperature (˚C)	Maximum current density (mA/cm^2)	Maximum power density (mW/cm^2)
30	138	54.8
40	206.2	83.6
50	295.8	117.6
60	426.4	185.8
70	516.6	234.4
80	623.3	334.4

4. Conclusion

The effects of membrane treatment had influence on DGFC performance alkaline condition with NaOH solution provide higher average current density and maximum power density than that membrane treated under acid condition with H_2SO_4 solution. High kinetic of chemical reaction was obtained when the cell operated at high temperature. Therefore, it can aid improvement the performance of DGFCs.

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Design and Development of Pen Vending Machine Using Arduino UNO R3 Microcontroller

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Keywords: vending machine, Arduino UNO R3, microcontroller

Abstract. The objective of the research was to design and development of pen vending machine which has 120 c.m. x 60 c.m. x 45 c.m. as width x length x height, respectively. Our designed machine has arduino UNO R3 microcontroller as the sensor and compressor to control the motor in the pen release set and the pen detects set. The experimental results show that the controlling of the pen release set according to the situation. Furthermore, the controlling of the pen detects set according to the number of coins and can withdraw coins correctly when the coin is withdrawn.

1. Introduction

Nowadays, there are many automatic vending machines. Vending machines are one example of an automation business process in which it utilizes meager supervision of the user. There are a lot of variety and forms of automation using vending machines [1].

Vending machines refers to a machine which accepts payments in the form of coin, token or card, and dispenses a product. Most often the vending machines used depend on coin intake with currency recognition techniques like image subtraction techniques [2-4].

The photocopy shop here in the Rajamangala University of Technology Phra Nakhon offers diverse services to its clientele. Primarily it offers documents photocopying and printing services. But sometimes customers need other learning materials such as paper pens etc. Therefore, if there is a machine that can facilitate the sale of teaching and learning equipment, it can make customers more comfortable.

This research aims to design and development automated piece-by-piece retail of common pens. This vending machine will dispense blue pens and red pens. This machine utilized coin slot to accommodate 1 baht, 2 baht, 5 baht and 10 baht. The buyer will simply select the type of pens and insert coins. This machine will not dispense change in amount.

2. Methodology

The design concept relates to the design of the model of the pen vending machine that accepts the pen currency by controlling it with arduino UNO R3 microcontroller through the use of the command input switch. Then the motor rotates the pen according to the order. The system will detect the pen when the specified amount has been reached, the display screen, the number of pens remaining and the amount, as shown in Fig. 1.

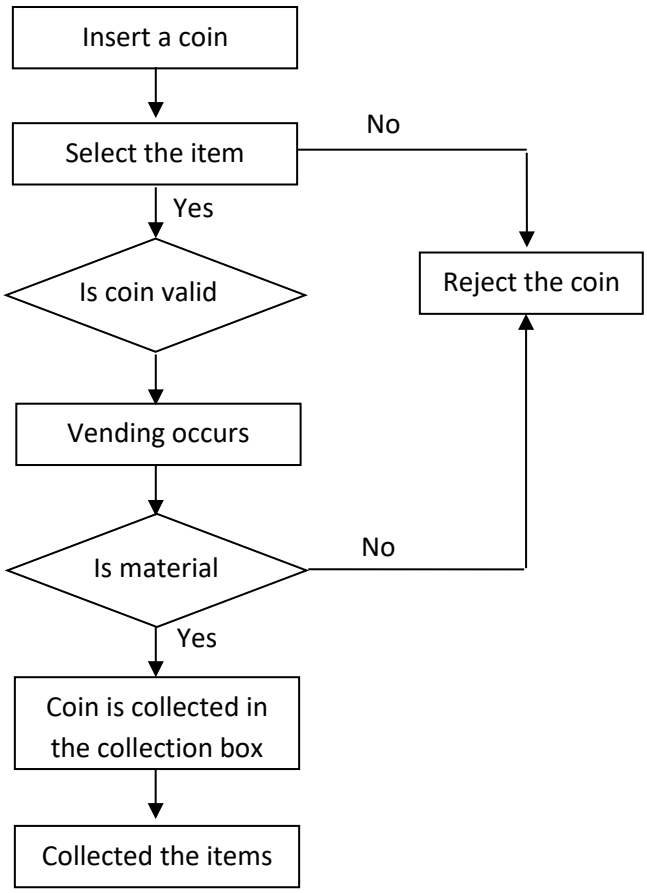

Fig. 1 The block diagram of the research concept

The mechanical structure of the pen vending machine. The prototype model is implemented by the help of the mechanical design setup. The various block present in the design are mentioned below, as shown in Fig. 2.

1. Lock and key
2. Display LCD
3. Coin insertion slot
4. Selection of the item
5. Control unit
6. Coin rejection slot

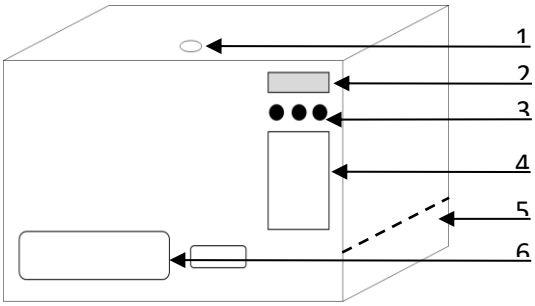

Fig. 2 Mechanical structure of the pen vending machine

The machine was evaluated using descriptive statistics utilizing the five-point rating scale that measures the acceptability of the pen vending machine in accordance to the following criteria:

- Functionality, technical evaluation of the machine in terms of its accurateness of dispensing, maintenance and power.
- Cost effectiveness, evaluation of the prototype as a stand-alone machine while rendering other services.
- Mobility, evaluation of the prototype considering its space occupancy and weight.
- Overall impression, in terms of aesthetics and marketability.

3. Results and Discussion

The machine stands 120 c.m. width, 60 c.m. length, and 45 c.m. height. The designed dimensions were sufficient in accommodating improvised printer dispensing section that is modified to be the storage and dispensing mechanism to pull the desired number of pen as shown in Fig. 3.

Fig. 3 The output prototype of the pen vending machine

The experimental results show that the controlling of the pen release set according to the situation. This machine can use the coin slot to accommodate 1 baht, 2 baht, 5 baht and 10 baht and have precise withdrawal as shown in Table 1.

Table 1 The test of accuracy of pen dispensed

Item	Total pen		Require pen		Select coin	Remaining pens		Withdrawal
	Blue pen	Red pen	Blue pen	Red pen		Blue pen	Red pen	
1	24	24	1	-	5	23	24	-
2	23	24	1	1	10	22	23	-
3	22	23	2	2	20	20	21	-
4	20	21	3	3	30	17	18	-
5	17	18	-	1	6	17	17	1
6	17	17	1	-	10	16	17	5

Table 1 shows the test of accuracy of pen dispensed found that the pen vending machine can choosing the pen type can supply the number of pens exactly.

The evaluation was in a form of questionnaires with specific points to assess the total functionality of the pen vending machine. The rating is from a 1 to 5 scale. Scale 5 to be the highest rate as shown in Fig. 4.

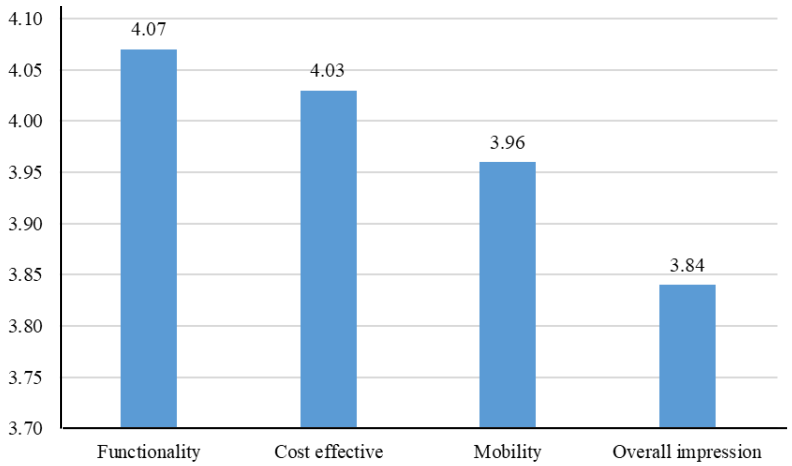

Fig. 4 the result of the assessment

Fig. 4 shows the result of the assessment. Both the mobility and overall impression parameters were quite a bit low, this is due to the usage of machine parts is large and the insertion of the pen is quite difficult was an issue in this prototype then. The functionality and cost effective clearly point out the demand for the prototype to be incorporated in the conduct of business among these shops.

4. Conclusion

The proposed system is the design of prototype model for pen vending machine. The controller part of the vending machine was working according to the specifications for which it was designed. The prototype model was designed for the implementation of the pen vending machine structure which can choose the pen type according to the amount of coins effectively. Coins were inserted and items were vended successfully. In future, pen vending machine of maximum accuracy and efficiency can be achieved with better design and faster control equipment's.

5. Acknowledgments

I would like to thank the Faculty of Industrial Education, Rajamangala University of Technology Phra Nakhon fund in supporting this research study.

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Development of Demonstration for Hydro Power Generation

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Keywords: Demonstration, Hydro power generation**,** Inverter circuit

Abstract. The objective of the research were to development of demonstration for hydro power generation and study of the inverter circuit. The experiment found that development of demonstration for hydro power $220V_{AC}$ by using $12V_{DC}$ electric motor to the current and voltage to the inverter, convert current and voltage to $12V_{DC}$ to current and voltage $220V_{AC}$, by using 24 V_{DC} water pump to suck water in a 20 liter tank. And create pressure to water in the PVC pipe, sending power to the motor rotor to rotate, generate electricity and send to the battery charger circuit, charge current and voltage from the motor to generate electricity to store in the battery. After that send current and voltage to the inverter circuit, convert $12V_{DC}$ battery voltage to $220V_{AC}$.

The results showed that the development of demonstration for hydro power generation able to produce 220 volts AC power for 25 minutes, static electricity without electricity increasing or decreasing all the time working, but the working period will be stable or decrease according to the percentage of usage.

1. Introduction

Nowadays, the number of households and residents in households has increased every year and thus increasing the demand for using electricity as well. Causing the need to produce electricity in various forms, whether produce hydro power generator, Electricity from wind, Energy from gas, Energy from coal, especially from coal, all of which can create pollution that affects the environment. [1-2]

Cause of power outage is the state in which the electricity stops flowing. Caused by the need for electricity from the electricity transmission line Excessive, short circuit in the transmission line. And problems with the transmission line, such as a falling electric pole or an explosion transformer. Which results in the inability to supply power from electricity. [3] Therefore, hydro power is an alternative energy that is possible to be used as an alternative fuel for electricity production. [4]

This research aims to design and development of demonstration for hydro power generation. It is necessary to focus on hydro power generator studies in order to be able use water energy efficiently. And maximize benefits we therefore choose to use the tap water that is used in everyday life. Because when using water in daily life, it will get electricity from using water to store it as a backup power.

2. Methodology

Starting with the study of related theories. Design a demonstration set. Providing equipment and assembling work pieces. Conducting a demonstration of demonstration for hydro power

generation 220 V_{AC}. Test the operation of the demonstration. To collect the data of the working voltage and current produced. Analyze the experimental results of the work of the demonstration for hydro power generation power and summarize results, as shown in Fig. 1.

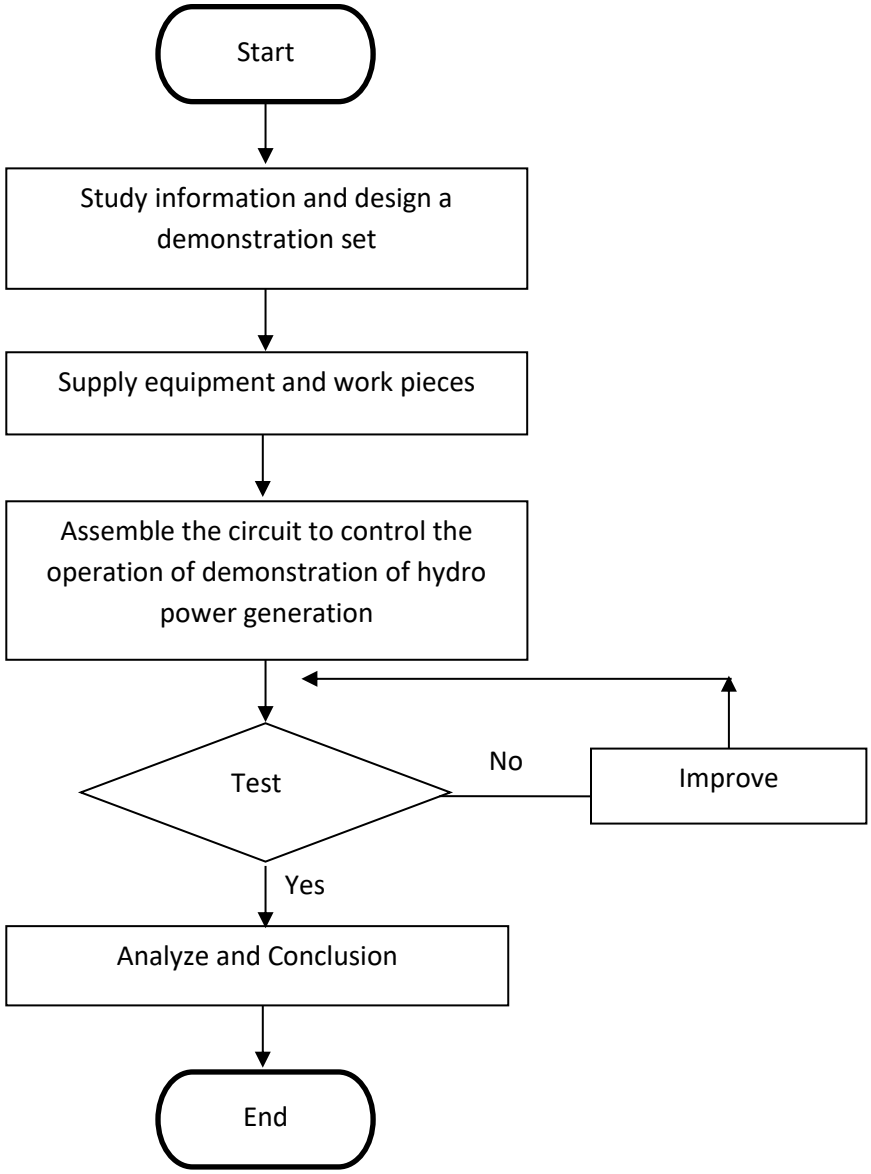

Fig. 1 The block diagram of the research concept

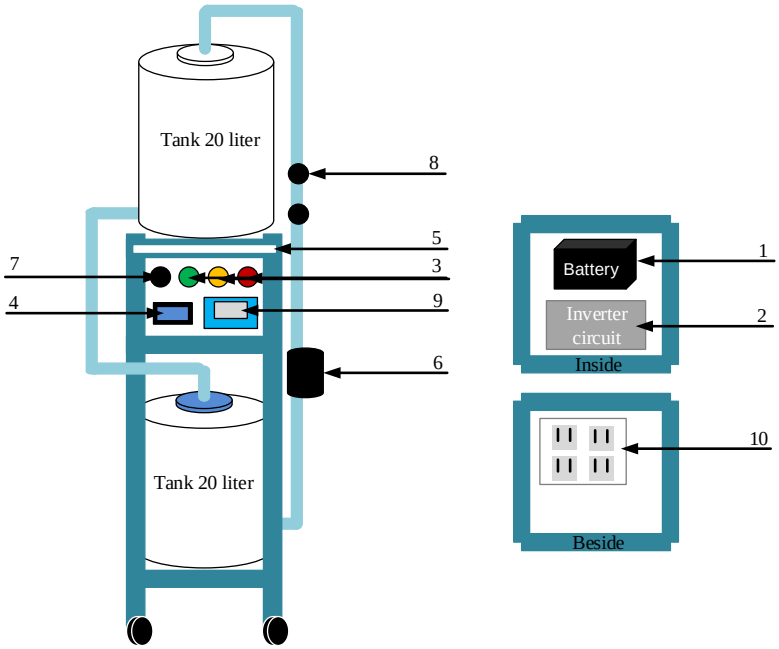

Fig. 2 Structure of demonstration for hydro power generation

Structure of demonstration for hydro power generation. The model is implemented of the design setup. The various block present in the design are mentioned below, as shown in Fig. 2.

1. Battery
2. Inverter circuit
3. One way switch circuit
4. Meter LCD digital dual display
5. LED SMD
6. Voltage regulator circuit 12-24 V_{DC}
7. Volume adjustable water pump motor 24 V_{DC}
8. Generator
9. Battery charger circuit 12V_{DC}
10. Four power plug

The work of the demonstration set starts with the battery supplying current and voltage of 12 V_{DC} to the current and voltage adjustment of 12 V_{DC} to 24 V_{DC} to adjust the current and voltage to suit the usage. Adjust the volume of the 24 V_{DC} water pump motor to get the proper water pressure flowing through the 12 V_{DC} generator. Then bring the electricity that the motor produces 12 V_{DC} to connect to the battery charge circuit. To send current and voltage to charge the battery. The battery is supplying current and voltage 12 V_{DC} to the inverter to convert current and voltage from 12 V_{DC} to 220 V_{AC} and then connect the current and voltage to 220 V_{AC} to the meter to display. Therefore will supply current and voltage to the 220 V_{AC} power plug.

3. Results and Discussion

Power generation from hydro power by using a 12V_{DC} electric motor to enter the current and voltage to the inverter, convert current and voltage to 12V_{DC} to current and voltage 220V_{AC}, by using a 24 V_{DC} water pump to suck water in a 20 liter tank and create pressure to water in the PVC pipe, sending power to the rotor in the motor to generate electricity, rotate to generate electricity and send to the battery charging circuit. Charge current and voltage obtained from the motor to generate electricity to store on the battery, then send the current and the voltage that has been given to the

inverter. Convert the voltage from the $12V_{DC}$ battery to $220V_{AC}$. Then the current and voltage obtained will be displayed via the voltmeter, ammeter, digital LCD meter, shows the electricity charge from the inverter, as shown in Fig. 3.

Fig. 3 Demonstration for hydro power generation

The experimental results show that the demonstration for hydro power generation. Experimental results that are non-load as shown Table 1.

Table 1 The test non-load experiment with a 25 minute timer.

Time (Minutes)	1	3	5	7	10	13	15	17	20	25
Voltage (V)	220	220	220	220	220	220	220	220	220	220
Current (Ah)	1	1	1	1	1	1	1	1	1	1

Table 1 shows the test non-load experiment with a 25 minute timer. The current and pressure obtained from the experiment are constant, without decreasing or increasing the current and pressure throughout the 25 minute timer. In conclusion, the demonstration for hydro power generation producing has a constant current and voltage.

Table 2 The test per-load experiment to use 4 power outlets with 5 and 10 minutes timer.

Count	1	2	3	4	5	6	7	8	9	10
Time (Minutes)	5	5	5	5	5	10	10	10	10	10
Voltage(V)	218	216	215	215	214	215	215	216	215	214
Current(Ah)	0.14	0.15	0.14	0.17	0.22	0.14	0.16	0.16	0.17	0.23
Power(W)	8.8	11.8	12.0	14.0	28.2	12.0	13.4	13.7	15.3	31.1

Table 2 shows the test per-load experiment to use 4 power outlets with 5 and 10 minutes timer. After using with different types of electrical equipment. The power will increase according to the usage time and the number of devices. The voltage is reduced by the timing and amount of equipment.

The current value will not change much. But will increase according to the amount of electrical equipment used.

4. Conclusion

The proposed of using the device to connect, load and non-load with 3 different time periods, 5, 10 and 25 minutes. To see the work of development demonstration for hydro power generation 220 V_{AC}. The current and voltage obtained from experiments that are not loaded are constant. There is no reduction or increase in current and pressure throughout the timer. And per-load experiment will increase the power according to the increasing use of the device as well as the current value, and the voltage is reduced by the amount of equipment used.

5. Acknowledgments

I would like to thank Faculty of Industrial Education, Rajamangala University of Technology Phra Nakhon fund in supporting this research study.

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The Real-Time Analysis for the Mechanical Properties of Red Bean Grains

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Keywords: Real-time mechanical properties, Red bean grains, Pneumatic mechanical tools, Breaking force

Abstract. Data on the physical and mechanical properties of seeds have significantly importance for most machinery and process equipment design. These properties include breaking force, deformation and stress-strain as well. This study was conducted to investigate the real-time mechanical properties of red bean grains via our design of the pneumatic mechanical tools (PnMT). The significant data revealed that the red bean grains mostly contained with moisture content of 20% w.b.. The average length, width and thickness were 6.2, 5.4 and 5.1 mm respectively. The results showed that breaking force, deformation and stress-strain were different in both features and values in most different applied force directions. In addition, we observed there were the relationship between strain and time. This followed the significant power law in all different force directions.

1. Introduction

The mechanical properties of agricultural materials such as applies force, deformation and stress-strain are important for the prediction of their load-deformation behavior [1, 2]. Investigating these technological characteristics can aid in the design of processing machineries and other post-harvest operations. The advantage of these mechanical properties may be used by manufacturers in order to design harvesting machines or other mechanical devices such as sorting and separating machines. However, in the previous studies [3], the mechanical strengths of red bean grains were typically reported as average values, or based on limited experimental data from the instrument and/or a few good kernels, which would hardly reflect the significant variations in physical and mechanical properties especially in terms of real-time deformation and dynamics. Our research has been conducted to propose the Real-time Pneumatic Mechanical Tools [1] for investigating the mechanical properties such as breaking forces and stress-strain properties of red bean grains. In addition, we were investigated the characteristic of these properties in the different force directions.

2. Materials and Methods

2.1 Sample preparation

The red bean grain used in this study was obtained from a local market in Bangkok, a central province in Thailand (shown in **Fig. 1**). The grains were cleaned manually and the foreign matter, as stones, straw and dirt were removed. Initial moisture content (MC) of samples was determined by drying them in an oven according to ASAE [4]. Initial MC of red bean grains were approximately

20% (w.b.). The red bean grain samples were finally sealed in double plastic bags and stored at approximately 4°C before conducting the experiments.

2.2 Real-time Pneumatic Mechanical Tools

The schematic of our system was shown in **Fig. 1**. The mechanical stress-strain measurements were supported by force, rotation and pressure sensor. The deformation rate was set at 0.2 mm/s. This system corresponds to the compression test of food materials of convex shape [5].

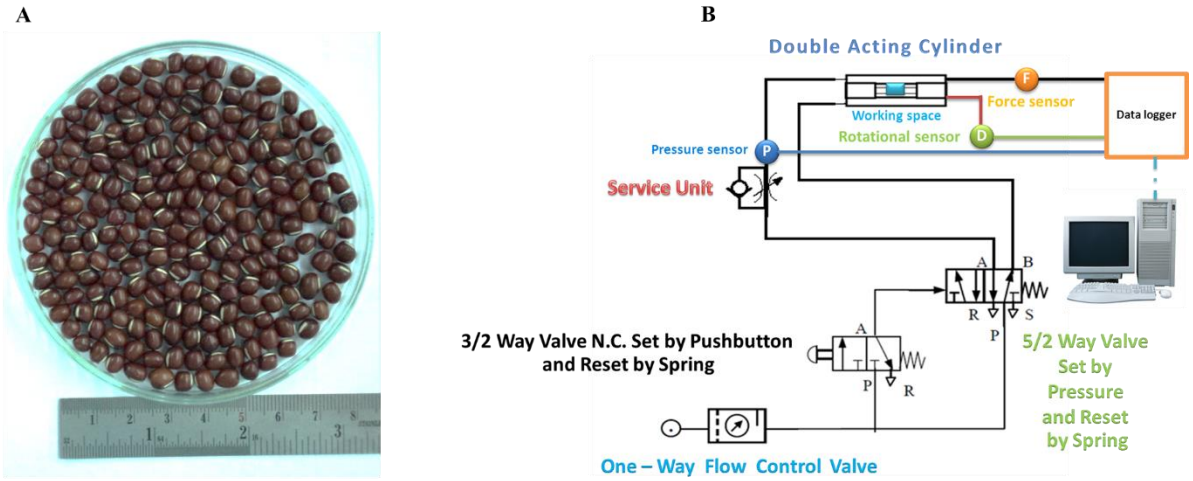
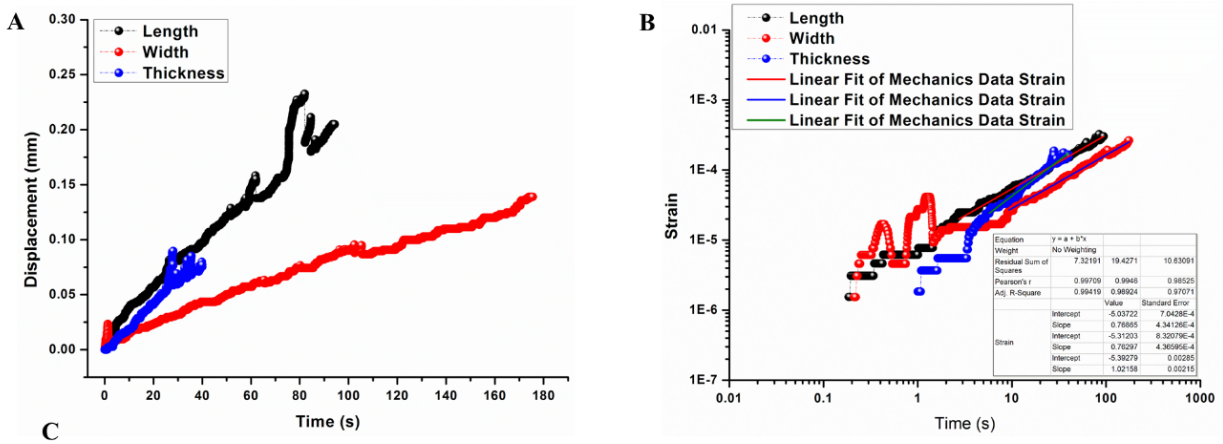


Fig. 1 The red bean grain and the pneumatic mechanical tools (PnMT) in this study. **(A)** The red bean grains in this study. **(B)** The schematic of Real-time Pneumatic Mechanical Tools.

3. Results and Discussion

The relation of kernel deformation and strain with respect to time for 3 applied forces (along length, width and thickness) as shown in **Fig. 2.** These results shown that the difference of applied force direction shown the characteristic time scale dynamics of red bean deformation. We could be led to distinguish the responsibility of red bean on the applied force directions by using the breaking time and breaking force. Moreover, we found that the slopes of Strain-time relation showed to be 0.77±4.34E-04 s^{-1}, 0.76±4.37E-04 s^{-1} and 1.02±0.00215 s^{-1} for the force direction along length, width and thickness, respectively, indicating a more strain rate for the thickness of red bean kernels. This is the first time for the reported results of mechanical properties including breaking time, breaking force, and slopes of Strain-time relation, which study in the red bean kernels that were found to compare with the results obtained in this study. However, these mechanical properties of the different agricultural materials are close to those reported by Bargale and Irudayaraj (1995) for barley kernels, Burubai et al. (2008) for African nutmeg (*Monodora myristica*), Baryeh (2000) for Avocado pear and Zhang et al. (2005) for rice [6-9].



C

	Breaking Time (s)	Breaking Time SD	Breaking Force (N)	Breaking Force SD	Strain-Time Slope (s^{-1})	Strain-Time Slope SD
Length	94.37	34.79	24.80	17.68	0.77	4.34E-04
Width	175.57	18.07	80.30	14.21	0.76	4.37E-04
Thickness	40.08	11.74	15.70	13.48	1.02	0.00215

Fig. 2 The time evolution of red bean kernels in terms of deformation and strain with 3 force directions **(A)** Displacement and time relation of red bean kernels that presented along length, width and thickness. **(B)** Strain-time relation of red bean kernels that calculated from (A). **(C)** Table mechanical parameters include breaking time, breaking force, and strain-time slope.

In addition, the modulus of red bean grains that applied the different force directions as shown in **Fig. 3**. We found that the transition point for changing to rupture behavior reflected the different acquiring energy for damage kernels. These results indicated that thickness has high strain energy for a red bean kernel undergoes fracture.

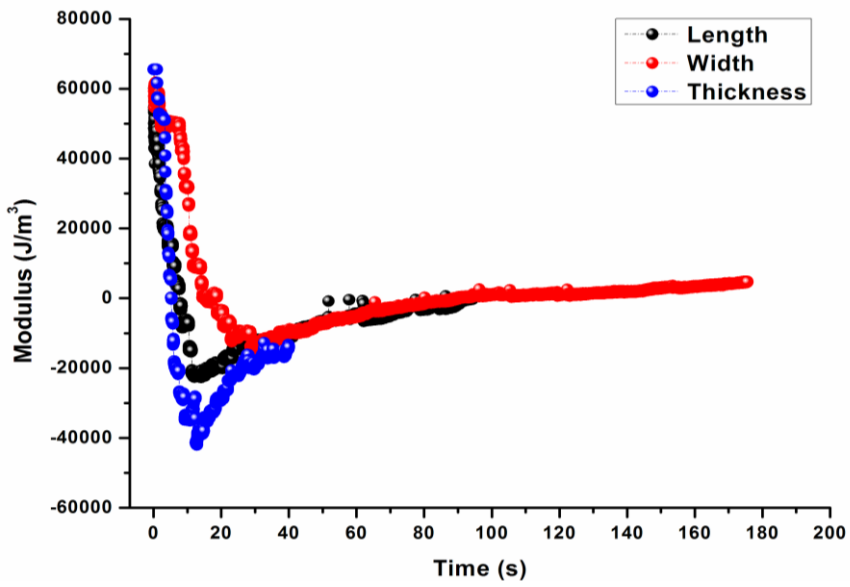


Fig. 3 The modulus-time relation of red bean kernels in terms of deformation and strain with 3 force directions which presented along length, width and thickness.

4. Summary and Conclusion

The real-time Pneumatic Mechanical Tools could be appropriated to study the mechanical properties of red bean grain especially the real-time dynamics. We were measured these properties in terms of average breaking force, breaking time, and stress-strain. Deformations and stress-strain properties of red bean grains generally differenced in the different applied force direction.

5. Acknowledgements

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Designed Environment for People with Baby Aphasia Case Studie : Babies Aphasia and Intelligence. "Nontawith Home Landscape," Pak Kret District Some Markets. Nonthaburi

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Keywords: Design environment, Baby aphasia, Babies aphasia and intelligence.

1. Introduction

With an average rate increase of 1 million people per year, most disabled people with health problems and a higher risk of health problems than the general population. A key element of the problem is that people with disabilities have limited health care. Including the ability to take care of themselves than people who have normal body. There are certain types of people with disabilities who have the disorder than the general disability. Need to be taken care of than normal. The cause and the problem. The government has set up an agency or shelter. Care to assist those with disabilities. Agencies are spread out geographically in order to assist and care for the disabled, these thoroughly. To be able to care for and help themselves. Improves quality of life and care as a fundamental right which should be given.

Babies aphasia and intelligence. "Nontawith home landscape," Pak Kret district some markets. Nonthaburi Has opened a service and support for a long period and infant aphasia. Use a lot of The current state of disrepair The area should be designed and updated as appropriate. This will allow service providers infant aphasia. Quality of life improved Education about the need for space applications is an important issue. Spatial needs to be aligned to real world applications. This will entail improving the quality of life in Babies aphasia and intelligence. Nonthaburi

In this study, Oriented education to fix problems in the design environment to respond to applications for Babies aphasia and intelligence. Nonthaburi researchers used data collection methods used by the target group. Focus on the involvement of people living in the area. By the authorities and local residents to participate in all activities in the research.

2. Literature References

Researchers have studied the concepts and theories related to research and research methodologies. The story is divided into four definitions of disability. The type of disability and welfare. The participatory design and design institutions and design standards thoroughfare for people with disabilities.

2.1 Definition of disability

Patip Assawaphom (2013) argue that the disabled population is important to the nation in many ways, but the well-being of people with disabilities is often neglected by the society. The environment

is not easy to live. Attitude of people in society toward the disabled. Understanding disability is essential. As a result, the society recognizes the importance of such matters. This will lead to help and support to enable people with disabilities to live like people.

Act for Empowerment of Persons with Disabilities 2550 under Article 4 provides that the definition of disability. "People who have limitations in performing everyday activities. Or participated in social activities. Due to impaired sight, hearing, mobility, communication, behavioral, mental, emotional intelligence, learning disabilities, or any other. The barriers in areas with special needs to receive help one area to be able to practice. In everyday life or social involvement with guests ".

From the above definition The medical definition of disability in terms of looking at the problems and difficulties of the life of ordinary people. The attitudes about people with disabilities as people who have been helping care. But the definition of disability in the social dimension. That disabled people have the same rights to everyone. It is the duty of society to encourage people with disabilities to participate in these activities as individuals.

2.2 The type of disability and welfare

Currently, there are many types of disabilities. Each type of defect are different away. The aid scheme is different. Sorting disabled by the Ministry of Social Development and Human Security disability guidelines on the type and No. 2, 2555 were categorized into 7 types of disabilities, including the visually impaired. Hearing disability or meaningful. Disability or physical movement. Disability, mental disability or behavioral intelligence. Learning Disabilities Disabilities and autism. (Ministry of Social Development and Human Security, 2555).

Babies aphasia and intelligence. Nonthaburi set up with the purpose of restoring health. Mental health advocacy and mentally. Neglected and abandoned by their families and society. The optimum age for dependents aged 60 years and over who have contagious diseases. And people with physical disabilities or brain, and can help them in their daily lives. homeless Can not live with the family The orphanage will assist with the development and rehabilitation in areas they can help themselves. The purpose of this educational institution. Is another way to help designers understand the special nature and needs of different people. This will be used to guide the design.

2.3 Design involved

Design participatory process that encourages stakeholders to exchange ideas. Offer solutions and create an understanding of the design work together. This will lead to the creation of partnerships for all parties. The decision to participate in the design process Information must come from those who actually use the building. This will create a sense of ownership. It also provides an opportunity to use the space to display their own needs. This approach brings to the design and the solutions to the problems which have come from the real needs (Nuntiya Hatanuwat and Narong Hutanuwat,2004).

2.4 Designed to foster and design standards for the disabled

Babies aphasia and intelligence. Nonthaburi A key element to take into account the 4: 1) the composition of the primary users and secondary users such as service providers and service recipients, 2) component activities include the type of activity. Patterns of user behavior and characteristics in building support for activities 3) the location and the surrounding environment. Access to the building The social, cultural, and 4) economic factors include the budget to invest in building new or

remodeling. Sources of funding The design may be more specific to different environmental conditions conducive to use. And facilities

Guide "recommendations designed facilities for all" has been identified as a thoroughfare that allows disabled people who can use it easily. To focus on two issues, namely the width of the path and the slope of the thoroughfare. The proper width will range between 0.90 to 1.50 meters and a slope of at least 1:12 if the ramp is longer than 2.50 meters must have handrails on both sides. If the length of the ramp-up period of 6.00 meters to a landing ramp has a width of 1.50 m, the surface of the ramp must not slip material is suitable for use. (Association of Siamese Architects under Royal Patronage, 2015).

3. Method

In the process of data collection, the researchers conducted a survey of the physical Babies aphasia and intelligence, Nonthaburi. The interviews are the primary means of storing data with the stored image and to create an environment within the actual project. The research was conducted into space three times. The physical storage conditions and issues twice co-designed with participation once again.

The first survey was held on December 7, 2018 with the objective to explore and visit. The Babies aphasia and intelligence. Nonthaburi accommodation Executive agencies Procurement Officer Nurses Mentors, as well as provide more frequent. To get information about the place and the overall issues in general. The information is used to guide the research and plans are preliminary. Later on, January 21, 2018, the researcher has studied the area again to collect insights. By means of interviews and participatory observation in detail. To understand the current conditions. Joint analysis of the problems and the needs of the staff interviewed in all sectors. The data were analyzed in order to design an environment for young children in primary aphasia to be used in preparing the detailed design. Plan to join the staff of the Social Welfare Development Center for the Elderly in the final stag

The sample consisted of interviews, the researchers selected a group of nurses, nurse aides, office staff and maintenance technicians within Babies aphasia and intelligence. Nonthaburi because groups to take place in practice. Information can be clearly Unlike patients who are handicapped and disabled. These groups, which are limited in their ability to provide information on the spatial applications. The researchers relied on data from the first sample-based.

On the 3rd day of March 27, 2018 were prepared and used in the design of the important issues that are involved. Using the information from the interview. Observations used as a guide to create the drawing. Environment Design of New Babies aphasia and intelligence. Nonthaburi and take such an approach to be presented with the information from the various departments. The audience follows: An executive agency of the first group of 12 providers and patients or those with disabilities who can communicate for 3 people. All participants can provide feedback and share ideas and make suggestions. To be used in the design environment. The layout of the building The new system, roaming the area with investigators. To make sure that all the parties. The draft development plan for the project can be used to cause a real interest in the work. It also can be used as documentation for presentation development budget Babies aphasia and intelligence. Nonthaburi province to the executive decision to go.

4. Case study

Babies aphasia and intelligence, Nonthaburi has a total area of 54 acres with 14 buildings, including building a building, service building and seven other buildings of 7 buildings with a single-

storey building. All the information in Dalmatia Derived from a joint survey and interview with nurse mentors and service providers in Babies aphasia and intelligence. Nonthaburi

5. Results and Discussions

Barriers that result in inconvenience and delay in providing and receiving services today can be split into two major areas: the problems and difficulties of the route within the area of the building.

5.1 Obstacles caused by road traffic.

The main obstacle occurs within the area. To modify applications that change based on actual usage in the area. The use of the building, according to the circumstances of each activity such as the increase of residential buildings infant aphasia showed that the number of disabled people more. Issue of a thoroughfare for vehicles and pedestrians occur immediately. The main route for commuters currently used mainly by vehicles. Nature trails current bridge is narrow and steep. As a result, access to Babies aphasia and intelligence. Nonthaburi continued inconvenience. The road project is currently not linked to each other where possible. Make car travel Not accessible in all areas within the project. The land is still at large. Roads within the current size of just 4 meters, resulting in the car can run only one lane. It is impossible to access the building thoroughly. If there is an emergency such as a fire. It may be that the fire will not reach the scene within a timely manner.

The pedestrian route traffic within the area is currently not designed to respond to the truly active, such as the link between building design is not contiguous. Both those with disabilities and service providers need to walk down the street with the car at some point. Although the wide walkways are wide enough for the use of disabled people, especially disabled people who use wheelchairs. The areas covered. Nevertheless, the slope of the ramp does not meet the standards for the use of disabled people in general. Some of the slopes are steep, the proportion of 1: 6, which is steeper than that disabled people are able to move up and down by themselves. Many point to rail Route traffic across multiple paths to serve as general entrance buildings 1 and 2 will be used together with the transfer of food. Or disabled access to buildings, hospitals need to walk down the road to get access to services and so on.

5.2 The tower is not appropriate

Located in the main building, in the improper access by Center staff and kitchen staff as the food can lead to the building. Building nurse disabled should be easily accessible and safe, but it is far greater than the disabled, remember to arrive in time. If there is an emergency Access the ambulance was not very convenient.

The research showed that the main problem of Babies aphasia and intelligence. The jobs issue is the placement of buildings and road projects. This issue affects the providers and recipients of services within the area. The research focus is on the design of the project is to improve the layout. To determine the distance between buildings. Should put an end to the new building. By requiring them to be in the proper position. Taking into account the needs of each service provider and the recipient. Routing and roaming through the various trails in the area. Traffic routes should have continuous access to various points of Babies aphasia and intelligence. Nonthaburi is easy Not interfere with each other and are safe if there is an emergency.

6. Conclusion

Data from the surveys and interviews, physical involvement with the informant. The research data were analyzed and the draft Plan. The placement of the building System traffic Design environment for the handicapped and disabled. The design approach can be summarized as follows environments.

1) The position of the hospital building - physiotherapy. Buildings and facilities such as the kitchen, office buildings and activities.

2) Route traffic within the Social Welfare Development elderly home Khae. Must be linked together and can shorten the travel time from the overall layout of the original. Either in the form of vehicular traffic and on foot. The width of the thoroughfare for vehicles to facilitate access for large vehicles. For quick service and assistance at the time.

The Rail Master Plan and design a new layout. Representing all sectors of Babies aphasia and intelligence. Nonthaburi province has offered a detailed layout of a building using segmented by type of building applications. To make it easier to understand Connect and respond to the needs of service providers, all within easy reach. Can be divided into zones as follows.

1) The building's accessibility, which classify disabilities are 2 types of people with disabilities and disability. To facilitate the allocation is appropriate to the nature of their disability. Each building will be classified according to specific characteristics such as disability disabled handicapped seat next to the bed with a wheelchair.

2) Office building, the staff work department. Both the Administration Procurement and Maintenance. In addition, the area is welcome to visit the project.

3) Building nursing and physiotherapy. Placement near a residential building of the handicapped and disabled. Also connected to each other.

4) The kitchen and dining area. Placed in the area to cater for services in various parts of the hotel.

5) The area in front of the project, Auditorium and recreational buildings, can accommodate a group of visitors in a group then. It is also an area for the joint activities of the disabled and those who visit them.

6) The shelter's staff and the service provider are separated from the service to ensure the privacy of its duties.

7) Size is 2 lane roads surrounding the project to facilitate and improve the trails for the project to be constantly connected.

The detailed requirements for each of the sub-group of buildings. Need to be designed to contribute more. Since each building is a form of active and functional requirements specific to different away. However, a draft of the Master Plan for Babies aphasia and intelligence. Nonthaburi Is the starting point for the development of the quality of life of those involved in the area. This will lead to the development of future projects.

7. Acknowledgment

Thanks to the staff as well as clients in Babies aphasia and intelligence, Nonthaburi, informative and educational activities to help guide this review article, which allows participation occurs in almost all stages of education and encourage studies of this success well.

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Designed Environment for People with Disabilities and Disability. Case Studies : Improving the Environment of the Center for Social Welfare Development Elderly Home Khae. Bangkok.

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1. Introduction

With an average rate increase of 1 million people per year, most disabled people with health problems and a higher risk of health problems than the general population. A key element of the problem is that people with disabilities have limited health care. Including the ability to take care of themselves than people who have normal body. There are certain types of people with disabilities who have the disorder than the general disability. Need to be taken care of than normal. The cause and the problem. The government has set up an agency or shelter. Care to assist those with disabilities. Agencies are spread out geographically in order to assist and care for the disabled, these thoroughly. To be able to care for and help themselves. Improves quality of life and care as a fundamental right which should be given.

Center for Social Welfare Development elderly home Khae. Bangkok Has opened a service and support for a long period and with disabled people and disability used a lot. The current state of disrepair The area should be designed and updated as appropriate. This will allow service providers. Disabled disability and quality of life improved. Education about the need for space applications is an important issue. Spatial needs to be aligned to real world applications. This will entail improving the quality of life in elderly homes Social Welfare Development Center Khae. Bangkok

In this study, Oriented education to fix problems in the design environment to respond to applications for people with disabilities and disability in elderly homes Social Welfare Development Center Khae. Bangkok, researchers used data collection methods used by the target group. Focus on the involvement of people living in the area. By the authorities and local residents to participate in all activities in the research.

2. Literature References

Researchers have studied the concepts and theories related to research and research methodology. The content is divided into four categories: the definition of disability. Types of disability and welfare Participatory design and design of shelters and standards for road design for people with disabilities

2.1 Definition of disability

Patip Assawaphom (2013) argue that the disabled population is important to the nation in many ways, but the well-being of people with disabilities is often neglected by the society. The environment is not easy to live. Attitude of people in society toward the disabled. Understanding disability is essential. As a result, the society recognizes the importance of such matters. This will lead to help and support to enable people with disabilities to live like people.

The Act on Promotion and Development of the Quality of Life of People with Disabilities, 2007, under Section 4, provides the meaning of the disabled. "Individuals who have limited access to daily activities. Or take part in social activities. They have a deficiency in seeing, hearing, movement, communication, mind, emotion, behavior, intelligence, learning, or other defect. There are obstacles in various areas and there is a special need to get help on one side in order to be able to practice activities. In daily life or in social participation as guests.

From the definition above. Medical definition in the view of disability is a problem and obstacle in the normal life. The attitude about the disabled is that people need special care. But definition of disability in the social dimension. I think that people with disabilities are human equal to everyone. It is the duty of the society to promote the participation of people with disabilities in public activities.

2.2 The type of disability and welfare

Currently, there are many types of disabilities. Each type of defect is different away. The aid scheme is different. Sorting disabled by the Ministry of Social Development and Human Security disability guidelines on the type and No. 2, 2555 were categorized into 7 types of disabilities, including the visually impaired. Hearing disability or meaningful. Disability or physical movement. Disability, mental disability or behavioral intelligence. Learning Disabilities Disabilities and autism. (Ministry of Social Development and Human Security, 2555).

Center for Social Welfare Development elderly home Khae. Bangkok Set up with the purpose of restoring health. Mental health and rights of people with disabilities. The optimum age of neglected and abandoned by their families and society. The optimum age for dependents aged 60 years and over who have contagious diseases. And people with physical disabilities or brain and can help them in their daily lives. Homeless can not live with the family. The orphanage will assist with the development and rehabilitation in areas they can help themselves. The purpose of this educational institution. Is another way to help designers understand the special nature and needs of different people. This will be used to guide the design. Improve the environment as well as allowing service providers to prioritize applications, which can increase the performance and speed of access to the service appropriately fast.

2.3 Design involved

Design participatory process that encourages stakeholders to exchange ideas. Offer solutions and create an understanding of the design work together. This will lead to the creation of partnerships for all parties. The decision to participate in the design process Information must come from those who actually use the building. This will create a sense of ownership. It also provides an opportunity to use the space to display their own needs. This approach brings to the design and the solutions to the problems which have come from the real needs (Nuntiya Hatanuwat and Narong Hutanuwat,2004).

2.4 Designed to foster and design standards for the disabled

Design Development Center for Social Welfare of the elderly. A key element to take into account the 4: 1) the composition of the primary users and secondary users such as service providers and service recipients, 2) component activities include the type of activity. Patterns of user behavior and characteristics in building support for activities 3) the location and the surrounding environment. Access to the building, the social, cultural, and 4) economic factors include the budget to invest in building new or remodeling. The design may be more specific to different environmental conditions conducive to use and facilities depending on sources of funding.

Guide "recommendations designed facilities for all" has been identified as a thoroughfare that allows disabled people who can use it easily. To focus on two issues, namely the width of the path and the slope of the thoroughfare. The proper width will range between 0.90 to 1.50 meters and a slope of at least 1:12 if the ramp is longer than 2.50 meters must have handrails on both sides. If the length of the ramp-up period of 6.00 meters to a landing ramp has a width of 1.50 m, the surface of the ramp must not slip material is suitable for use. (Association of Siamese Architects under Royal Patronage, 2015).

3. Method

In the process of data collection, the researchers conducted a survey of data center physical development, social welfare, the elderly home Khae, Bangkok. The interviews are the primary means of storing data with the stored image and to create an environment within the actual project. The research was conducted into space three times. The physical storage conditions and issues twice. Co-designed with participation once again.

The survey for the first time on December 4, 2018 with the objective to explore and visit. The Center for Social Welfare Development elderly home Khae includes executive agencies. Procurement Officer Nurses Mentors, as well as provide more frequent. To get information about the place and the overall issues in general. The information is used to guide the research and plans are preliminary. Later on, January 12, 2018, the researcher has studied the area again to collect insights. By means of interviews and participatory observation in detail. To understand the current conditions. Joint analysis of the problems and the needs of the staff interviewed in all sectors. The data were analyzed in order to design an environment for people with disabilities and disability in the initial stage to be used in preparing the detailed design.

The sample consisted of interviews, the researchers selected a group of nurses, nurse aides, office staff and maintenance technicians within the Social Welfare Development Center for the Elderly. Because the sample is based on those who work in practice. Information can be clearly Unlike patients who are handicapped and disabled. These groups, which are limited in their ability to provide information on the spatial applications. The researchers relied on data from the first sample-based.

On the 3rd day of March 27, 2561 were prepared and used in the design of the important issues that are involved. Using the information from the interview. Observations used as a guide to create the drawing. Environment Design Center's new home Khae Social Welfare Development elderly. And such an approach to be presented with the information from the various departments. The audience follows: An executive agency of the first group of 12 providers and patients or those with disabilities who can communicate for 3 people. All participants can provide feedback and share ideas and make

suggestions to the design environment, the layout of the building, the new system, roaming the area with investigators to make sure that all the parties. The draft development plan for the project can be used to cause a real interest in the work. It also can be used as a document presenting the budget to develop a Social Welfare Development elderly Khae home for senior decision further.

4. Case study

Center for Social Welfare Development elderly home Khae. Bangkok has a total area of 67 acres with buildings for a total of 18 buildings divided into seven buildings, service buildings and other buildings of 11 buildings, all with a single-storey building. All the information in Dalmatia Derived from a joint survey and interview with nurse mentors and service providers in the Social Welfare Development Center for the Elderly Home Khae. Bangkok

5. Results Conclusions

Barriers that result in inconvenience and delay in providing and receiving services today can be split into two major areas: the problems and difficulties of the route within the area of the building.

5.1 Obstacles caused by road traffic.

The main obstacle occurs within the area. To modify applications that change based on actual usage in the area. The use of the building, according to the circumstances of each activity such as the increase in residential building handicapped and disabled. It was found that there were more people with disabilities. Issue of a thoroughfare for vehicles and pedestrians occur immediately. The main route for commuters currently used mainly by vehicles. Nature trails current bridge is narrow and steep. The center provides access to the Social Welfare Development elderly home Khae continued inconvenience. The road project is currently not linked to each other where possible. Make car travel Not accessible in all areas within the project. The land is still at large. Roads within the current size of just 4 meters, resulting in the car can run only one lane. It is impossible to access the building thoroughly. If there is an emergency such as a fire. It may be that the fire will not reach the scene within a timely manner.

Both those with disabilities and service providers need to walk down the street with the car at some point. Although the wide walkways are wide enough for the use of disabled people, especially disabled people who use wheelchairs. The areas covered. Nevertheless, the slope of the ramp does not meet the standards for the use of disabled people in general. Some of the slopes are steep, the proportion of 1: 6, which is steeper than that disabled people are able to move up and down by themselves. Many points to rail Route traffic across multiple paths to serve as general entrance buildings 1 and 2 will be used together with the transfer of food. Or disabled access to buildings, hospitals need to walk down the road to get access to services and so on.

5.2 The tower is not appropriate

Located in the main building, in the improper access by Center staff and kitchen staff as the food can lead to the building. Building nurse disabled should be easily accessible and safe, but it is far greater than the disabled, remember to arrive in time. If there is an emergency access, the ambulance was not very convenient.

Based on the information received and state issues. The researchers found that the primary focus of the Center for Social Welfare Development elderly home Khae problem is the placement of

buildings and road projects. This issue affects the providers and recipients of services within the area. The research focus is on the design of the project is to improve the layout. To determine the distance between buildings. Should put an end to the new building. By requiring them to be in the proper position, considering the needs of each service provider and the recipient, routing and roaming through the various trails in the area. Traffic routes should have continuous access to the center of Ban Bang Khae Social Welfare Development elderly friendly, not interfere with each other and are safe if there is an emergency.

6. Conclusions

Data from the surveys and interviews, physical involvement with the informant. The research data were analyzed and the draft Plan. The placement of the building System traffic Design environment for the handicapped and disabled. The design approach can be summarized as follows environments.

1) The position of the hospital building - physiotherapy. Buildings and facilities such as the kitchen, office buildings and activities.

2) route traffic within the Social Welfare Development elderly home Khae. Must be linked together and can shorten the travel time from the overall layout of the original. Either in the form of vehicular traffic and on foot. The width of the thoroughfare for vehicles to facilitate access for large vehicles. For quick service and assistance at the time.

The Rail Master Plan and design a new layout. Representing all sectors of the Center for Social Welfare Development elderly home Khae. Bangkok has offered a detailed layout of a building using segmented by type of building applications. To make it easier to understand Connect and respond to the needs of service providers, all within easy reach. Can be divided into zones as follows.

1) The building's accessibility. Which classify disabilities are 2 types of people with disabilities and disability. To facilitate the allocation is appropriate to the nature of their disability. Each building will be classified according to specific characteristics such as disability disabled handicapped seat next to the bed with a wheelchair.

2) Office building, the staff work department. Both the Administration Procurement and Maintenance. In addition, the area is welcome to visit the project.

3) Building nursing and physiotherapy. Placement near a residential building of the handicapped and disabled. Also connected to each other.

4) The kitchen and dining area. Placed in the area to cater for services in various parts of the hotel.

5) Auditorium and recreational buildings, the area in front of the project It can accommodate a group of visitors in a group then. It is also an area for the joint activities of the disabled and those who visit them.

6) The shelter's staff and the service provider are separate from the service. To ensure the privacy of its duties.

7) Size is 2 lane roads surrounding the project to facilitate and improve the trails for the project to be constantly connected.

The detailed requirements for each of the sub-group of buildings. Need to be designed to contribute more. Since each building is a form of active and functional requirements specific to different away. However, a draft of the Master Plan for Social Welfare Development Center for the Elderly Home Khae is the starting point for the development of the quality of life of those involved in the area. This will lead to the development of future projects.

7. Acknowledgment

Thanks to the staff as well as service centers in Bang Khae Social Welfare Development elderly home, the friendly, informative and educational activities to help guide this review article, which allows participation occurs in almost all stages of education and encourage studies of this success well.

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The design space for recreational activities for disability. Case studies: Improve the physical of the center for social welfare development. Pathum Thani

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1. Introduction

With an average rate increase of 1 million people per year, most disabled people with health problems and a higher risk of health problems than the general population. A key element of the problem is that people with disabilities have limited health care. Including the ability to take care of themselves than people who have normal body. There are certain types of people with disabilities who have the disorder than the general disability. Need to be taken care of than normal. The cause and the problem. The government has set up an agency or shelter. Care to assist those with disabilities. Agencies are spread out geographically in order to assist and care for the disabled, these thoroughly. To be able to care for and help themselves. Improves quality of life and care as a fundamental right which should be given.

Welfare Development Center for the Elderly Pathum Thani, has opened a service and support for a long period, and the elderly to use the service a lot. The current state of disrepair The area should be designed and updated as appropriate. This will allow service providers. Seniors' quality of life improved. Education about the need for space applications is an important issue. Spatial needs to be aligned to real world applications. This will entail improving the quality of life in elderly Welfare Development Center. Pathum Thani

In this study, Oriented education to fix problems in the design environment to respond to applications for the elderly Welfare Development Center. Pathum Thani Researchers used data collection methods used by the target group. Focus on the involvement of people living in the area. By the authorities and local residents to participate in all activities in the research.

2. Literature References

Researchers have studied the concepts and theories related to research and research methodologies. The story is divided into four definitions of disability. The type of disability and welfare. The participatory design and design institutions and design standards thoroughfare for people with disabilities.

2.1 Definition of disability

Patip Assawaphom (2014) Said that disabled people are vital to the nation in many ways, but the well-being of people with disabilities are often socially omissions. Whether the environment is no easy life. The attitude of the society towards the disabled. Understanding about disability is essential. The society recognizes the importance of the matter. This will lead to the help and support so that people with disabilities can live like normal people.

Act for Empowerment of Persons with Disabilities 2550 under Article 4 provides that the definition of disability. "People who have limitations in performing everyday activities. Or participated in social activities. Due to impaired sight, hearing, mobility, communication, behavioral, mental, emotional intelligence, learning disabilities, or any other. The barriers in areas with special needs to receive help one area to be able to practice. In everyday life or social involvement with guests ".

From the above definition The medical definition of disability in terms of looking at the problems and difficulties of the life of ordinary people. The attitudes about people with disabilities as people who have been helping care. But the definition of disability in the social dimension. That disabled people have the same rights to everyone. It is the duty of society to encourage people with disabilities to participate in these activities as individuals.

2.2 The type of disability and welfare

Currently, there are many types of disabilities. Each type of defect are different away. The aid scheme is different. Sorting disabled by the Ministry of Social Development and Human Security disability guidelines on the type and No. 2, 2555 were categorized into 7 types of disabilities, including the visually impaired. Hearing disability or meaningful. Disability or physical movement. Disability, mental disability or behavioral intelligence. Learning Disabilities Disabilities and autism. (Ministry of Social Development and Human Security, 2013).

Welfare Development Center for the Elderly Pathum Thani Set up with the purpose of restoring health. Mental health and the rights of the elderly. Neglected and abandoned by their families and society. The optimum age for dependents aged 60 years and over who have contagious diseases and people with physical disabilities or brain and can help them in their daily lives. Homeless who can not live with the family, the orphanage will assist with the development and rehabilitation in areas until they can help themselves. The purpose of this educational institution is another way to help designers understand the special nature and needs of different people. This will be used to guide the design. Improve the environment as well as allowing service providers to prioritize applications, which can increase the performance and speed of access to the service appropriately fast.

2.3 Design involved

Design participatory process that encourages stakeholders to exchange ideas. Offer solutions and create an understanding of the design work together. This will lead to the creation of partnerships for all parties. The decision to participate in the design process Information must come from those who actually use the building. This will create a sense of ownership. It also provides an opportunity to use the space to display their own needs. This approach brings to the design and the solutions to the problems which have come from the real needs.

2.4 Designed to foster and design standards for the disabled

Welfare Development Center for the Elderly Pathum Thani A key element to take into account the 4: 1) the composition of the primary users and secondary users such as service providers and service recipients, 2) component activities include the type of activity. Patterns of user behavior and characteristics in building support for activities 3) the location and the surrounding environment access to the building, the social, cultural, and 4) economic factors include the budget to invest in building new or remodeling sources of funding. The design may be more specific to different environmental conditions conducive to use and facilities.

Guide "recommendations designed facilities for all" has been identified as a thoroughfare that allows disabled people who can use it easily. To focus on two issues, namely the width of the path and the slope of the thoroughfare. The proper width will range between 0.90 to 1.50 meters and a slope of at least 1:12 if the ramp is longer than 2.50 meters must have handrails on both sides. If the length of the ramp-up period of 6.00 meters to a landing ramp has a width of 1.50 m, the surface of the ramp must not slip material is suitable for use. (Association of Siamese Architects under Royal Patronage, 2015).

3. Method

In the process of data collection, the researchers collected survey data on the physical side of Welfare Development Center for the Elderly. Pathum Thani. The interviews are the primary means of storing data with the stored image and to create an environment within the actual project. The research was conducted into space three times. The physical storage conditions and issues twice. Co-designed with participation once again.

The survey for the first time on December 1, 2017, aims to explore the place and met. The Welfare Development Center for the Elderly. Pathumthani include Executive agencies Procurement Officer Nurses Mentors, as well as provide more frequent. To get information about the place and the overall issues in general. The information is used to guide the research and plans are preliminary. Later on January 19, 2018, the researcher has studied the area again to collect insights. By means of interviews and participatory observation in detail. To understand the current conditions. Joint analysis of the problems and the needs of the staff interviewed in all sectors. The data obtained were analyzed for use in the design environment for seniors. In the preliminary stage, to be used in preparing the detailed design.

The sample consisted of interviews, the researchers selected a group of nurses, nurse aides, office staff and maintenance technicians within the Development Center for Welfare seniors. Pathum Thani Because the sample is based on those who actually work in practice. Information can be clearly Unlike patients who are handicapped and disabled. These groups, which are limited in their ability to provide information on the spatial applications. The researchers relied on data from the first sample-based.

On the 3rd day of March 4, 2560, the research provides the information and issues important to the design part. Using the information from the interview. Observations used as a guide to create the drawing. The new design environment's Welfare Development Center for the Elderly. Pathum Thani and take such an approach to be presented with the information from the various departments. The audience follows: Executive Agency Number 1 Provider Group of 12 and Group of the clients or those with disabilities who can communicate for 3 people, all participants can provide feedback and

share ideas and make suggestions. To be used in the design environment. The layout of the building the new system, roaming the area with investigators. To make sure that all the parties. The draft development plan for the project can be used to cause a real interest in the work. It also can be used as documentation for presentation development budget Development Center for Welfare seniors. Pathumthani the executive decision to go.

4. Case study

Center for Social Welfare Development elderly home Khae. Bangkok With a total area of 48 acres with buildings for a total of 7 buildings divided into five buildings, service buildings and other buildings of 2 buildings, all with a single-storey building. All the information in Dalmatia Derived from a joint survey and interview with nurse mentors and service providers in the Social Welfare Development Center for the Elderly Home Khae.

5. Results and discussion

Barriers that result in inconvenience and delay in providing and receiving services today can be split into two major areas: the problems and difficulties of the route within the area of the building

5.1 Obstacles caused by road traffic.

The main obstacle occurs within the area. To modify applications that change based on actual usage in the area. The use of the building, according to the circumstances of each activity such as the increase of residential buildings for disabled children. It was found that there were more people with disabilities. Issue of a thoroughfare for vehicles and pedestrians occur immediately. The main route for commuters currently used mainly by vehicles. Nature trails current bridge is narrow and steep. As a result, access to the orphanage for disabled children in Pak Kret. Nonthaburi Continued inconvenience The road project is currently not linked to each other where possible. Make car travel Not accessible in all areas within the project. The land is still at large. Roads within the current size of just 4 meters, resulting in the car can run only one lane. It is impossible to access the building thoroughly. If there is an emergency such as a fire.

The pedestrian route traffic within the area is currently not designed to respond to the truly active, such as the link between building design is not contiguous. Both those with disabilities and service providers need to walk down the street with the car at some point. Although the wide walkways are wide enough for the use of disabled people, especially disabled people who use wheelchairs. The areas covered. Nevertheless, the slope of the ramp does not meet the standards for the use of disabled people in general. Some of the slopes are steep, the proportion of 1: 6, which is steeper than that disabled people are able to move up and down by themselves. Many point to rail Route traffic across multiple paths to serve as general entrance buildings 1 and 2 will be used together with the transfer of food. Or disabled access to buildings, hospitals need to walk down the road to get access to services and so on.

5.2 The tower is not appropriate

Located in the main building, in the improper access by Center staff and kitchen staff as the food can lead to the building. Building nurse disabled should be easily accessible and safe, but it is far greater than the disabled, remember to arrive in time. If there is an emergency Access the ambulance was not very convenient.

Based on the information received and state issues. The researchers found that the primary focus of the Center for Social Welfare Development elderly home Khae. Nonthaburi problem is the placement of buildings and road projects. This issue affects the providers and recipients of services within the area. The research focus is on the design of the project is to improve the layout. To determine the distance between buildings. Should put an end to the new building. By requiring them to be in the proper position. Taking into account the needs of each service provider and the recipient. Routing and roaming through the various trails in the area. Traffic routes should have continuous access to the center of Ban Bang Khae Social Welfare Development elderly friendly. Not interfere with each other and are safe if there is an emergency.

6. Conclusions

Data from the surveys and interviews, physical involvement with the informant. The research data were analyzed and the draft Plan. The placement of the building System traffic Design environment for the handicapped and disabled. The design approach can be summarized as follows environments.

1) The position of the hospital building - physiotherapy. Buildings and facilities such as the kitchen, office buildings and activities. Should be located at the center of a building project by the recipient of the service is enclosed to the easy access of the client and increase the speed of service and the assistance of a service provider.

2) Route traffic within the Social Welfare Development elderly home Khae. Must be linked together and can shorten the travel time from the overall layout of the original. Either in the form of vehicular traffic and on foot. The width of the thoroughfare for vehicles to facilitate access for large vehicles. For quick service and assistance at the time.

The Rail Master Plan and design a new layout. Representing all sectors of the Elderly Welfare Development Center. Pathum Thani has offered a detailed layout of a building using segmented by type of building applications. To make it easier to understand Connect and respond to the needs of service providers, all within easy reach. Can be divided into zones as follows.

1) The building's accessibility. Which classify disabilities are 2 types of people with disabilities and disability. To facilitate the allocation is appropriate to the nature of their disability. Each building will be classified according to specific characteristics such as disability disabled handicapped seat next to the bed with a wheelchair.

2) Office building, the staff work department. Both the Administration Procurement and Maintenance. In addition, the area is welcome to visit the project.

3) Building nursing and physiotherapy. Placement near a residential building of the handicapped and disabled. Also connected to each other.

4) The kitchen and dining area. Placed in the area to cater for services in various parts of the hotel.

5) Auditorium and recreational buildings, the area in front of the project It can accommodate a group of visitors in a group then. It is also an area for the joint activities of the disabled and those who visit them.

6) The shelter's staff and the service provider are separated from the service. To ensure the privacy of its duties.

7) Size is 2 lane roads surrounding the project. To facilitate and improve the trails for the project to be constantly connected.

The detailed requirements for each of the sub-group of buildings. Need to be designed to contribute more. Since each building is a form of active and functional requirements specific to different away. However, a draft of the Master Plan for the Welfare Development Center for the Elderly. Pathum Thani Is the starting point for the development of the quality of life of those involved in the area. This will lead to the development of future projects.

7. Acknowledgment

Thanks to the staff as well as clients in Welfare Development Center for the Elderly. Pathumthani everyone for the friendly, informative and educational activities to help guide this review article, which allows participation occurs in almost all stages of education and encourage studies of this success well.

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The design space for recreational activities for childen with disabilities. Case studie : The environment of the orphanage for disabled children in Pak Kret. Nonthaburi

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Keywords: Design environment, Baby aphasia, Disabled children, foster children Pak.

1. Introduction

With an average rate increase of 1 million people per year, most disabled people with health problems and a higher risk of health problems than the general population. A key element of the problem is that people with disabilities have limited health care. Including the ability to take care of themselves than people who have normal body. There are certain types of people with disabilities who have the disorder than the general disability. Need to be taken care of than normal. The cause and the problem. The government has set up an agency or shelter. Care to assist those with disabilities. Agencies are spread out geographically in order to assist and care for the disabled, these thoroughly. To be able to care for and help themselves. Improves quality of life and care as a fundamental right which should be given.

Children with disabilities Pak Jobs are available and help a long time and have children with disabilities. Use a lot of the current state of disrepair The area should be designed and updated as appropriate. This will allow service providers. Disabled children have a better life. Education about the need for space applications is an important issue. Spatial needs to be aligned to real world applications. This will entail improving the quality of life in the orphanage for disabled children in Pak Kret. Nonthaburi

In this study, Oriented education to fix problems in the design environment to respond to applications for children with disabilities. Children with disabilities in Pak Nonthaburi researchers used data collection methods used by the target group. Focus on the involvement of people living in the area. By the authorities and local residents to participate in all activities in the research.

2. Literature References

Researchers have studied the concepts and theories related to research and research methodologies. The story is divided into four definitions of disability. The type of disability and welfare. The participatory design and design institutions and design standards thoroughfare for people with disabilities.

2.1 Definition of disability

Patip Assawaphom (2014) Said that disabled people are vital to the nation in many ways, but the well-being of people with disabilities are often socially omissions. Whether the environment is no easy life. The attitude of the society towards the disabled. Understanding about disability is essential. The society recognizes the importance of the matter. This will lead to the help and support so that people with disabilities can live like normal people.

Act for Empowerment of Persons with Disabilities 2550 under Article 4 provides that the definition of disability. "People who have limitations in performing everyday activities. Or participated in social activities. Due to impaired sight, hearing, mobility, communication, behavioral, mental, emotional intelligence, learning disabilities, or any other. The barriers in areas with special needs to receive help one area to be able to practice. In everyday life or social involvement with guests ".

From the above definition, the medical definition of disability in terms of looking at the problems and difficulties of the life of ordinary people. The attitudes about people with disabilities as people who have been helping care. But the definition of disability in the social dimension. That disabled people have the same rights to everyone. It is the duty of society to encourage people with disabilities to participate in these activities as individuals.

2.2 The type of disability and welfare

Currently, there are many types of disabilities. Each type of defect are different away. The aid scheme is different. Sorting disabled by the Ministry of Social Development and Human Security disability guidelines on the type and No. 2, 2555 were categorized into 7 types of disabilities, including the visually impaired. Hearing disability or meaningful. Disability or physical movement. Disability, mental disability or behavioral intelligence. Learning Disabilities Disabilities and autism. (Ministry of Social Development and Human Security, 2013).

Children with disabilities Pak Nonthaburi set up with the purpose of restoring health. Mental health and rights of children with disabilities. Neglected and abandoned by their families and society. The optimum age for dependents aged 60 years and over who have contagious diseases. And people with physical disabilities or brain and can help them in their daily lives. Homeless can not live with the family, the orphanage will assist with the development and rehabilitation in areas they can help themselves. The purpose of this educational institution. Is another way to help designers understand the special nature and needs of different people. This will be used to guide the design. Improve the environment as well as allowing service providers to prioritize applications, which can increase the performance and speed of access to the service appropriately fast.

2.3 Design involved

Design participatory process that encourages stakeholders to exchange ideas. Offer solutions and create an understanding of the design work together. This will lead to the creation of partnerships for all parties. The decision to participate in the design process Information must come from those who actually use the building. This will create a sense of ownership. It also provides an opportunity to use the space to display their own needs. This approach brings to the design and to solve problems which come from the real needs are.

2.4 Designed to foster and design standards for the disabled

Children with disabilities Pak Nonthaburi important element to take into account the 4: 1) the composition of the primary users and secondary users such as service providers and service recipients, 2) component activities include the type of activity. Patterns of user behavior and characteristics in building support for activities 3) the location and the surrounding environment. Access to the building the social, cultural, and 4) economic factors include the budget to invest in building new or remodeling. Sources of funding the design may be more specific to different environmental conditions conducive to use. And facilities

Guide "recommendations designed facilities for all" has been identified as a thoroughfare that allows disabled people who can use it easily. To focus on two issues, namely the width of the path and the slope of the thoroughfare. The proper width will range between 0.90 to 1.50 meters and a slope of at least 1:12 if the ramp is longer than 2.50 meters must have handrails on both sides. If the length of the ramp-up period of 6.00 meters to a landing ramp has a width of 1.50 m, the surface of the ramp must not slip material is suitable for use. (Association of Siamese Architects under Royal Patronage, 2015).

3. Method

In the process of data collection, The researchers collected survey data on the physical side of the foster children with disabilities Pak. Nonthaburi using the interview as the primary means of storing data with the stored image and to create an environment within the actual project. The research was conducted into space three times. The physical storage conditions and issues twice. Co-designed with participation once again.

The first survey on December 9th, 2017, aims to explore the place and met. Children with disabilities Pak Nonthaburi accommodation Executive agencies Procurement Officer Nurses Mentors, as well as provide more frequent. To get information about the place and the overall issues in general. The information is used to guide the research and plans are preliminary. On January 4, 2019 that the researcher has studied the area again to collect insights. By means of interviews and participatory observation in detail. To understand the current conditions. Joint analysis of the problems and the needs of the staff interviewed in all sectors. The data obtained were analyzed for use in the design environment for children with disabilities. In the preliminary stage, to be used in preparing the detailed design. Plan to join the staff of the orphanage for disabled children in Pak Kret. In the final stage

The sample consisted of interviews, the researchers selected a group of nurses, nurse aides, office staff and maintenance technicians within the orphanage for disabled children in Pak Kret. Nonthaburi because groups to take place in practice. Information can be clearly Unlike patients who are handicapped and disabled. These groups, which are limited in their ability to provide information on the spatial applications. The researchers relied on data from the first sample-based.

On the 3rd day of March 12, 2018 were prepared and used in the design of the important issues that are involved. Using the information from the interview. Observations used as a guide to create the drawing. Environment Design of workhouse children Pak. Nonthaburi and take such an approach to be presented with the information from the various departments. The audience follows: Executive Agency Number 1 Provider Group of 12 and Group of the clients or those with disabilities who can

communicate for 3 people, all participants can provide feedback and share ideas and make suggestions. To be used in the design environment. The layout of the building The new system, roaming the area with investigators. To make sure that all the parties. The draft development plan for the project can be used to cause a real interest in the work. It also can be used as documentation for presentation development budget orphanage for disabled children in Pak Kret. Nonthaburi The executive decision to go.

4. Case study

Children with disabilities Pak Nonthaburi With a total area of 12 acres with buildings for a total of 11 buildings divided into five buildings, service buildings and other buildings with a total of 6 buildings, one-storey building. All the information in Dalmatia Derived from a joint survey and interview with nurse mentors and service providers in the orphanage for disabled children in Pak Kret. Nonthaburi

5. Results and discussion

Barriers that result in inconvenience and delay in providing and receiving services today can be split into two major areas: the problems and difficulties of the route within the area of the building.

5.1 Obstacles caused by road traffic.

The main obstacle occurs within the area. To modify applications that change based on actual usage in the area. The use of the building, according to the circumstances of each activity such as the increase of residential buildings for disabled children. It was found that there were more people with disabilities. Issue of a thoroughfare for vehicles and pedestrians occur immediately. The main route for commuters currently used mainly by vehicles. Nature trails current bridge is narrow and steep. As a result, access to the orphanage for disabled children in Pak Kret. Nonthaburi Continued inconvenience The road project is currently not linked to each other where possible. Make car travel Not accessible in all areas within the project. The land is still at large. Roads within the current size of just 4 meters, resulting in the car can run only one lane. It is impossible to access the building thoroughly. If there is an emergency such as a fire.

The pedestrian route traffic within the area is currently not designed to respond to the truly active, such as the link between building design is not contiguous. Both those with disabilities and service providers need to walk down the street with the car at some point. Although the wide walkways are wide enough for the use of disabled people, especially disabled people who use wheelchairs. The areas covered. Nevertheless, the slope of the ramp does not meet the standards for the use of disabled people in general. Some of the slopes are steep, the proportion of 1: 6, which is steeper than that disabled people are able to move up and down by themselves. Many point to rail Route traffic across multiple paths to serve as general entrance buildings 1 and 2 will be used together with the transfer of food. Or disabled access to buildings, hospitals need to walk down the road to get access to services and so on.

5.2 The tower is not appropriate

Located in the main building, in the improper access by Center staff and kitchen staff as the food can lead to the building. Building nurse disabled should be easily accessible and safe, but it is far greater than the disabled, remember to arrive in time. If there is an emergency Access the ambulance was not very convenient.

Based on the information received and state issues. The researchers found that the primary focus of the Center for Social Welfare Development elderly home Khae. Nonthaburi problem is the placement of buildings and road projects. This issue affects the providers and recipients of services within the area. The research focus is on the design of the project is to improve the layout. To determine the distance between buildings. Should put an end to the new building. By requiring them to be in the proper position. Taking into account the needs of each service provider and the recipient. Routing and roaming through the various trails in the area. Traffic routes should have continuous access to the center of Ban Bang Khae Social Welfare Development elderly friendly. Not interfere with each other and are safe if there is an emergency.

6. Conclusions

Data from the surveys and interviews, physical involvement with the informant. The research data were analyzed and the draft Plan. The placement of the building System traffic Design environment for the handicapped and disabled. The design approach can be summarized as follows environments.

1) The position of the hospital building - physiotherapy. Buildings and facilities such as the kitchen, office buildings and activities.

2) Route traffic within the Social Welfare Development elderly home Khae. Must be linked together and can shorten the travel time from the overall layout of the original. Either in the form of vehicular traffic and on foot. The width of the thoroughfare for vehicles to facilitate access for large vehicles. For quick service and assistance at the time.

The Rail Master Plan and design a new layout. Representing all sectors of the orphanage for disabled children in Pak Kret. Nonthaburi It offers a detailed layout of a building using segmented by type of building applications. To make it easier to understand Connect and respond to the needs of service providers, all within easy reach. Can be divided into zones as follows.

1) The building's accessibility. Which classify disabilities are 2 types of people with disabilities and disability. To facilitate the allocation is appropriate to the nature of their disability. Each building will be classified according to specific characteristics such as disability disabled handicapped seat next to the bed with a wheelchair.

2) Office building The staff work department. Both the Administration Procurement and Maintenance. In addition, the area is welcome to visit the project.

3) Building nursing and physiotherapy. Placement near a residential building of the handicapped and disabled. Also connected to each other.

4) The kitchen and dining area. Placed in the area to cater for services in various parts of the hotel.

5) Auditorium and recreational buildings, the area in front of the project It can accommodate a group of visitors in a group then. It is also an area for the joint activities of the disabled and those who visit them.

6) The shelter's staff and the service provider are separated from the service. To ensure the privacy of its duties.

7) Size is 2 lane roads surrounding the project to facilitate and improve the trails for the project to be constantly connected.

The detailed requirements for each of the sub-group of buildings. Need to be designed to contribute more. Since each building is a form of active and functional requirements specific to different away. However, a draft of the Master Plan for Children with Disabilities Pak. Nonthaburi Is the starting point for the development of the quality of life of those involved in the area. This will lead to the development of future projects.

7. Acknowledgment

Thanks to the staff as well as clients in the orphanage for disabled children in Pak Kret. Nonthaburi province every courtesy, informative and educational activities fot helping guide this review article, which allows participation occurs in almost all stages of education and encourage studies of this success well.

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ECONIMICS

Relationship between Media of Integrated Marketing Communications and Purchasing Decisions for Food & Beverage Products of Durian by Gender

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Keywords: Integrated Marketing Communications (IMC), Purchasing decisions, Food & beverage products, Durian

Abstract. With the goal of making the most efficient use of advertising for food and beverage products of durian, the focus of this study is to examine the relationship between media of Integrated Marketing Communications (IMC) and purchasing decisions for food and beverage products of durian by gender. The population for this study comprises consumers of durian products, who lived in Thailand in 2019. Quota sampling is used to select for a sample size of 138 subjects. The results show that most in the sample group agree on preference of purchase of durian products, in order, from all media, social media and television and radio, with newspapers and magazines appearing last. In addition, the Chi-square test indicates there is no relationship between media of IMC and purchasing decisions for food and beverage (F&B) products of durian by gender, hence there is no need for different advertising policy for this factor.

1. Introduction

Durian is regarded in Southeast Asia as “The King of Fruits”, and it is known for its thorn covering, unique taste and distinctive aroma. Global trade in durian is dominated by two countries: Thailand dominates exports and China dominates imports. In 2016, the two largest exporters of durian were Thailand and Malaysia with 403 million kilograms and 18 million kilograms respectively, with these two countries accounting for 99% of global exports [1]. Durian products typically can be divided into four key market segments - raw durian fruit, desserts, snacks and beverages [2]. Demand for durian has been rising due to Chinese consumers in terms of the fresh fruit and from food and beverage (F&B) companies [3]. In addition, major global corporations such as Nestle, F&N, McDonald's and Pizza Hut have all released durian-based products in an effort to capture this growing market [2]. As a result, the demand for durian has been growing enormously with new durian-based products continuously being developed.

The data from the Commerce Ministry show that each year Thailand rakes in serious income from durian exports. For example, from January to November in 2017, durian accounted for US$616 million, or 26.4%, of the country's $2.32 billion worth of fruit and vegetable exports [4]. Despite Thai

durian being exported to several markets, before rising consumption by China from 2012 to 2016, there was an oversupply which caused a fall in price [5]. To avoid this problem in the future, fresh durian can be processed to become one kilogram of the value-added product, which means producers can generate a net margin on processed durian products. It is very interesting concerning how to promote food and beverage products, when fresh durian is very famous. This paper investigates the relationship between media of Integrated Marketing Communications (IMC) and purchasing decisions for food and beverage (F&B) products of durian by gender.

2. Methods and Procedures

- Conceptual Framework

There are many options in choosing advertising that will spread the word about products and services, attract customers and generate sales. The most suitable advertising option for business depends on knowing the target audience and selecting the relevant media source. Currently, Integrated Marketing Communication (IMC) provides a new concept in marketing communication involving not only advertising but also other marketing tools such as events, public relations, sales promotion, direct marketing, data base, and research service in order to communicate a marketing message from company to target market [6]. IMC represents integration in terms of media, incorporating performance through a mix of traditional media [7] and a mix of communications initiated both by consumers and organizations [8]. Our study focuses on the main media of IMC including social media, television (TV) and radio, newspaper and magazine and all media.

However, many studies indicate that there is difference between males and females in purchasing decisions. This is because males and females, due to their different upbringing and socialization along with various other social, biological and psychological factors depict different types of behaviour in various situations [9]. With a view to extracting the maximum benefit from advertising, this study focuses on exploring the connection between influence of IMC through various types of media advertising and purchasing decisions for F&B products of durian by gender (see Fig. 1).

Fig.1 Conceptual Framework

- Data

In our study, questionnaires provide a quantitative method of data gathering. The population is Thai consumers of F&B products of durian in 2019, and quota sampling is used to select for a sample size of 138 subjects. Our survey consists of multiple choice questions and one comment section for subjects to complete.

3. Empirical Results

In relation to the questionnaires, 138 were completed by 72 males and 66 females. Results show that most in the sample group believe in social media more than TV and radio, and newspapers and magazines. However, IMC, with all media, has a strongest influence on purchasing decisions for F&B products of durian for both males and females (see Table 1).

Table 1 Test of Significant Relationship between Media of Influence of Integrated Marketing Communications and Purchasing Decisions for Food & Beverage Products of Durian by Gender

Media	Purchasing Decision by Gender			Total	X^2 value	X^2 prob
		Male	Female			
Social Media	count	18	16	34	5.778	.123
	% within gender	25.4%	23.9%	24.6%		
	% within media	52.9%	47.1%	100.0%		
TV + Radio	count	13	16	29		
	% within gender	18.3%	23.9%	21.0%		
	% within media	44.8%	55.2%	100.0%		
Newspaper + Magazine	count	2	8	10		
	% within gender	2.8%	11.9%	7.2%		
	% within media	20.0%	80.0%	100.0%		
All Media	count	38	27	65		
	% within gender	53.5%	40.3%	47.1%		
	% within media	58.5%	41.5%	100.0%		
Total	count	71	65	138		
	% within gender	100.0%	100.0%	100.0%		
	% within media	51.4%	48.6%	100.0%		

- Hypothesis

H_O: There is no relationship between media of IMC and purchasing decisions for food and beverage products of durian by gender

H_1: There is a relationship between these variables.

Table 1 shows the chi-square p-value (0.12) is higher than our α value (0.05). Therefore, H_O is not rejected indicating that there is no relationship between media of IMC and purchasing decisions for food and beverage products of durian by gender.

4. Discussion

The results show that all media in descending order of popularity are the most effective in convincing clients to buy F&B products of durian, whereas newspapers and magazines are not so highly rated by the sample group. Therefore, producers and media agencies should give consideration to restricting the advertising of F&B products of durian to IMC for all outlets, especially for social media, TV and radio. This is because of their ability to reach large audiences, and their communication advantage through text, image, sound and movement. In addition, paid social media advertising is a cost-effective way to promote business, especially small business. Finally, there is no difference

between males and females in making purchasing decisions for food and beverage products from media advertising, hence there is no need for different advertising policies for gender.

5. Conclusion

This study explores the relationship between media of IMC (social media, TV and radio, newspapers and magazines and all media) and purchasing decisions for F&B products of durian by gender through a sample group of 138 Thai subjects in 2019. The results show a preference, in order, for purchasing Food and Beverage products of durian from social media, TV and radio rather than from newspapers and magazines. However, IMC, with all media, has a strongest influence on purchasing decisions for F&B products of durian. In addition, we found that there is no relationship between media of IMC and purchasing decisions for food and beverage products of durian by gender, so there is no need for different advertising policies in this regard. In addition, we found that all media in descending order of popularity are the most effective in convincing Thai consumers to purchase food and beverage products of durian.

6. Acknowledgements

The authors are grateful to Rajamangala University of Technology Phra Nakhon (RMUTP). In addition, the authors would like to thank Mr. Robin Neill for comments and suggestions.

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The Future of Thailand Income Distribution

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Abstract. The Thai government has an important role in improving distribution of income and wealth, as the relationship between income disparity and economic growth has been a critical issue in economics over several decades. The purpose of this paper involves explaining and forecasting the value of the Thailand Gini index, which is a measure of statistical dispersion intended to represent distribution of national income, and is the most commonly used measurement of inequality. This study employs mixed methods research that includes documentary research and time series analysis on Thailand's Gini index. The result indicates that the Gini index will slightly decrease from 2016 to 2020. However, policy makers should still redistribute income from those with more income to those with less to improve income distribution, which could challenge Thailand's public policies.

1. Introduction

National income distribution is how a nation's total Gross Domestic Product (GDP) is distributed amongst its population, and it has always been a central concern for economic policy makers. Ostry and Berg proposed that inequality in economic growth is like "two sides of the same coin". They believed that the impact of unequal income distribution is divided into two sides. Inequality has influence on growth negatively and becomes a problem for society. For example, it can obstruct human capital accumulation, especially in education [1]. In addition, it was noted that income disparity can also lead to political and economic instability, resulting in corruption, resource misallocation, and nepotism [2]. On the other hand, inequality can also improve the economy since it provides incentives for people to invest and save for their future livelihood, and also for entrepreneurs to run their businesses successfully [3].

The relationship between unequal income distribution and economic growth has been an analytical issue in macroeconomics for all countries. Thailand is one of the developing countries that requires economic growth to improve its standard of living, to reduce poverty, as well as to survive in the modern economy [4]. The World Bank reported that Thailand had made remarkable progress in social and economic development over the last four decades. The country moved from a low-income country to an upper-income country in less than a generation. In addition, Thailand's Gini coefficient, which is a measured for income inequality, declined less so in recent years. However, although regional income grew in the Central, North and South regions, it shrank in the Northeast region and Bangkok [5]. For effectively targeted policies, governments need the tools to fully understand country conditions and the expectations of their citizens. The aim of this paper is to forecast income inequality in Thailand between 2016 and 2020, which could challenge public policies.

2. Methodology

The Gini coefficient or Gini index is a statistical tool of income distribution developed by the Italian statistician, Corrado Gini, in 1912. It is often used as an indicator of economic inequality, measuring income distribution or, less commonly, wealth distribution among a population [6]. This study employs mixed methods research that includes documentary research and time series analysis on yearly data of Thailand's Gini index. There are four components of this analysis - trend, cyclical variation, seasonal variation, and irregular variation [7]. Firstly, secular trend (Tt) is the long-term trend of Thailand clothing export in ASEAN. Secondly, cyclical variation (Ct) is a typical business cycle consisting of a period of prosperity followed by periods of recession, depression, and then recovery with no fixed duration of the cycle. As a result, there are fluctuations unfolding over more than one period in time above and below the secular trend. Thirdly, seasonal variation (St) is series fluctuating with the seasons. Finally, irregular variation (It) cannot be calculated, because it is unpredictable and cannot be projected into the future.

The equation of Thailand Gini index can be written as:

$$Gini = T_t * C_t * S_t$$

The linear equation to describe growth in the Thailand Gini index is:

$$T = a + bt$$

where:

Gini'	is the projected values for a selected value of *t*.
a	is the Y-intercept. It is the estimated value of net *Gini'* when $t = 0$.
b	is the slope of the line, or the average change in *Gini'* for each change of one unit in *t*.
t	is any value of time that is selected. The unit of time reported in this paper is monthly.

The Thailand Gini index every two years from 1988 to 2015 is provided by The World Bank. In this time series analysis, we estimate the data of the Thailand Gini index from 2016 to 2020.

3. Results

Documentary research indicates that Thailand has experienced rapid growth and a structural transformation since the 1950s. However the country has also experienced growth without equity. Thailand's Gini index has been above 0.40 for the last 30 years, which is high when compared with other ASEAN countries [8]. Moreover, the debate on income and wealth inequality has been heating up following the Credit Suisse Global Wealth Report of 2018. The report showed that Thailand is the least equitable country in the world, because the richest 1 per cent hold 66.9 per cent of the country's total wealth [9]. However, The World Bank confirmed that, inequality of income has been declining in Thailand. Even though the Gini index is still high, the poor have been gaining at a faster rate than the rich, and this is the most striking feature of economic and social development in Thailand today [10].

For time series analysis, the model summary of linear trend, is given by the equation

$$T = a + bt$$

To obtain the coordinates of the point on the line for the Thailand Gini index from 2016 to 2020, insert the t value in the equation. Then, we have

$$T_t = 45.895 - 0.458(t).$$

The results are presented for every two years as shown in Table 1. The values of the Thailand Gini index from 1988 to 2015 are used to forecast values for 2016 to 2020. It can be seen that Thai garment exports will continue to decrease slightly (see Table 1).

Table 1 The Thailand Gini index from 1988 to 2015 and the forecast values 2016 to 2020.

			Y_t		baseline	Y_t / CMA		Y_t / S_t		
t	Year		GINI index	MA	CMA	S_t, I_t	S_t	Deseasonalize	T_t	forcast
1	1988	1	43.80				1.00	43.67	45.44	45.57
2	1990	2	45.30				1.00	45.38	44.98	44.90
3	1992	3	47.90	45.13	45.01	1.06	1.02	46.73	44.52	45.63
4	1994	4	43.50	44.90	44.43	0.98	0.98	44.24	44.06	43.32
5	1996	1	42.90	43.95	43.35	0.99	1.00	42.77	43.61	43.74
6	1998	2	41.50	42.75	42.66	0.97	1.00	41.57	43.15	43.07
7	1999	3	43.10	42.58	42.45	1.02	1.02	42.05	42.69	43.76
8	2000	4	42.80	42.33	42.45	1.01	0.98	43.53	42.23	41.52
9	2002	1	41.90	42.58	42.41	0.99	1.00	41.78	41.77	41.90
10	2004	2	42.50	42.25	41.88	1.01	1.00	42.58	41.32	41.24
11	2006	3	41.80	41.50	41.30	1.01	1.02	40.78	40.86	41.88
12	2007	4	39.80	41.10	40.74	0.98	0.98	40.48	40.40	39.72
13	2008	1	40.30	40.38	40.08	1.01	1.00	40.18	39.94	40.06
14	2009	2	39.60	39.78	39.49	1.00	1.00	39.67	39.49	39.42
15	2010	3	39.40	39.20	39.08	1.01	1.02	38.44	39.03	40.00
16	2011	4	37.50	38.95	38.73	0.97	0.98	38.14	38.57	37.92
17	2012	1	39.30	38.50	38.20	1.03	1.00	39.18	38.11	38.23
18	2013	2	37.80	37.90	37.71	1.00	1.00	37.87	37.65	37.59
19	2014	3	37.00	37.53			1.02	36.10	37.20	38.13
20	2015	4	36.00				0.98	36.62	36.74	36.12
21	2016	1					1.00		36.28	36.39
22	2017	2					1.00		35.82	35.76
23	2018	3					1.02		35.37	36.25
24	2019	4					0.98		34.91	34.32
25	2020	5					1.00		34.45	34.55

4. Discussion

From Fig. 1, the forecasting results every two years of time series analysis for the period 1988 to 2015 shows that the Thailand Gini index will decrease significantly from 2016 to 2020. Even though this is a good sign for Thailand's economy, in order to develop the country, policy makers should increase in governmental aid to poor people have been concentrated in nonmoney benefits such as education, medical and food. Second, an increasing proportion of wage-earners' total compensation goes to health insurance and pension benefits. Third, taxes rich people modify the income distribution.

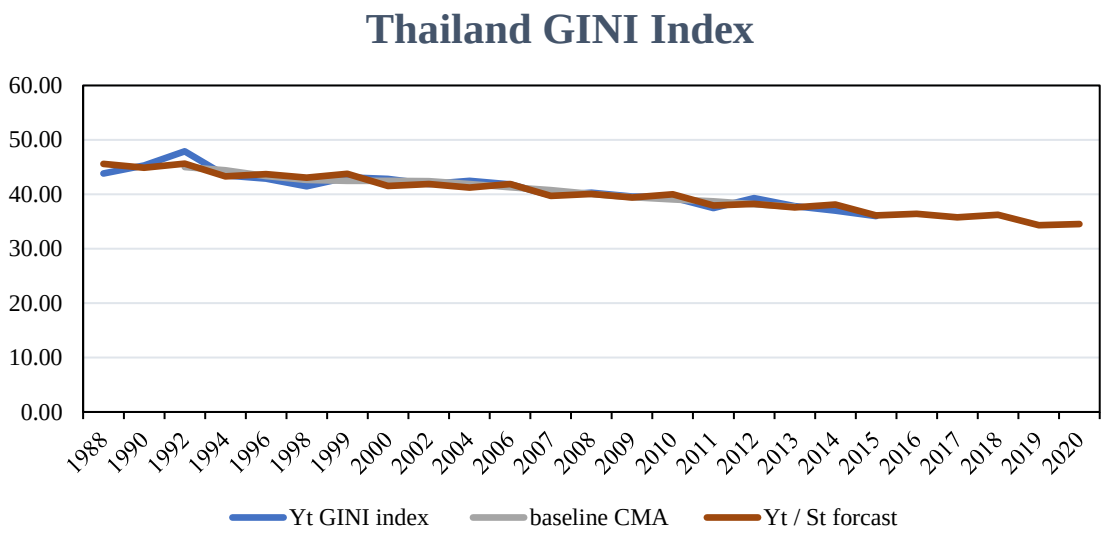

Fig. 1 The Thailand Gini index from 1988 to 2015 and the forecast values 2016 to 2020.

5. Conclusion

The purpose of this paper involves explaining and forecasting the Thailand Gini index using a mixed-method research approach. From documentary research, the Credit Suisse Global Wealth Report of 2018 mentions that Thailand is the world's least equitable country in this regard. However, The World Bank confirmed that inequality of income has been declining in Thailand. Even though the Gini index is still high, the poor have been gaining at a faster rate than the rich, and this is the most striking feature of economic and social development in Thailand. Even so, the forecasting result of time series analysis for the period 1988 to 2015 indicates that the Thailand Gini index will experience significant decrease from 2016 to 2020. Although this is a positive sign for Thailand's economy, the government's agenda needs to focus on improving income distribution within the country.

6. Acknowledgements

The authors are grateful to Rajamangala University of Technology Phra Nakhon (RMUTP). In addition, the authors would like to thank Mr. Robin Neill for comments and suggestions.

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