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Contributed Paper

Effect of Microbial Consortia on Photosynthetic Efficiency of *Arabidopsis thaliana* under Drought Stress

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ABSTRACT

Photosynthetic efficiency of *Arabidopsis thaliana* plants has been investigated under drought stress conditions by several studies, but not in response to effective microorganisms (EM) application. Thus, our study aims to examine the effect of EM on growth and photosynthetic efficiency of *Arabidopsis thaliana* under water stress condition. Arabidopsis plants were treated with 3 EM concentrations (1, 5 and 10% v/v) on the same day of drought stress application. Another 2 experimental variants/combinations were also provided: control treatment (watered plants and without EM) and drought treatment (non-watered plants and without EM). After 2 weeks of drought application, photosynthetic efficiency, fresh and dry weight of *Arabidopsis* plants were measured and compared. Based on the data obtained from chlorophyll fluorescence measurements, the results showed negative effect of water stress on photosynthesis efficiency of *Arabidopsis* plants. This was confirmed by a notable decrease of fresh and dry mass of *Arabidopsis* plants exposed to drought. EM application enhanced plants tolerance to drought. That was proved by the increase of chlorophyll content and photosynthetic efficiency. The highest increase of chlorophyll fluorescence parameters was observed in stressed plants treated with EM concentration 1% v/v. Our research indicated that, EM treatment caused a stimulation of photosynthetic efficiency

of *Arabidopsis* plant due to enhancement of energy transfer efficiency within photosystem II (PSII) under drought stress.

Keywords: bentonite, thermal and acid activation (TAA), methylene blue, kinetics, thermodynamic

1. INTRODUCTION

Growth and development of plants depend on environmental conditions. Drought stress is one of the major factors that affect crop production. To survive under drought stress, plants have evolved morphological, physiological, and biochemical responses. Photosynthesis and cell growth are the primary processes affected by stress [1]. Drought stress effects on photosynthetic apparatus are well known. They typically start with mostly stomatal effects at moderate drought intensity, and culminate in metabolic and structural changes caused by severe or long-lasting drought stress [2]. Chlorophyll fluorescence measurements reformulate protection of photosystem II (PSII) and Photosystem I (PSI) photochemistry under drought conditions by adjusting the energy distribution between those photosystems and by activating alternative electron sinks [3].

The Photosynthetic machinery of plants is very sensitive to changes in their overall state [4]. Changes in the environmental conditions trigger stress reactions and different adaptive mechanisms in the plants organism [5]. Evaluation of the photosynthetic process can give information about the presence of stress factors, the plants response to them, their tolerance and protective mechanisms, about their vitality and productivity during different conditions [6]. One of the most perspective approaches to investigate of photosynthetic efficiency is measuring of the chlorophyll *a* fluorescence emitted by plants when illuminated with actinic (photosynthetic active) light [7]. Chlorophyll fluorescence (ChlF) measurements have recently become a widely adopted to evaluate the impact of stress factors on photosynthesis [8]. They represent a simple, non-destructive, inexpensive and rapid tool for analyzing light-dependent photosynthetic reactions and for indirectly estimating chlorophyll content within the same sample tissue [9].

Many different microbial bio-fertilizers are available in the market for agricultural use. The products claim to enhance plant growth and yields and to improve soil fertility [10]. One bio-fertilizer that has received a lot of attention is the so called microbial inoculum 'Effective Microorganisms' (EM) which was developed by Prof. Teruo Higa at the University of Ryukyus, Okinawa, Japan [11]. The improvement of crops productivity using EM has been reported. The studies were conducted under normal cultivation condition in many crops around the world, for example, cotton [12], maize [13,14] paddy rice [15] and tomato [16].

However, the effect of the application of EM on plants under unfavorable condition is not well-studied yet, thus this study was conducted. *Arabidopsis thaliana*, a winter annual

plant with a relatively short life cycle and a popular model organism in plant biology and genetics was used in this study. Currently, the photosynthetic efficiency of *A. thaliana* was investigated under salt stress in response to EM application [17]. The same technique will be used aiming to study the effect of the different levels of EM treatment on growth, drought tolerance ability and photosynthetic efficiency of *A. thaliana* under water stress (drought condition).

2. MATERIALS AND METHODS

2.1 Arabidopsis Plants

Arabidopsis plants were obtained by geminating seeds of *Arabidopsis thaliana* (Columbia) under dark condition, in multi-cell plastic plant trays containing soil and sand mixture (2:1 V/V) in order to provide enough space in soil for growth and respiration of roots. After germination, seedlings were relocated to phytotron and grew under the following conditions: temperature 20°C day and 15°C night; light: 16 h day (ca. 200 µmol photons m^{-2} s^{-1} light intensity); air humidity: approximate 70%. Multi-cell plastic plant trays were covered with plastic foil to maintain proper soil humidity. Three weeks old seedlings were individually transferred to new multi-cell plastic plant trays (one seedling/cell). After 1 week, seedlings were moved to 1 L plastic pots containing 1.7 kg of the above described soil, then allowed to grow 2 weeks more to ensure that the plants will mature enough to perform the measurements. Plants were watered with 100 ml of distilled water every day to maintain soil near field capacity in order to avoid any water stress.

2.2 Effective Microorganisms (EM) Preparation

EM used in this experiment was prepared by fermentation of sugarcane molasses initiated with consisting of 12 species which belong to 5 groups of microorganisms usually found in EM including: **photosynthetic bacteria** (*Rhodopseudomonas palustris*), **Lactic acid bacteria** (*Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* subsp. *Bulgaricus*, *L. fermentum*, *L. plantarum*, *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus*), **yeast** (*Saccharomyces cerevisiae*), **Actinobacteria** (*Bifidobacterium animalis*, *B. longum*) and **endospore producing bacteria** (*Bacillus subtilis*) [18].

Fermentation process was carried out in airtight container for 2 weeks until the propagation of micro-flora in EM reached stable density around 7.2-7.6 log10 CFU/ml and pH drop below 4.0, EM was then applied in drought stress experiment. 10 ml of EM solution of 1 or 5 or 10% v/v concentration (10, 50 and 100 ml EM/L water, respectively) was applied once on the same day of drought stress application. For control plants (without EM), distilled water was apply instead of EM.

2.3 Drought Application

Six week after germination, fully grown Arabidopsis plants were selected for drought stress experiment, the plants were divided in to 5 series as following: control plants (10 plants), drought plants (10 plants) and drought plants supplemented with EM 1, 5 and 10 % v/v (5 plants for each EM treatment). Drought stress experiment was conducted for 2 weeks, during this period control plants were fully watered with distilled water (sub-irrigation achieved by placing pots into flats or trays, allowing proper drainage of the soil), while drought plants and drought plants supplemented with 1, 5 and 10% EM were not watered.

2.4 Chlorophyll Content, Chlorophyll *a* Fluorescence, Fresh, Dry Weight Measurement

Chlorophyll content was estimated by Minolta SPAD 502 Meter (Spectrum Technologies, Inc., USA) and chlorophyll *a* fluorescence by FMS-2 Modulated Chlorophyll Fluorescence System (Hansatech Instruments Ltd., UK). Three measurements were done on 3 fully developed leaves of each cultivated plant. Throughout the experiment, the measurements were performed three times, once before drought stress application and twice during drought stress application (at 0, 1, and 2 week of drought stress). In this study, three fluorescence parameters included maximum fluorescence signal of light-adapted plants (*Fm*'), steady state fluorescence yield (*Fs*) and quantum efficiency of PSII *(OPSII)* were used to determine the effect of EM on drought tolerance of Arabidopsis plants. At the end of the experiment, fresh mass of plants was assessed and the plants were placed in hot air oven at 120 °C for 48 h to get dry mass values.

2.5 Statistical Analysis

The obtained data were statistical analyzed using Statistica ver. 10 Software (Statsoft, Tulsa, USA). One-way ANOVA) was used to analyze the variance associated with the treatments. Fischer's test was used to compare mean in order to determine statistically differences (p = 0.05) between each treatment.

3. RESULTS AND DISCUSSIONS

3.1 Chlorophyll Content

Comparing between control plants and plants under drought stress condition, the result indicated that drought does not affect chlorophyll content at early period of drought stress application as can be seen in Figure 1 that after a week under drought stress, chlorophyll content of plants in both conditions are still increasing as plants are growing. However, a considerable reduction of chlorophyll content of stress plants around 70% comparing to control was observed after 2 weeks of drought application. The reduction of chlorophyll content of drought stress plants was detected in all treatments especially after 2 weeks under drought condition. In general, EM application alleviated the drought effects under both drought stress and well-watering conditions. The beneficial effect of EM was more evident 2 weeks after drought application. Chlorophyll content of plants treated with different concentration of EM was showed in Figure 2, demonstrated the remarkable positive effect on plants treated with EM 1% v/v by increasing its chlorophyll content by 90% comparing with drought stress plant without EM supplement. The application of EM 5 and 10 % v/v also gave the positive effect on drought stress plant but there are no significantly different between these 2 treatments.

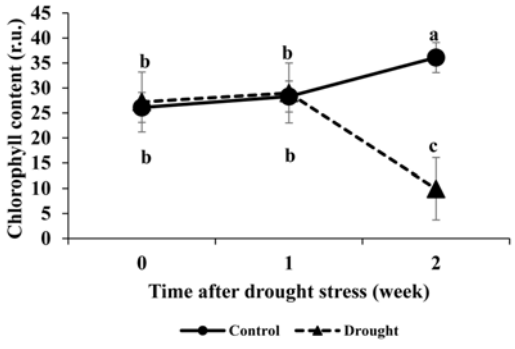

Figure 1. Change of chlorophyll content of *A. thaliana* under well-watering (●) and drought stress conditions (▲).

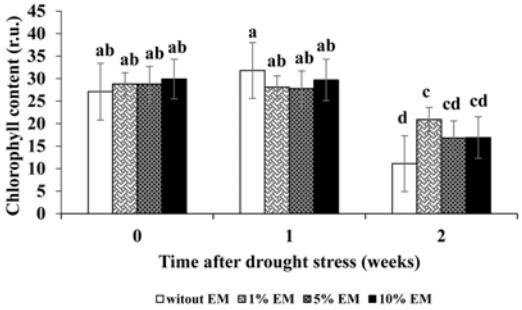

Figure 2. Change of chlorophyll content of *A. thaliana* under drought stress conditions in response to different levels of EM application.

Estimation of photosynthetic efficiency of plants by measuring chlorophyll content and chlorophyll fluorescence parameters, can explore the influence of the environmental stress on growth and yield, since these traits were closely correlated with the rate of carbon exchange [19-20]. Our results confirmed the negative effects of drought stress on chlorophyll content and chlorophyll fluorescence, only after two weeks of its application.

Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate [21]. Chlorophyll content of Arabidopsis plants showed similar changes to actual photosynthetic efficiency measured by chlorophyll fluorescence parameters when drought stress was applied (Figure 1). The reduction of chlorophyll content under stress conditions could be additional reason for the drop of photosynthetic efficiency of Arabidopsis plants. Lower chlorophyll content could provide to create smaller energy antenna which absorb light energy or reduction of its numbers. Moreover, less chlorophyll content means reduction in reaction centers (RC) number (dimmer of chlorophyll *a*) which are playing main role in light energy transfer.

3.2 Chlorophyll *a* Fluorescence

Steady state fluorescence yield (F_s) showed in Figure 3 and 4. An overall trend of F_s value of stress plants is decreasing as drought application continued. The reduction of F_s value of drought stress plants around 14% compared to control plants was observed at 2 weeks after the stress condition.

The results indicated no significantly different between F_s value of drought stress plants without and with complement of EM at different concentration at the early period of stress. After 2 weeks under drought stress condition, drastically increasing of F_s of stressed plants was recorded in stressed plants treated with EM 5% v/v (Figure 4).

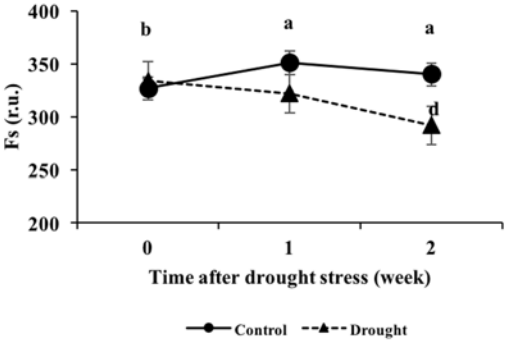

Figure 3. Change of steady state fluorescence yield (F_s) of *A. thaliana* under well-watering conditions (●) and drought stress conditions (▲).

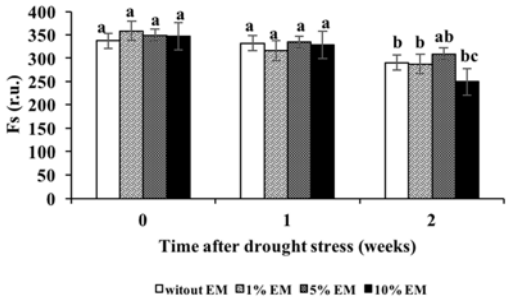

Figure 4. Change of steady state fluorescence yield (F_s) of *A. thaliana* under drought stress conditions in response to different levels of EM application.

The results of the maximum fluorescence signal of light-adapted plants (F_m') showed similar trend as chlorophyll content. F_m' value of control plants increased with time and F_m' of drought stress plant considerably decreased (about 55%) at 2 weeks after drought application (Figure 5). There is no significantly different between F_m' value of drought stress plant without and with EM supplement at the first 2 weeks of drought application. The positive effect of EM treatment on drought stress plant was recorded after 2 weeks under stress condition. The results showed that F_m' value of drought stress plant treated with 1 and 5 % EM V/V are increased 28 and 12% respectively comparing to drought stress plant without EM supplement (Figure 6).

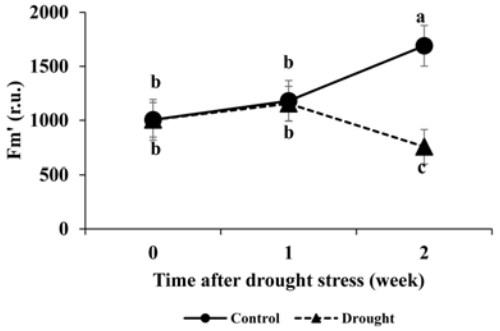

Figure 5. Change of maximum fluorescence signal of light-adapted plants (F_m') of *A. thaliana* under well-watering conditions (●) and drought stress conditions (▲).

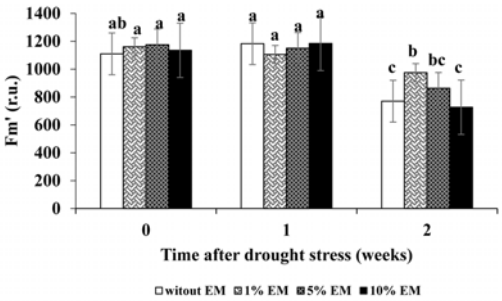

Figure 6. Change of maximum fluorescence signal of light-adapted plants (F_m') of *A. thaliana* under drought stress conditions in response to different levels of EM application.

Quantum efficiency of PSII (ΦPSII) of control plants slightly increased during plant development. Drought negatively affected the ΦPSII of plants at two weeks after its application as it decreased 27% compared to control (Figure 7). The effects of EM application on the ΦPSII was not considerable before and 1 week after drought treatment. Definitely, 2 weeks after drought application, the significantly increasing of the ΦPSII value of drought stress plant treated with EM was observed in drought stress plant treated with 1% EM v/v (Figure 8). While he ΦPSII value of drought stress plant treated with EM 5 and 10 % v/v are slightly higher than control (12 and 5% respectively).

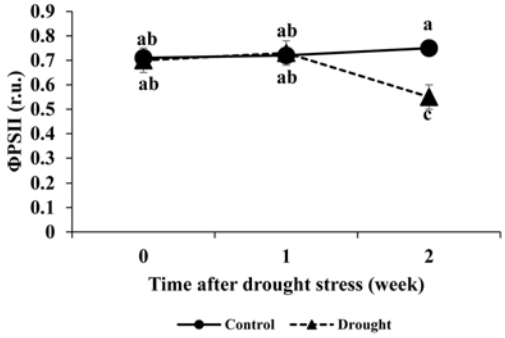

Figure 7. Change of Quantum efficiency of PSII (ΦPSII) of *A. thaliana* under well-watering conditions (●) and drought stress conditions (▲).

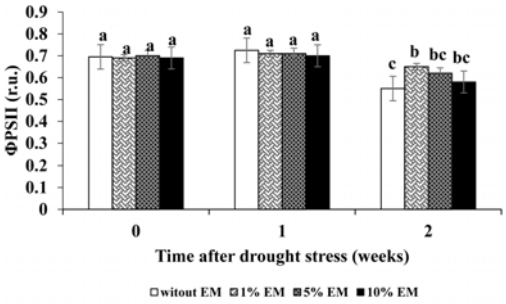

Figure 8. Change of Quantum efficiency of PSII (ΦPSII) of *A. thaliana* under drought stress conditions in response to different levels of EM application.

The functional state of photosynthesis has been considered an ideal physiological efficiency to monitor the health and vitality of plants [22]. In this study, Arabidopsis plants tolerate drought stress conditions moderately during the first two weeks of the stress application. However, on third week, plants seemed to lose this tolerance. That was expressed by reduction of measured chlorophyll fluorescence parameters (Fs, Fm', ΦPSII) (Figure 2-4). The highest reduction was noted for Fm' parameter (ca. 55% comparing to control plants) (Figure 3). This indicated that, drought stress caused inhibition of photosynthetic efficiency of Arabidopsis plant due reduction of energy transfer efficiency within photosystem II. It mostly due that drought affects oxygen evolving complex (OEC) thus; less electrons are available for photochemistry process. This in turn will cause reduction of the rates of photophosphorylation and photoinduced NADP+ reduction as well as of CO_2 assimilation. On other hand, drought (low water availability) causes stomata closer and then less CO_2 will be available for assimilation. Both effects (less energy for CO_2 assimilation and less intercellular CO_2 concentration) bring about reduction of photoassimilates production.

3.3 Fresh and Dry Weight Measurement

Under drought condition, both fresh and dry weight of Arabidopsis plants are significantly decreased comparing to fully watering conditions. Application of EM tend to has positive effect as the result demonstrated the improvement of fresh and dry mass and water content of plants under drought stress. The result turned out that EM 1% v/v had the most beneficial effect on fresh and dry mass and water content of plants (Table 1).

Table 1. Fresh and dry mass and water content of *A. thaliana* under fully watered and drought stress conditions in response to different levels of EM application.

Treatment	Fresh mass (g)	Dry mass (g)	Water content
Control	13.1^{ab}	1.20^{b}	12.0^{b}
Drought	8.70^{c}	1.74^{a}	7.00^{c}
Drought + EM 1 % v/v	16.1^{a}	1.67^{ab}	14.9^{a}
Drought + EM 5 % v/v	13.8^{ab}	1.65^{ab}	12.8^{ab}
Drought + EM 10 % v/v	11.0^{b}	1.02^{c}	$9.80b^{c}$

*Different letters in each column indicate significant difference at $p \leq 0.05$.

The decreasing of fresh and dry mass of drought treated Arabidopsis plants confirms that drought stress can cause the inhibition of photosynthetic efficiency resulting in the reduction of photoassimilates production.

The application of effective micro-organisms (EM) proved to enhance many plants yield [12-16, 23]. Numerous studies indicate beneficial influence EM application on the condition of the soil and plants. EM application positively influencing on the soil density and porosity as well as water permeability [24]. The use of beneficial microorganisms may also impact on increasing the availability of minerals such as phosphorus and potassium [25] and initiate changes in microflora composition [18, 26]. All these

factors affect the condition of subsequent crops. In this study, it was observed that, EM alleviates the effect of drought on Arabidopsis plants photosynthetic efficiency and growth which is clearly seen from the increasing of fresh and dry mass of stressed plants treated with EM comparing to stressed pant without EM supplement in accordance with the increasing of plant chlorophyll (Figure 2) and water content (Table 1), and photosystem II efficiency (Figure 6) in drought stressed plant treated with EM solution. This is in agreement with Shokouhian [27] which indicated that the application of the EM had positive impact in water stress condition on growth of almond seedlings cultivated under water stress condition in respect of plant growth, number of leaf, leaf area, fresh and dry weight, storage chlorophyll, N, P and K in leaves.

Photosynthetic bacteria, in EM produce the essential substances for plant and other beneficial microorganisms growth such as organic matters. Furthermore, there is the possibility that some strains of lactic acid bacteria in EM may form biofilm, high water-holding capacity substance. Microbial biofilm tends to play role in protecting the root cells from dehydrated. These allow plants to survive long enough to adapt to tolerate drought stress. The presence of yeast and actinomycetes in EM applied play great role in improving drought tolerance ability of Arabidopsis plant by synthesizing bioactive substances and plant hormones which help promote plant growth and photosynthetic efficiency [18] and also enhance the modification of plant root system such as increasing of number and length of roots In agreement with Lasudee *et al.* [28], and Kechid *et al.* [29] which suggested that plants hormones such as indoleacetic acid (IAA) and auxin produced by beneficial microorganisms helps enhancing growth and development of plants' roots. This would lead to better absorption capacity, resulting in greater drought tolerance ability of plants comparing with plants untreated with EM.

4. Conclusion

As far as we know, this is the first report to use chlorophyll fluorescence technic to monitor the effect of EM on Arabidopsis plant under water stress condition. Our results provide evidence of EM to promote growth and photosynthetic efficiency of *Arabidopsis* plant under water stress condition. Beneficial microbes within the EM could insert positive effects on stressed *Arabidopsis* plant through various approaches including production of useful substances such as plant hormones and increase nutrient availability. Based on our study, the ability to promote plant growth, drought tolerance ability and photosynthetic efficiency of *Arabidopsis* plant support the possibility of EM application for agricultural propose to minimize the negative effects of unfavorable climate condition.

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References

[1] Lotfi R., Pessarakli M., Gharavi-Kouchebagh P. and Khoshvaghti H., *Crop J.*, 2015; **3**: 434-439. DOI 10.1016/j.cj.2015.05.006.

[2] Jedmowski C., Ashoub A. and Brüggemann W., *Acta Physiol. Plant*, 2013; **35(2)**: 345-354. DOI 10.1007/s11738-012-1077-9.

[3] Zivcak M., Brestic M., Balatova Z., Drevenakova P., Olsovska K., Kalaji H.M., Yang X. and Allakhverdiev I.S., *Photosynth. Res.*, 2013; **117**: 529-546. DOI 10.1007/s11120-013-9885-3.

[4] Blankenship R.E., *Molecular Mechanisms of Photosynthesis*, John Wiley and Sons, 2013.

[5] Strasser R.J. and Stirbet A.D., *Math. Comput. Simulat.*, 1998; **48(1)**: 3-9. DOI 10.1016/S0378-4754(98)00150-5.

[6] Dimitrova S., Dankov K., Paunov M., Pavlova B., Goltsev V. Velikova V., Tsonev T., Kalaji H.M. and Strasser R., *J. BioSci. Biotechnol.*, 2015; **1**: 103-113.

[7] Schansker G., Tóth S.Z., Holzwarth A.R. and Garab G., *Photosynth. Res.*, 2014; **120(1-2)**: 43-58. DOI 10.1007/s11120-013-9806-5.

[8] Kalaji H.M., Goltsev V., Bosa K. Allakhverdiev, I.S., Strasser R.J. and Govindjee, *Photosynth. Res.*, 2012; **114**: 69-96. DOI 10.1007/s11120-012-9780-3.

[9] Kalaji H.M., Schansker G., Brestic M., Bussotti F., Calatayud A., Ferroni L., Goltsev V., Guidi L., Jajoo A., Li P., Losciale P., Mishra V.K., Misra A.N., Nebauer S.G., Pancaldi S., Penella C., Pollastrini M., Suresh K., Tambussi E., Yanniccari M., Zivcak M., Cetner M.D., Samborska I.A., Stirbet A., Olsovska K., Kunderlikova K., Shelonzek H., Rusinowski S. and Baba W., *Photosynth. Res.*, 2017; **132(1)**: 13-66. DOI 10.1007/s11120-016-0318-y.

[10] Schenck zu Schweinsberg-Mickan M. and Müller T., *J. Plant Nutr. Soil Sci.*, 2009; **172**: 704-712. DOI 10.1002/jpln.200800021.

[11] Higa T., *Proceedings of the 1st International Conference on Kyusei Nature Farming*, Washington DC, United States of America, 1991; 8-14.

[12] Khaliq A., Abbasi M.K. and Hussain T., *Bioresour. Technol.*, 2006; **97**: 967-972. DOI 10.1016/j.biortech.2005.05.002.

[13] Jilani G., Akram A., Ali R.M., Hafeez F.Y. Shamsi I.H., Chaudhry A.N. and Chaudhry A.G., *Ann. Microbiol.*, 2007; **57**: 177-184. DOI 10.1007/BF03175204.

[14] Priyadi K., Abdul H., Siagian T.H., Nisa C., Azizah A., Raihani N. and Inubushi K., *Soil Sci. Plant Nutr.*, 2005; **51**: 689-691. DOI 10.1111/j.1747-0765.2005.tb00092.x.

[15] Iwaishi S., *J. Crop Prod.*, 2000; **3**: 269-273. DOI 10.1300/J144v03n01_22.

[16] Xu H., Wang R. and Mridha A.U., *J. Crop Prod.*, 2000; **3**: 173-182. DOI 10.1300/J144v03n01_15.

[17] Kalaji H.M., Cetner M.D., Samborska I.A., Lukasik I., Oukarroum A., Rusinowski S., Pietkiewicz S., Swiatek M. and Dlbrowski P., *Ann. Warsaw Univ. Life Sci. - SGGW*, 2016; **48**: 153-163. DOI 10.1515/sggw-2016-0012.

[18] Asian-Pacific Natural Agriculture, Application manual for APNAN Countries, 2nd Edn., Asian-Pacific Natural Agricultural Network. Bangkok, 1996.

[19] Guo P.G. and Li R.H., *Acta Bot. Sin.*, 2000; **42**: 13-18. [In Chinese]

[20] Fracheboud Y., Jompuk C., Ribaut J.M., Stamp P. and Leipner J., *Plant Mol. Biol.*, 2004; **56**: 241-253. DOI 10.1007/s11103-004-3353-6.

[21] Guo P. and Li M., *J. Trop. Subtrop. Bot.*, 1996; **4**: 60-65. [In Chinese]

[22] Clark A.J., Landolt W., Bucher J.B. and Strasser R.J., *Environ. Pollut.*, 2000; **109**: 501-507. DOI 10.1016/S0269-7491(00)00053-1.

[23] Yamada K. and Xu H., Properties and Applications of An Organic Fertilizer Inoculated with Effective

Microorganisms, - in: Xu H., Parr J.F. and Umemura H., eds, *Nature Farming and Microbial Applications*, The Haworth Press, New York, 2000: 255-268.

[24] Kaczmarek Z., Wolna-Maruwka A. and Jakubus M., *J. Res. Appl. Agric. Eng.*, 2008; **53(3)**: 122-127. [In Polish]

[25] Kocoñ A. and Jadczyszyn T., *Polish J. Agron.*, 2015; **21**: 11-18. [In Polish]

[26] Okorski A. and Majchrzak B., *Acta Botanica*, 2007; **60(1)**: 113-121.

[27] Shokouhian A.A., Davarynejad G.H., Tehranifar A., Imani A. and Rasoulzadeh A., *J. Basic Appl. Sci. Res.*, 2013; **3(2s)**: 86-92.

[28] Lasudee K., Tokuyama S., Lumyong S. and Pathom-aree W., *Chiang Mai J. Sci.*, 2017; **44(1)**: 94-101.

[29] Kechid M., Desbrosses G., Rokhsi W., Varoquaux F., Djekoun A. and Touraine B., *New Phytol.*, 2013; **198**: 514-524. DOI 10.1111/nph.12158.