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The effects of rice seed dressing with *Paenibacillus yonginensis* and silicon on crop development on South Korea's reclaimed tidal land

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ABSTRACT

A field trial to investigate the effects of plant growth promoting bacteria (PGPB) *Paenibacillus yonginensis* ($DCY84^T$) and/or SiO_2 seed coating on rice growth (*Oryza sativa* L.) was carried out on reclaimed tidal land in Taean County in South Korea. The field test was performed twice between May–October 2014 and May–October 2015, in a randomized complete block (RCB) design with three replications. Treatments consisted of: Mock, $DCY84^T$-treated seeds, SiO_2-coated seeds and $DCY84^T$-SiO_2-treated seeds and each treatment area covered approximately 300 m^2. During the early developmental period of rice seedlings, the SiO_2 coating without $DCY84^T$ led to the most favorable 30 DAS rice seedling parameters. Moreover, the combination of $DCY84^T$ and SiO_2 treatments resulted in 2-fold greater fresh and dry weights of 60 DAS rice seedlings compared to Mock seedlings. $DCY84^T$ and SiO_2, both individually and together, produced a greater grain yield and a greater total yield; specifically, $DCY84^T$ and SiO_2 treatments yielded a 73% and 70% increase in mass compared to Mock plants, respectively. Rice treated with both $DCY84^T$ and SiO_2 treatment contained the highest amount of Al, Fe, Ca and Mg, which were 54%, 169%, 42% and 67% higher than the Mock rice, respectively. Remarkably, $DCY84^T$ treatment had the most phosphate [P], potassium [K] and total nitrogen [T–N]. $DCY84^T$ and/or SiO_2 treatment highly increased the whole kernel percentage. Thus, lower its broken kernel percentage to 9.60–24.58%. The protein content of the grain with both treatments was 7.2%, which was greater than that of the Mock grain (6.0%). The content of chlorophyll a, b and carotinoid in the rice leaves which were treated with silica and DCY84 has increased more than the mock without treatment. After harvest, the GABA content of brown rice was increased to 1.9-fold (2014), 1.5 fold (2015) compared to mock grain, respectively. Overall, $DCY84^T$ treatment and SiO_2 coating can be useful methods for promoting growth of rice under conditions of saline stress. Results from other laboratory trials and greenhouse experiments are also provided.

1. Introduction

The exponential growth of the human population demands more food and thus enhanced crop production. During the 1960's, the Green Revolution was largely responsible for increasing food supply. Currently, two alternatives exist for increasing cereal production: (1) expanding farming into areas that are currently not used for food production or (2) attaining a higher yield per unit of land area in existing agricultural areas (Linquist et al., 2011). In South Korea, because the available farmland had been decreasing due to urbanization and industrialization, farming on reclaimed tidal land is one of the country's efforts to produce more food. However, agricultural activities on reclaimed tidal land are generally difficult due to high salinity and low amounts of nutrients (Cho et al., 2008). A "saline soil" is usually defined as soil in which the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone is more than 4 dS m^{-1} at 25 °C (equal to 40 mM NaCl) and the exchangeable sodium is less than 15%. The yield of most crop plants is reduced at this EC_e, though many crops exhibit yield

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reduction at lower EC_es (Jamil et al., 2011). Indeed, excess saline has been known to suppress plant growth (Paul, 2012); it affects almost all aspects of plant development (germination, vegetative growth, reproductive development, etc.,), through osmotic and oxidative stress (Bano and Fatima, 2009), which can decrease enzyme activity (Seckin et al., 2009); inhibit DNA, RNA and protein synthesis (Javid et al., 2011; Tabur and Demir, 2010); cause ion toxicity and lead to nutrient deficiency (*e.g.*, N, Ca, K, P, Fe and Zn) (Bano and Fatima, 2009).

Indirect stimulation of Plant growth-promoting bacteria (PGPB) is related to inducing the plant's systemic resistant responses against biotic (as biocontrol agents) and abiotic stress (as plant strengthener) (Ahmad et al., 2008; Yang et al., 2009). In addition, PGPB also can be used to achieve enhancement of plant growth (as plant growth promoter) and nutrient management (as biofertilizers) (Nia et al., 2012; Ramadoss et al., 2013). The use of microorganism inoculant continues to be an area of rapidly growing research because it is both ecologically and economically relevant (Berg, 2009). An excellent review about these bacteria inoculants has recently been published (Pérez-Montãno et al., 2014). Meanwhile, *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, some members of the *Enterobacteriaceae* family and, recently, *Paenibacillus* are known for their ability to promote plant growth (Bashan et al., 2004; Hayat et al., 2010). The genus *Paenibacillus* has been shown to fix atmospheric nitrogen, produce siderophores and solubilize minerals in soil (Timmusk 2003). To date, several *Paenibacillus* species have been reported to be PGPB such as *Paenibacillus polymyxa* (Lebuhn et al., 1997; Timmusk and Wagner, 1999; Raza et al., 2009), *Paenibacillus brasiliensis* (von der Weid et al., 2002), *Paenibacillus borealis* (Elo et al., 2001; Egamberdiyeva, 2005), *Paenibacillus illinoisensis* (Jung et al., 2005), *Paenibacillus elgii* (Das et al., 2010), *Paenibacillus ehimensis* (Naing et al., 2013) and recently *Paenibacillus yonginensis* (Sukweenadhi et al., 2015). Numerous field experiments have demonstrated the potential for single strain inoculant biofertilizers to reduce fertilizer requirements and enhance yields of rice, including applications of azospirilla, pseudomonas, enterobacteria and rhizobia such as: *Azospirillum brasilense* Wb3 and *Azospirillum lipoferum* N4 (Malik et al., 2002), *Pseudomonas fluorescens* Pf1 (Vidhyaskaran et al., 1997), *Burkholderia vietnamiensis* TVV75 (Trân Van et al., 2000) and *Rhizobium leguminosarum* bv trifolii E11 (Yanni et al., 2001).

Silicon (Si) is a primary constituent of the Earth's crust that can easily be found in almost all soil types. However, Si uptake by plants is faster than the Si cycle in nature (Guntzer et al., 2012). Si-deficient plants are often anatomically weaker and exhibit abnormal growth; thus, they are more susceptible to biotic and abiotic stress compared with Si-rich plants (Mitani and Ma, 2005). The beneficial effect of Si has been demonstrated by many studies using pots, hydroponic, and field experiments. A review by Ma nicely describes how Si helps plants overcome biotic stress and abiotic stress (Ma, 2004). Plants can be supplemented with Si via a seed coating. Seed coating is one of seed dressing technique in which a thin and uniform polymer layer is deposited on the seed surface (Corlett et al., 2014). Its protective material is applied in a formulated amount and with minimal influence on the environment (Baudet and Peres, 2004). Several previous studies have reported satisfactory effects of seed coating on seed germination, seedling growth, leaf area, root and shoot growth, dry biomass and yield gain (Gevrek et al., 2012; Tavares et al., 2012).

The recognition of PGPB as potential implements for stimulating plant growth, relieving environmental stress and increasing crop yield has progressed over the past decade; today, researchers are able to reliably use them in field experiments. Among major cereals crops, rice is one of the most important commodities in the world, as it serves as food for about 50% of the world's population (Ladha et al., 1997). In South Korea, rice is a main traditional food crop. Growth attributes, nutrient content and yield enhancement of rice crops by using PGPB strains as bacterial inoculants have been reported as well as of potato, sugar beet, radish and sweet potato (Manivannan, 2011; Singh et al., 2011; Farzana et al., 2009). *P. yonginensis* DCY84^T showed promising plant growth promoting bacteria *in vitro* (Sukweenadhi et al., 2014) and showed induced systemic resistance against saline stress *in planta* using *Arabidopsis* model (Sukweenadhi et al., 2015). Silicon can alleviates the effects of other abiotic stresses including salt stress, drought stress, radiation damage, nutrient imbalance, high temperatures and freezing (Ma, 2004). Seed coating technique often employed to increase seed size, improve seed shape and texture and facilitate direct sowing. Thus, we investigated the effects of *P. yonginensis* DCY84^T, combined with silicon seed coating on rice seeds to check its potential to increase rice productivity under saline stress conditions in pots, in a greenhouse and in field experiments on reclaimed tidal land. The non-protein amino acid, gamma-aminobutyric acid (GABA) rapidly accumulates in plant tissues in response to biotic and abiotic stress, and nutritional starvation stresses and regulates plant growth (Ramesh et al., 2015). The content of GABA, glutamic acid and the activity of the glutamate decarboxylase (GAD) and GABA transaminase during germination were investigated. GABA is associated with primary nitrogen and carbon metabolism and is tightly linked to the TCA cycle.

2. Materials and methods

2.1. Materials

A total of 6 different rice seed cultivars, Junam, Odae, Hiami, Samgwang, Chuchung and Koshihikari, were obtained from the Korean Rice Collection Center. The SiO_2 coating method for rice seeds was conducted at Saturn Biotech in Seoul, Korea. Approximately 500 g SiO_2 (Zeolite) was mixed with 2 kg dried rice seed and rotated at 4 rpm for 2 min. Then, the mixture was sprayed with 300 ml binder solution and dried for 3 min. The coating process was repeated a second time with an additional 500 g dried Zeolite powder, the seeds were sprayed with 300 ml binder solution and then they were dried for 2 min. For the finishing step, 500 g dried Zeolite powder was added, the mixture was sprayed with 200 ml sprayed binder solution and then it was dried. The ability to promote plant growth by *P. yonginensis* DCY84^T, a strain recently reported by Sukweenadhi et al. (2014) was evaluated; it also can be obtained from the Korean Collection for Type Cultures (as KCTC 33428^T) and the Japan Collection of Microorganisms (as JCM 19885^T).

2.2. The effect of DCY84^T isolates on rice seed germination

P. yonginensis DCY84^T strain was grown at 30 °C on Trypticase soy broth (TSB) for 16 h. The culture broth was centrifuged at 3000 × *g* for 15 min and the precipitated cells were dissolved in saline water. The bacteria suspension was centrifuged again at 3000 × *g* for 15 min and dissolved in saline water; centrifugation and reconstitution were repeated until the cells reached 10^8 CFU ml^{-1}. Subsequently, 6 uncoated rice and coated rice seeds (Junam, Odae, Hiami, Samgwang, Chuchung and, Koshihikari) were surface sterilized using 0.02% sodium hypochlorite for 2 min and then rinsed using sterile distilled water. The seeds were then inoculated by soaking them in the bacteria suspension for 30 min (Bhatia et al., 2014). Saline water was used as a negative control treatment (Mock). The soil used in this experiment was collected from the coast of Taean, South Korea. Chemical characteristics of the soil are shown in Supplementary Table 1. The soil was sterilized by autoclaving it at 121 °C for 1 h on three consecutive days. A total of 20 rice seeds were planted for each treatment in a soil pot and

Table 1
Effect of DCY84T and SiO2 coating during germination and early developmental seedlings (15DAS).

Treament and growth parameter	Rice cultivar (uncoated)					
	Junam	Hiami	Samgwang	Odae	Chuchung	Kosihikary
Control						
Shoot length (cm)	16.98 ± 0.31	11.23 ± 0.97	10.3 ± 1.39	12.31 ± 1.53	12.51 ± 1.35	11.43 ± 2.15
Roots number	5.67 ± 0.18	4.23 ± 0.49	4.5 ± 1.27	4.37 ± 1.24	4.2 ± 1.06	4.51 ± 1.21
Roots length (cm)	6.07 ± 0.41	3.6 ± 0.12	2.72 ± 1.57	4.57 ± 1.54	4.3 ± 1.05	3.45 ± 1.05
Fresh weight (g)	53.93 ± 1.86	48.35 ± 2.12	49.5 ± 2.01	53.6 ± 2.26	45.45 ± 1.71	50.31 ± 2.02
Germination rate (%)	45 ± 0.6	36 ± 0.25	47 ± 0.3	39 ± 1.5	38 ± 1.1	38 ± 0.7
Vigor index	1037.25 ± 27	533.88 ± 35	611.94 ± 25	658.32 ± 38	638.78 ± 11	565.44 ± 24
Paenibacillus yonginensis DCY84^T						
Shoot length (cm)	19.42 ± 0.28	15.31 ± 0.52	16.42 ± 2.31	13.81 ± 2.57	13.11 ± 0.91	15.24 ± 3.61
Roots number	8.2 ± 0.18	5.2 ± 0.16	6.4 ± 0.25	5.23 ± 0.71	5.7 ± 1.2	4.1 ± 0.91
Roots length (cm)	7.6 ± 0.46	5.45 ± 0.23	5.12 ± 1.54	7.23 ± 1.12	7.01 ± 1.56	7.5 ± 1.36
Fresh weight (g)	59.24 ± 1.08	53.21 ± 2.29	61.74 ± 2.87	58.61 ± 2.94	66.4 ± 2.58	57.64 ± 3.21
Germination rate (%)	47 ± 0.2	40 ± 2.2	52 ± 1.53	53 ± 0.5	49 ± 0.6	50 ± 0.5
Vigor index	1269.94 ± 23	830.4 ± 22	1120.08 ± 41	1115.12 ± 32	985.88 ± 42	1137.02 ± 19

Treament and growth parameter	Rice cultivar (coated)					
	Junam	Hiami	Samgwang	Odae	Chuchung	Kosihikary
Control						
Shoot length (cm)	19.3 ± 0.58**	12.63 ± 1.23*	13 ± 0.59**	11.43 ± 0.42*	11.95 ± 0.17	11.38 ± 0.65
Roots number	6.1 ± 0.14	4.33 ± 0.46	5.4 ± 0.25	4.67 ± 0.34	5.5 ± 0.16	5.75 ± 0.11*
Roots length (cm)	4.1 ± 0.07***	3.7 ± 0.09	3.02 ± 0.07	5.27 ± 0.55*	5.5 ± 0.09	3.85 ± 0.48
Fresh weight (g)	66.2 ± 1.81***	51.37 ± 3.59*	53.74 ± 1.81*	54.4 ± 1.18	45.85 ± 0.57	60.3 ± 3.63**
Germination rate (%)	43 ± 0.2*	38 ± 0.3*	48 ± 0.3	38 ± 0.2	37 ± 0.2	40 ± 0.3*
Vigor index	1003 ± 14***	620.67 ± 31**	768.96 ± 17*	786.6 ± 21*	645.65 ± 14	609 ± 19*
Paenibacillus yonginensis DCY84^T						
Shoot length (cm)	23.3 ± 0.42***	20.28 ± 0.4**	19.84 ± 1.06**	14.28 ± 1.15*	13.96 ± 1.35	14.94 ± 1.46
Roots number	9.0 ± 0.32*	7.5 ± 0.29*	7.4 ± 0.3*	7 ± 0.27**	6.67 ± 0.52*	5.8 ± 0.29**
Roots length (cm)	9.2 ± 0.08**	7.65 ± 0.16**	5.8 ± 0.17*	8.67 ± 0.51**	8.53 ± 0.45**	10.2 ± 0.73**
Fresh weight (g)	85.7 ± 2.25***	78.5 ± 1.89***	73.44 ± 3.48**	64.36 ± 3.99*	72.6 ± 4.35**	63.36 ± 2.98**
Germination rate (%)	52 ± 0.3***	48 ± 0.2***	50 ± 0.3*	52 ± 0.2	48 ± 0.3	52 ± 0.2*
Vigor index	1690 ± 9***	1340.4 ± 10***	1282 ± 38**	1192.88 ± 49*	1080 ± 52*	1307.28 ± 24**

Coated seeds treatment is compared with uncoated seeds, respectively with or without bacteria treatment.

** Statistical significance was assigned at $P < 0.01$.

*** Statistical significance was assigned at $P < 0.001$.

incubated under the following conditions: 16 h days at 28 °C and 8 h nights at 20 °C at 60% relative humidity. The rice seeds were watered daily and no additional fertilizer was used. The germination rate was recorded 5 days after sowing (DAS) while the shoot length, root length, root number, fresh weight and dry weight were recorded 15 DAS (Yoshida et al., 1976). The seedlings were dried using a 60 °C oven for 3 days to determine the dry weight. The seedling vigor index (VI) was also calculated using the formula: VI = [mean root length + mean hypocotyl length] × % germination. All of these data are shown in Table 1. Three independent replicate experiments were performed.

2.3. Greenhouse experiment evaluating DCY84^T treatment and SiO_2 coating on rice seeds under saline stress

A concentrated suspension of *P. yonginensis* DCY84^T was prepared as previously described. Exactly 100 SiO_2-coated and 100 uncoated Junam cultivar rice seeds were co-cultured with the bacteria suspension and allowed 3 days to germinate on a petri dish (150 × 150 mm) with moistened sterile tissue paper. Saline water was used as a negative control (Mock). Then, 50 well-germinated seeds were transferred to a soil pot and cultivated inside the greenhouse with 14 h days at 30 °C and 10 h nights at 20 °C, 90% relative humidity. After 1 week, 32 similar-sized rice seedlings were transferred to bigger pots. Saline stress was introduced at 15 DAS to 16 seedlings for each treatment by watering them with a 250 mM NaCl solution for 5 days. On the 5th day, the water was changed back to normal water to allow the rice seedlings to recover. After 2 weeks of recovery (at 34 DAS), the recovery level of the rice seedlings was observed. The recovery level was judged by placing the plants into 1 of 3 categories: Good = At least 3 leaves are green; Mild = 1–2 leaves are green; None = All leaves are withered (the plant is dead). A total of 3 independent replications were performed and the data are shown in Fig. 1. A more thorough description of the salt stress treatment at the greenhouse is provided in Supplementary Fig. S1.

2.4. Field site and climate conditions

The field experiments were conducted on farmland near the Taean coastal area, South Korea (36°39′39″N, 126°20′17″E, map in Supplementary Fig. S2). The landscape of the experimental area consisted of a gently sloping alluvial plain that was artificially filled with soil in 2010 (tidal land reclamation) (Choi, 2012). This region experiences warm and humid weather with an average temperature ranging between 15–25 °C during May–October 2014 and 12–28 °C during May–October 2015. An average annual rainfall is 800 mm and 600 mm during 2014 and 2015, respectively (http://www.accuweather.com/en/kr/south-korea-weather). The monthly maximum and minimum temperatures and rainfall during the field trials is shown in Supplementary Fig. S3. The chemical characteristics of the upper soil layer (30 cm) were: pH (of 1:5 soil:water), 4.9; electrical conductance, 2.42 dS m^{-1}; total organic matter, 1.10%; total N, 0.54%; available P, 40.9 mg kg^{-1} and available K, 217.6 mg kg^{-1}. All of these data rep-

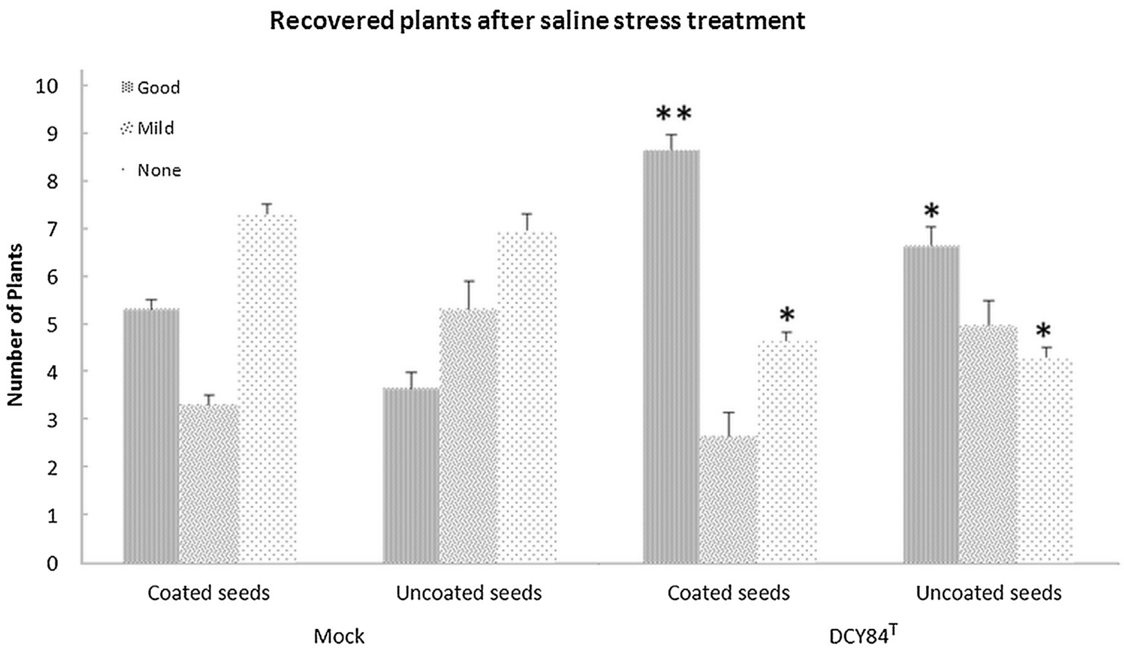

Fig. 1. Recovered rice seedlings after the saline stress treatment in the greenhouse. The recovery was categorized into 1 of 3 levels; Good = At least 3 leaves are green; Mild = At least 1 leaf is green; None = All leaves are withered (the plant is dead). Statistical significance was assigned at "*" for $P < 0.05$, "**" for $P < 0.01$ and "***" for $P < 0.001$.

resent the average value from 5 representative samples as shown in Supplementary Table 1.

2.5. Soil chemical analysis

Soil samples were air-dried and passed through a 2-mm sieve. The chemical properties of these samples were analyzed according to the standard methods of the Rural Development Administration, Korea (RDA-Rural Development Administration and Korea, 1988). Soil pH and electrical conductivity (EC) were measured using a soil-to-water ratio of 1:5 with a pH meter (Thermo, Orion 900 A) and an Orion 162 A conductivity meter, respectively. Soil organic matter (OM) content, phosphorus content, and the levels of available cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) were assessed using the Tyurin method, the Lancaster method, and the 1N-NH_4OAc (pH 7.0) method, respectively.

2.6. The field test for evaluating the effects of DCY84^T treatment and SiO_2 coating on the growth of Junam rice cultivars

The field test was performed between May until October 2014 and repeated on May until October 2015. The experiment used a randomized complete block (RCB) design with three replications. The size of the unit plot was 10 m × 10 m. In total, there was approximately 300 m^2 of separated area for each treatment: Mock, DCY84^T-treated seeds, SiO_2-coated seeds and DCY84^T-SiO_2-treated seeds. Approximately 4.5 kg seeds were used for each treatment and spread using a spreader machine for each 100 m^2 area. The farmland was regularly irrigated with water (pH 4.6, EC = 4.13 dS m^{-1}). The early developmental stage of seedlings was observed by sampling the rice seedlings at 30 and 60 DAS, as shown in Table 2. The shoot element analysis of 60 DAS seedlings is also provided in Table 3. Harvesting was performed at 160 DAS and yield attributes such as length of panicle, number of panicle m^{-1}, number of spikelets panicle^{-1}, normal spikelets panicle^{-1}, fertility percentage (normal spikelets panicle^{-1}/number of spikelets panicle^{-1} × 100%), 1000-grain wright, grain yield and total yield are shown in Table 4. A more thorough description of the field trial is provided in Supplementary Fig. S4.

2.7. Shoot element analysis

For nitrogen content of the plant, sulfuric acid was added then decomposed. It was analyzed by Kjeldahl distillation (Kjeltec sytem, FOSS 8400). 1 g dried rice leave were treated with 10 ml HNO_3 and decomposed by the Microwave digestion system (Mars 5, CEM) then phosphoric acid, potassium and lime were analyzed by the ICP (inductively coupled plasma analyzer, Optima 8400RL, PerkinElmer). 1 g sample was hydrolyzed by 3 ml ternary solution then filtered. The remaining residue was measured for the silicate content of the rice after it was decomposed for 6 h in an electric furnace of 600 °C.

2.8. Rice kernel characteristics and nutrient content analysis

Rice spikelets were hulled using a FC2 K friction machine (KETT, Japan) while milling work was performed using a MC-90A testing rice miller (Toyo Co., Tokyo, Japan) that milled spikelets to 91% of their weight ratio. Characteristics of the rice kernels were determined with a Cervitec 1625 Grain Inspector machine (FOSS, Sweden), protein content was measured with a component analyzer (Infractec 1241 Grain Analyzer) and palatability was measured with a rice taste analyzer. Rice kernel characteristics and its nutrient content for each treatment were also conducted and the results are shown in Fig. 2. Representative morphology of Junam rice cultivar grains and kernels is shown in Supplementary Fig. S5.

2.9. Content of chlorophyll a, b and carotinoid in the rice leaves

100 mg of fresh rice leaf sample was powdered with liquid nitrogen and then adding 50 ml of 80% cold acetone. Incubate in dark 4 °C for overnight, then centrifuge 2500 rpm 15 min in 4 °C. The supernatant was collected in new tube and read in different

Table 2
Effects of DCY84^T treatment and/or SiO$_2$ coating on seedlings in the early developmental stage after cultivation in the rice field; (A) 30 DAS rice seedlings, (B) 60 DAS rice seedlings A) 30 DAS rice seedlings.

(A) 30 DAS rice seedlings						
Treatment	Fresh weight (mg)	Dry weight (mg)	Shoot dry weight (mg)	Root dry weight (mg)	Shoot length (mm)	Root length (mm)
DCY84^T	475 ± 18.8[***]	83 ± 5.2[**]	45 ± 3.2	38 ± 4.5[**]	157 ± 4.6[***]	72 ± 5.0[*]
SiO2	585 ± 51.5[***]	98 ± 4.2[***]	57 ± 2.0[**]	41 ± 2.7[***]	167 ± 5.8[***]	53 ± 5.3
SiO2 + DCY84^T	518 ± 46.3[***]	84 ± 3.7[***]	51 ± 4.3	33 ± 3.8[**]	176 ± 7.4[***]	59 ± 4.9
Mock	296 ± 29.8	58 ± 5.1	42 ± 4.6	16 ± 3.7	114 ± 6.3	53 ± 6.1
(B) 60 DAS rice seedlings						
1st year						
DCY84^T	4211 ± 183.6[***]	1977 ± 78.5[***]	1357 ± 64.2[***]	620 ± 44.2[***]	483 ± 15.1[***]	160 ± 4.8[***]
SiO2	3665 ± 216.4[**]	1381 ± 65.9[***]	904 ± 48.6[***]	478 ± 32.2[***]	489 ± 9.6[***]	191 ± 7.6[***]
SiO2 + DCY84^T	4957 ± 140.4[***]	2097 ± 100.6[***]	1435 ± 72.7[***]	662 ± 51.3[***]	497 ± 11.6[***]	180 ± 5.3[***]
Mock	2319 ± 315.1	820 ± 46.0	596 ± 40.2	224 ± 23.4	410 ± 10.1	124 ± 6.3
2nd year						
DCY84^T	4472 ± 126.4[***]	1824 ± 17.4[***]	1375 ± 31.5[***]	449 ± 16.8[***]	425 ± 6.9[**]	165 ± 5.2[***]
SiO2	4104 ± 158.3[**]	1618 ± 37.1[***]	1156 ± 71.7[***]	461 ± 20.6[***]	429 ± 8.2[***]	153 ± 7.0[**]
SiO2 + DCY84^T	5044 ± 156.0[***]	2112 ± 31.3[***]	1743 ± 62.8[***]	369 ± 12.1[***]	438 ± 10.4[***]	168 ± 5.5[***]
Mock	3328 ± 133.3	1055 ± 14.7	843 ± 28.9	212 ± 27.0	410 ± 5.4	142 ± 6.2

All treatment parameter is compared to Mock.

[*] Statistical significance was assigned at $P < 0.05$.

[**] Statistical significance was assigned at $P < 0.01$.

[***] Statistical significance was assigned at $P < 0.001$.

Table 3
60 DAS shoot element analysis of DCY84^T treatment and/or SiO$_2$ coating in the Junam rice cultivar.

Element analysis	Treatment			
	DCY84^T	SiO$_2$	SiO$_2$ + CY84^T	Mock
Al (g/kg)	0.47 ± 0.007[**]	0.43 ± 0.020[*]	0.54 ± 0.006[***]	0.35 ± 0.016
Fe (g/kg)	0.54 ± 0.004[***]	0.67 ± 0.004[***]	0.77 ± 0.010[***]	0.29 ± 0.012
P (mg/kg)	0.87 ± 0.005[***]	0.84 ± 0.003[***]	0.81 ± 0.010[***]	0.65 ± 0.004
K (cmol/kg)	2.66 ± 0.109[**]	2.05 ± 0.034	2.45 ± 0.044[**]	1.85 ± 0.088
Ca (cmol/kg)	0.42 ± 0.007[***]	0.45 ± 0.008[***]	0.46 ± 0.009[***]	0.32 ± 0.006
Mg (cmol/kg)	0.62 ± 0.012[***]	0.65 ± 0.009[***]	0.68 ± 0.012[***]	0.41 ± 0.007
Na (cmol/kg)	1.68 ± 0.032[*]	2.02 ± 0.017[*]	1.88 ± 0.016	1.88 ± 0.031
T-N (%)	2.47 ± 0.060[*]	1.85 ± 0.187[***]	2.14 ± 0.083	1.02 ± 0.061

[*] Statistical significance was assigned at $P < 0.05$.

[**] Statistical significance was assigned at $P < 0.01$.

[***] Statistical significance was assigned at $P < 0.001$.

Table 4
Spikelets and yield characteristics of DCY84^T treatment and/or SiO$_2$ coating in the Junam rice cultivar.

Spikelets and yield Characteristics	Treatment			
	SiO$_2$ + DCY84^T	DCY84^T	SiO$_2$	Mock
1st year				
Length of panicle (cm)	18.37 ± 3.1[***]	17.74 ± 3.1[**]	15.65 ± 2.1[*]	16.79 ± 2.3
Number of panicle/m^2	105 ± 2.7[**]	103 ± 2.4[*]	92 ± 1.5	91 ± 2.2
Normal spikelets/panicle	50 ± 2.9[*]	57 ± 0.7[**]	57 ± 0.9[**]	48 ± 0.6
Total spikelets/panicle	114 ± 2.9[***]	109 ± 2.5[***]	86 ± 1.7[***]	97 ± 2.3
Fertility percentage (%)	91.8 ± 0.96	93.7 ± 0.47	94.4 ± 0.45	94.7 ± 0.25
1000-grain weight (gram)	156.9 ± 2.57[***]	121.8 ± 1.29[***]	115.0 ± 1.78[*]	111.0 ± 0.16
Total yield (kg/100 m^2)	109 ± 0.6[***]	88 ± 2.1[***]	75 ± 1.0[***]	64 ± 1.5
Grains yield (kg/100 m^2)	90 ± 0.9[***]	72 ± 1.7[***]	60 ± 0.9[**]	52 ± 1.5
2nd year				
Length of panicle (cm)	18.65 ± 5.5[*]	19.01 ± 6.4[**]	17.63 ± 3.0[*]	18.10 ± 3.9
Number of panicle/m^2	95 ± 1.8[**]	97 ± 2.5[**]	87 ± 1.1	85 ± 0.8
Normal spikelets/panicle	90 ± 2.6[***]	87 ± 3.1[***]	82 ± 2.6[**]	79 ± 2.4
Total spikelets/panicle	95 ± 3.2[***]	93 ± 3.8[***]	82 ± 3.1[*]	80 ± 3.8
Fertility percentage (%)	93.3 ± 1.29	92.5 ± 0.34	93.3 ± 1.18	95.5 ± 2.13
1000-grain weight (gram)	121.6 ± 2.15[**]	118.2 ± 1.38[**]	111.3 ± 2.08[*]	106.1 ± 1.78
Total yield (kg/100 m^2)	99 ± 2.7[**]	94 ± 2.2[**]	89 ± 4.9[*]	85 ± 2.9
Grains yield (kg/100 m^2)	87 ± 2.3[**]	81 ± 2.0[**]	79 ± 4.4[*]	67 ± 2.7

[*] Statistical significance was assigned at $P < 0.05$.

[**] Statistical significance was assigned at $P < 0.01$.

[***] Statistical significance was assigned at $P < 0.001$.

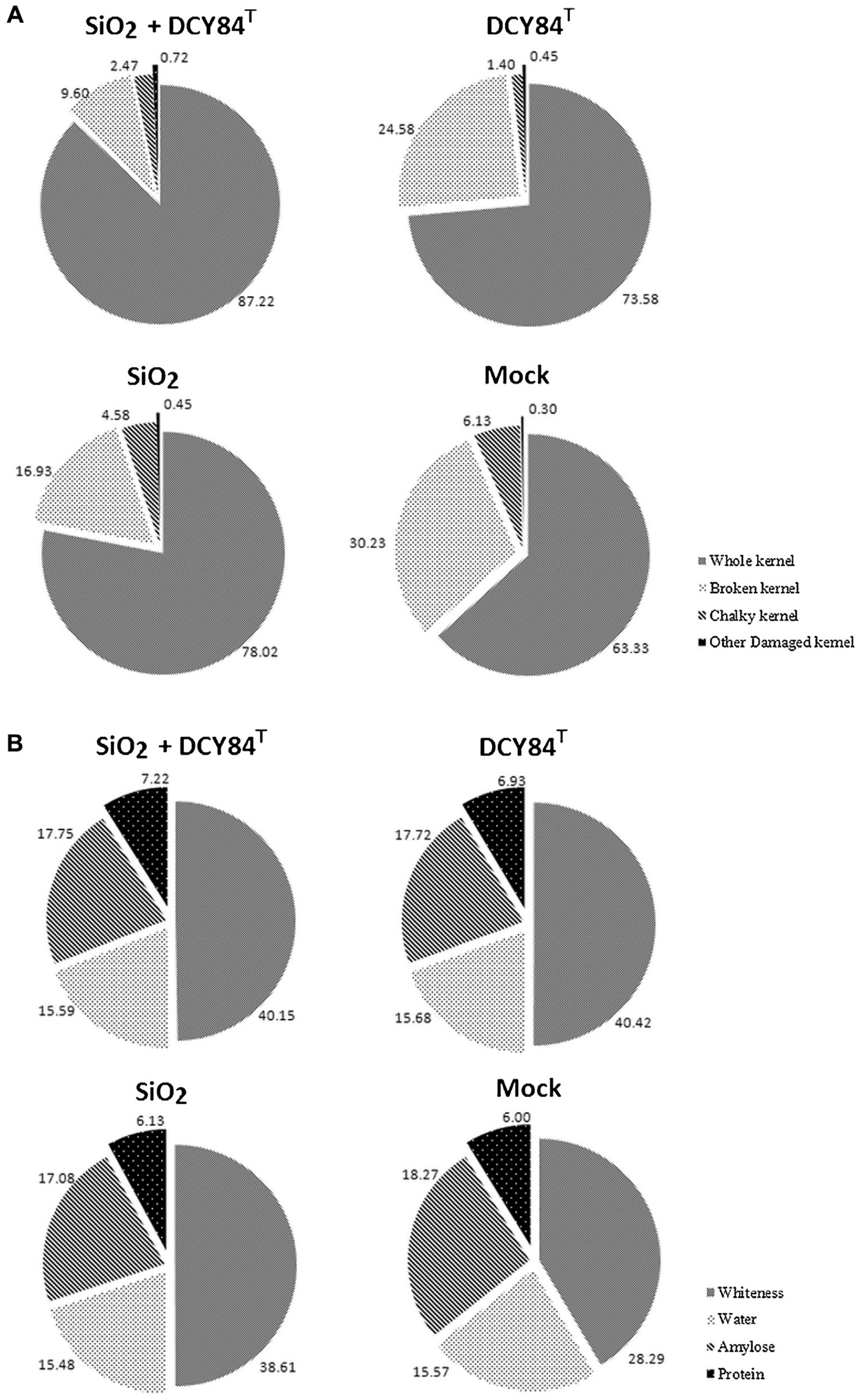

Fig. 2. A Grain characteristics and B Grain nutrient content of DCY84^T treatment and/or SiO_2 coating in the Junam rice cultivar.

absorbance 480, 645, 663 nm. There is some formula for chlorophyll and carotenoid calculation

$$\text{Chlorophyll } a = 12.72 \times \text{A663} - 2.59 \times \text{A645}, \text{ chlorophyll } b = 22.9 \times \text{A645} - 4.67 \times \text{A663}. \text{Total chlorophyll} = 20.31 \times \text{A645} + 8.05 \times \text{A663}, \text{ carotenoid} = \text{A480} + (0.114 \times \text{A663} - 0.638 \times \text{A645})$$

2.10. Analysis of GABA content in the brown rice grain

Standard GABA were purchased from Sigma (GABA 99%, Sigma–Aldrich; USA). All solvents were HPLC grade, water, acetonitrile were purchased from Burdick & Jackson, USA. Ammonium formate, formic acid were purchased from Sigma–Aldrich; USA. Samples were extracted by reciprocal shaker (SR-1, Taitec Co.; Japan) and centrifuged at 10,000 × *g* (Labogene 1736R, Korea). Shiseido Nanospace SI2 (API 3200, ABSciex, USA) were used for GABA analysis; Column was Intrada amino acid (3 mm × 100 mm, 3 μm), mobile phase was 40 mM ammonium formate in DW: 0.3% formic acid in ACN (75:25, v/v). Flow rate was 0.4 ml/min, injection volume was 5 μL. GABA was analyzed by LC/MS/MS. Ionization mode was ESI, positive ion source polarity, 1 mg GABA was dissolved in 1 ml 0.1 N HCl and was appropriately diluted for standard solution. 3 g homogenized sample was mixed with 2% formic acid in 70% ethanol and homogenized for 5 min, adjusted to 50 ml and then homogenized again for 30 min. After 30 min centrifugation at 13,000 rpm and the supernatant was analyzed after filtration with 0.2 μm syringe filter.

3. Results

3.1. The effects of DCY84^T and/or SiO_2 coating treatment on rice seed germination

Seedling vigor index can be estimated by means of seedling length or seedling dry weight methods to identify favorable environment for production of good quality seeds. Since field soil was used as plant media, the vigor index calculated from the length of the plant because it will be important traits for trial on field test later. The root length is important for strengthening the plant into ground, while the shoot length is important for quickly providing the nutrient accumulation by doing photosynthesis above of water surface. Similar report used this method especially when the effect of some treatment on seedling growth was investigated (Dhanda et al., 2004; He et al., 2014; Long et al., 2008).

As shown in Table 1, almost all measures of Junam rice seedlings were increase with the SiO_2 coating compared to control (without coating). A marked negative result was seen in root length, germination rate and vigor index. However, the SiO_2 treatment increased shoot length (19.3 cm) and fresh weight (66.2 g) of Junam cultivar; there was also no significant difference in the case of root number. Meanwhile, DCY84^T and SiO_2 coating treatment increased all seedlings growth parameter. Similar trend was showed on other cultivars regarding the increasing shoot length, root length and fresh weight at various significant levels, which resulted on increasing Vigor Index. Overall, SiO_2 coating treatment mostly affects the shoots while combination of DCY84^T and SiO_2 coating treatment affect both roots and shoots. For further experiments in the greenhouse and in the field, only Junam rice was used.

3.2. The greenhouse experiment of DCY84^T-treated and/or SiO_2-coated rice seeds under saline stress

Pilot greenhouse tests showed that the SiO_2 coating alone did not significantly affect the 34 DAS rice seedlings' recovery after saline stress (5.3 ± 0.19 plants vs 3.7 ± 0.33 plants). Seedlings treated with DCY84^T showed a slightly better recovery than Mock seedlings, as they had a higher number of plants in the "Good" category (6.7 ± 0.38 plants vs. 3.7 ± 0.33 plants). As shown in Fig. 1, the combination of DCY84^T and SiO_2 treatments produced the highest number of "Good"-recovered plants (8.7 ± 0.32 plants), a number that is much greater than the uncoated Mock counterparts. Regardless of the SiO_2 coating, the number of plants that did not recover (given a score of "None") was similar to the number of plants that received a score of "Good" when treated with DCY84^T. Moreover, the number of plants that did not recover (that were categorized as "None") was lower in the DCY84^T group compared to the Mock group regardless of coating (4.7 ± 0.18 plants in the DCY84^T plus SiO_2 group, 4.3 ± 0.20 plants in the DCY84^T group, 7.3 ± 0.17 plants in the SiO_2 group and 7.1 ± 0.30 plants in the Mock group). The number of plants placed in the "Mild" recovery category was similar for all treatments.

3.3. The effect of DCY84^T treatment and/or SiO_2 coating during rice field cultivation

The early developmental period of rice seedlings is summarized in Table 2. In Table 2A, 30 DAS Junam rice cultivar seedlings exhibited greater shoot length, fresh weight and dry weight when treated with DCY84^T and/or coated with SiO_2. Compared to Mock seedlings that had a shoot length of 114 ± 6.3 mm, the combination of DCY84^T and SiO_2 treatments resulted in the tallest shoots, which were 176 ± 7.4 mm, followed by those with the SiO_2 treatment (167 ± 5.8 mm) and those with the DCY84^T treatment (157 ± 4.6 mm). However, only the DCY84^T treatment led to significantly longer roots (72 ± 5.0 mm vs. Mock, 53 ± 6.1 mm). Both the DCY84^T and/or SiO_2 treatment had a heavier fresh weight (475 ± 18.8–585 ± 51.5 mg) compared to the Mock treatment (296 ± 29.8 mg) and a heavier dry weight (83 ± 5.2–98 ± 4.2 mg) compared to the Mock treatment (58 ± 5.1 mg). When focusing on dry weight, DCY84^T and/or SiO_2 treatment groups all resulted in a heavier root dry weight, but only the SiO_2 treatment group showed heavier shoot dry weights (57 ± 2.03 mg) compared to the Mock group (42.3 ± 4.62 mg). All in all, the SiO_2 coating without DCY84^T led to the most favorable 30 DAS rice seedling parameters. The measurement was done for the first year field trial.

As shown in Table 2B, DCY84^T treatment and/or SiO_2 coating significantly improved the growth of the seedlings in regard to many metrics compared to the Mock treatment group. For example, shoots (483 ± 15.1–497 ± 11.6 mm vs. Mock, 410 ± 10.1 mm) and roots (160 ± 4.8–191 ± 7.6 mm vs. Mock, 124 ± 6.3 mm) were longer than those in the Mock treatment group. The combination of DCY84^T and SiO_2 treatments resulted in the heaviest fresh (4957 ± 140.1 mg vs. 2319 ± 315.1 mg for Mock) and dry weight (2097 ± 100.6 mg vs. 820 ± 46.0 mg for Mock). Moreover, the heaviest shoot and root dry weights were observed with both DCY84^T treatment and SiO_2 coating; dry shoots and dry roots were 1435 ± 72.7 mg (vs. Mock, 596 ± 40.2 mg) and 662 ± 51.3 mg (vs. Mock, 224 ± 23.4 mg), respectively. These significant results also showed consistently at second year field trial, whereas the combination of DCY84^T and SiO_2 treatments resulted in the heaviest fresh (5044 ± 156 mg vs. 3328 ± 133 mg for Mock) and dry weight (2112 ± 31.3 mg vs. 1055 ± 14.7 mg for Mock), also significantly longer shoots (425 ± 6.9–438 ± 10.4 mm vs. Mock, 410 ± 5.4 mm) and roots (153 ± 7.0–168 ± 5.5 mm vs.

Mock, 142 ± 6.2 mm) consistently showed on treatment group. Again, DCY84^T treatment and SiO_2 coating combination treatment provide heaviest shoot and root dry weights which was 1743 ± 62.8 mg (vs. Mock, 843 ± 28.9 mg) and 369 ± 12.1 mg (vs. Mock, 212 ± 27.0 mg), respectively. Thus, the combination of DCY84^T and SiO_2 treatments resulted in the most favorable characteristics of 60 DAS rice seedlings.

3.4. Shoot element analysis

As shown in Table 3, plants treated with DCY84^T had the most phosphate [P] (0.87 ± 0.005 mg kg^{-1}), potassium [K] (2.66 ± 0.109 cmol kg^{-1}) and total nitrogen [T–N] (2.47 ± 0.06%). Plants treated with SiO_2 had the most sodium [Na] (2.02 ± 0.017 cmol kg^{-1}). Furthermore, plants treated with both DCY84^T and SiO_2 had the most aluminum [Al] (0.54 ± 0.006 g kg^{-1}), iron [Fe] (0.77 ± 0.010 g kg^{-1}), calcium [Ca] (0.46 ± 0.009 cmol kg^{-1}) and magnesium [Mg] (0.68 ± 0.012 cmol kg^{-1})

3.5. Yield characteristics

Surprisingly, DCY84^T and/or SiO_2 did not significantly affect the fertility percentage (Table 4). However, DCY84^T plus SiO_2 did result in a higher number of spikelets per panicle (2014: 114 ± 2.9 vs. Mock, 97 ± 2.3; 2015: 90 ± 2.6 vs. Mock, 79 ± 2.4) as shown in Table 4. SiO_2 treatment resulted in the lowest number of spikelets per panicle at first trial (81 ± 1.5) and at second trial observation (82 ± 3.1 vs. Mock, 80 ± 3.8). Furthermore, SiO_2 treatment also resulted in the shortest panicle, which was 16 ± 2.1 cm at 2014 trial and 18 ± 3.0 cm at 2015 trial, while the longest panicle was observed with the SiO_2 and DCY84^T treatment (18 ± 3.1 cm vs. Mock, 17 ± 2.3 cm). 1000-grain weight revealed either DCY84^T with SiO_2 (2014: 156.9 ± 2.57; 2015: 121.6 ± 2.15) or without SiO_2 (2014: 121.8 ± 1.29; 2015: 118.2 ± 1.38) gave significantly higher mass. SiO2Treatment with DCY84^T and/or SiO_2 gave a greater grain yield and a greater total yield; DCY84^T plus SiO_2 treatment yielded the highest mass, 90 kg for grain yield (vs. Mock, 52 kg) and 109 kg for total yield (vs. Mock, 64 kg), each from 100 m^2 harvested area. Second trial at 2015 resulted consistently, with 99 kg of total yield and 87 kg of grain yield for DCY84^T and/or SiO_2 treatment (vs. Mock, 85 kg and 67 kg, respectively). Morphology of normal and abnormal spikelet can be seen in Supplementary Fig. S5.

3.6. Rice kernel characteristics and grain nutrient analysis

As shown in Fig. 2A, the combination of DCY84^T and SiO_2 treatments resulted in the highest whole kernel percentage (87.2 ± 0.57%), followed by SiO_2 treatment (78.0 ± 0.81%) and DCY84^T treatment (73.6 ± 2.41%). As a consequence of having lowest whole kernel percentage (63.3 ± 0.38%), seedlings in the Mock treatment group had the highest broken kernel percentage, which was 35.2 ± 0.45%. Remarkably, plants in the SiO_2 treatment group had 4.6 ± 0.24% chalky kernels and the highest protein percentage (7.2 ± 0.64%), whereas those in the Mock treatment group had only 1.1 ± 0.03% chalky kernels and lowest protein percentage (6.0 ± 0.08%). Nutrient content analysis showed distinct results for each treatment (Fig. 2B). Grains in the Mock treatment group scored the poorest in all parameters except amylose percentage, for which it was the highest at 18.3 ± 0.05%.

3.7. Content of Chlorophyll a, b and carotinoid in the rice leave

The content of chlorophyll a, b and carotinoid in the rice leaves which were treated with silica and DCY84^T has increased more than the mock without treatment.

3.8. Analysis of GABA content in brown rice grain

The silica and PGPR treated rice has essential amino acid more higher than the Mock (control) that was not treated. Major amino acid in the rice are composed of glutamate, aspartic acid, leucine, and valine. GABA content was detected by LC/MS/MS, silica and microbial dressing on the sprouted rice show higher GABA content than mock. Gene expression via RNA sequencing, future verification are required to be confirmed. Based on the genome analysis result of the strain DCY84^T that several sets of glutamate decarboxylase, malate transporter gene which involved in GABA production pathway were presented (data not shown). Each samples were treated by triplicate. After harvest, the GABA content of brown rice grain showed a 1.9-fold increase (field trial in 2014), 1.5 fold increase (2015) compared to mock grain, respectively.

4. Discussion

Agriculture is extremely susceptible to global climate change. Temperature and precipitation disturbances have limited not only the farming areas that are possible for agriculture, but also its crop growth and yields. Among the several different techniques that contribute to increasing rice crop productivity, soil fertilization with nutrients is paramount and silicon (Si), specifically, is one of the most important nutrients for rice. It helps the plants overcome multiple stresses including biotic and abiotic stress (Mitani and Ma, 2005). Seeds can be supplemented with silicon through seed coating, a common practice especially for the seeds of horticulture plants, forest plants and ornamentals. This advanced technology allows the application of a combination of nutrients, fungicides, insecticides, herbicides and beneficial microorganisms to the seeds (Corlett et al., 2014).

Microbiological communities in the soil or rhizosphere contribute to plant growth by recycling nutrients and making them available to the plant, increasing root health through competition with root pathogens or enhancing nutrient uptake (Weller et al., 2002). Among them, a group of beneficial plant bacteria referred to as plant growth-promoting bacteria (PGPB) are beneficial to plants via nutrient acquisition (Ladha and Reddy, 2003), biocontrol (Walsh et al., 2001), production of plant hormone-like compounds, reduction of the plant ethylene level (Glick, 1995) and induction of systemic resistance (van Loon et al., 1998). *P. yonginensis* DCY84^T, a promising PGPB previously described by Sukweenadhi et al. (2014), has been found to have the ability to produce indole acetic acid, produce siderophores, solubilize phosphate and also give the Arabidopsis plant resistance against drought, saline and aluminum stress (Sukweenadhi et al., 2015).

Junam-byeo (*Oryza sativa* L. cv. Junam) was used as the main experimental cultivar in this study. It is the most widely cultivated rice strain in Korea and it was registered as a new japonica rice cultivar in 2006 (Kim and You, 2010). In the soil pot test, all seeds had a germination rate of not more than 55% regardless of the treatment (Table 1). With the EC value around 2.42 ± 0.257 dS m^{-1} (Supplementary Table S1), this soil condition provided enough salinity stress to inhibit the seedlings' growth as previously described on a rice crop field in California with an EC value range of 0.5–3.0 dS m^{-1} (Scardaci et al., 1996). As a salt-sensitive crop, young rice seedlings are at a stage that is particularly sensitive to salinity (Lutts et al., 1995). Therefore, the early developmental stage of rice seedlings was assessed to test the effectiveness of DCY84^T and/or SiO_2 treatments on rice growth under saline stress. We observed *in vitro* that SiO_2-coated seeds germinated later (3–4 days) while seeds in the Mock group took only 1–2 days (data not shown). Not only germination rate, coating treatment also significantly reduced root length but it increased the shoot length. Although several growth parameters were measured, the vigor index is an important parameter because it reflects the overall health of the seedling and the productivity of the plant. A higher vigor index predicts a better yield from the plant, since the young seedlings grow better on saline field. Seeds treated with the SiO_2 coating had an overall lower vigor index compared to the combination of $DCY84^T$ and SiO_2. This result corroborates another related study in that *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* promoted rice growth under saline conditions (Jha et al., 2011). Longer shoots and roots of SiO_2-treated seeds also confirmed previous studies that reported satisfactory effects of a seed coating on root and shoot growth (Tavares et al., 2012).

The pilot experiment at the greenhouse was performed by introducing salinity stress (250 mM NaCl) to young seedlings (15 DAS), not seeds. The seedling stage is one of the most sensitive stages to salt stress in rice, and a trial of salt tolerance during this stage can probably provide insight into the field test results. During the stress period, all treatments exhibited similar symptoms, either pale-green leaves or withered leaves. However, after a recovery period, seedlings in the $DCY84^T$ plus SiO_2 treatment group recovered significantly better than those in the Mock group while the SiO_2 coating itself did not have a strong influence on the recovery level (Fig. 1). It seems that pre-inoculation with $DCY84^T$ triggered resistance against salinity stress in the rice. This result corresponds with a previous study in which $DCY84^T$ pre-inoculation promoted plant tolerance against salt on *Arabidopsis thaliana* (Sukweenadhi et al., 2015). Following these greenhouse results, we performed a similar experiment in a rice field on a reclaimed tidal land. As one of South Korea's efforts to expand farming areas, an estuarine tidal flat was developed on the coast of the West Sea in South Korea. Around 30% of the land has been set aside for agriculture. Only 17% of these lands have a salinity level that is acceptable for crop cultivation and the remaining 83% of the land has been deemed unsuitable for crop growth due to high salt levels. This land also has several other drawbacks such as limited macro- and micronutrients, restricted organic matter and physical and chemical properties that do not promote a good crop yield (Kim et al., 2014). Thus, these areas will require an integrated management strategy. Biotechnology provides promising solutions to improve not only the quality of crop itself, but also the interaction of the roots with soil-dwelling microbial partners. Commercial applications of PGPB are being tested and are frequently successful (Farzana et al., 2009). As observed on the seedlings at 60 DAS (Table 2), both $DCY84^T$ plus SiO_2 treatments resulted in the best growth parameters, followed by the $DCY84^T$ treatment. This result corroborates a previous report of *Methylobacterium oryzae* CBMB20 and *Brevibacterium iodinum* RS16 on sorghum and maize on reclaimed land (Kim, 2014). However, at an earlier stage (30 DAS), the SiO_2 treatment led to greater shoot and root dry weights, demonstrating the importance of silicon during the germination stage of rice in saline soil. Rice plants usually take up Si from soil at levels several fold greater than essential macronutrients such as N, P and K. Therefore, rice is a specific silica-accumulator among higher plants. The Si in rice enhances resistance to biotic and abiotic stresses (Tsujimoto et al., 2014). Thus, a SiO_2 coating might be useful to help young seedlings (around 30 DAS) grow under saline conditions. Under salinity stress, salt injury symptoms were perceptible including leaf drying and burning; severely stunted plant growth was seen in the Mock rice plants, but not in the treated rice. As previously reported by Sarangi et al. (2015), at a high seed density ($40\ g{\cdot}m^{-2}$), the average seedling shoot length was 533 mm and the average seedling root length was 139 mm for 40 DAS seedlings, which was lower than the results with a low seed density ($25\ g\ m^{-2}$). Meanwhile, in this experiment, the average $DCY84^T$ and/or SiO_2-treated seedlings' shoot length and root length were 483–497 mm and 160–191 mm, respectively (measured in 60 DAS seedlings). Thus, the $DCY84^T$ and/or SiO_2 treatments might have helped the seedlings overcome the high density of the seedlings ($45\ g\ m^{-2}$). Meanwhile, previously, co-inoculation of PGPB during the early developmental stage on plants grown in Saemangeum reclaimed soil showed that plant growth-promoting *B. iodinum* RS16 and *M. oryzae* CBMB20 increased plant height, dry biomass accumulation and macro-nutrient accumulation of maize and a sorghum-sudangrass hybrid. Macronutrient accumulation (P, K, Ca and Mg) was higher with *M. oryzae* CBMB20 treatment compared to the non-inoculated treatment, while *B. iodinum* RS16 treatment increased N, Ca and Mg accumulation compared to the non-inoculated treatment (Kim et al., 2014). Another report found that inoculation with *Actinomycetes* NB_1, AVermi7, YB6y and NB_3 in combination with a full rate of fertilization significantly increased P uptake by 80 to 136% over the non-inoculated control (Cruz et al., 2014). This pattern of nutrient uptake and accumulation was also observed with $DCY84^T$ and/or SiO_2 treatment on the Junam rice cultivar after 60 days on reclaimed tidal land (Table 3). Remarkably, we observed that $DCY84^T$ treatment increased P accumulation by 34%, available K by 44% and 2.4-fold higher total N content compared to the Mock group while $DCY84^T$ plus SiO_2 treatments showed the highest amounts of Al, Fe, Ca and Mg (54%, 169%, 42% and 67% higher, respectively, compared to the Mock group). Previous report on saline-stressed barley, silicon found to enhance salt tolerance attributed to selective uptake and transport of potassium and sodium by reducing sodium but increasing potassium concentrations (Liang, 1999). Meanwhile, Gong et al. (2006) reported silicon deposition in the exodermis and endodermis reduced sodium uptake in rice (*Oryza sativa* L.) seedlings under NaCl stress through a reduction in byplass flow. However, at 60 DAS Junam rice seedlings, either sodium and potassium concentration were found to be higher than Mock. It seems that the Si-coating treated seeds have different mechanism of alleviating saline stress compared to Si-giving seedlings. Further study is needed to confirm this prejudice.

Plants treated with both $DCY84^T$ and SiO_2 had the lowest normal grain percentage, but they had the highest number of grains/panicles (Table 4). This result confirmed previous silicon fertilization treatment of upland rice, which reduced the number of blank (abnormal) spikelets per panicles and increased grain mass, but did not affect grain productivity (Mauad et al., 2003). $DCY84^T$ and/or SiO_2 treatments gave a higher grain yield and total yield compared to Mock crops, and treatment with $DCY84^T$ plus SiO_2 yielded the highest mass (in average grain yield was 44% greater and total yield was 43% greater). Das and Saha (2005) reported that PGPB inoculation with non-symbiotic N_2 fixing bacteria *Azotobacter* (strain DS3) and *Azospirillum* (strain DM10) significantly increased growth and yield (23.7%) of rice. Kernel analysis showed that $DCY84^T$ and/or SiO_2 treatment significantly increased the whole kernel percentage and decreased the broken kernel percentage. With a full rate of fertilization, inoculation with NB_3 and AVermi7 significantly increased grain yield and total yield by 62% and 48%, respectively, relative to non-inoculated treatment. The significant increase in grain yield by NB_3 and AVermi7 demonstrated the potential of these *Actinomycetes* as plant growth-promoting inoculants for upland rice (Cruz et al., 2014).

In case of grains quality, $DCY84^T$ and/or SiO_2 treatment highly increased the whole kernel percentage. Thus, lower its broken kernel percentage to 9.60-24.58% (Fig. 2A). Broken rice is generally at only 30 to 50% of whole grain and it considerably lower commercial value than whole, unbroken kernels; therefore, farmer strive to produce rice with the least amount of broken rice (Mutters, 1998). Grain formation process play important roles in this and previous report found that salt stress during reproduction stage can disrupt this process (Moradi et al., 2003). Lower broken ker-

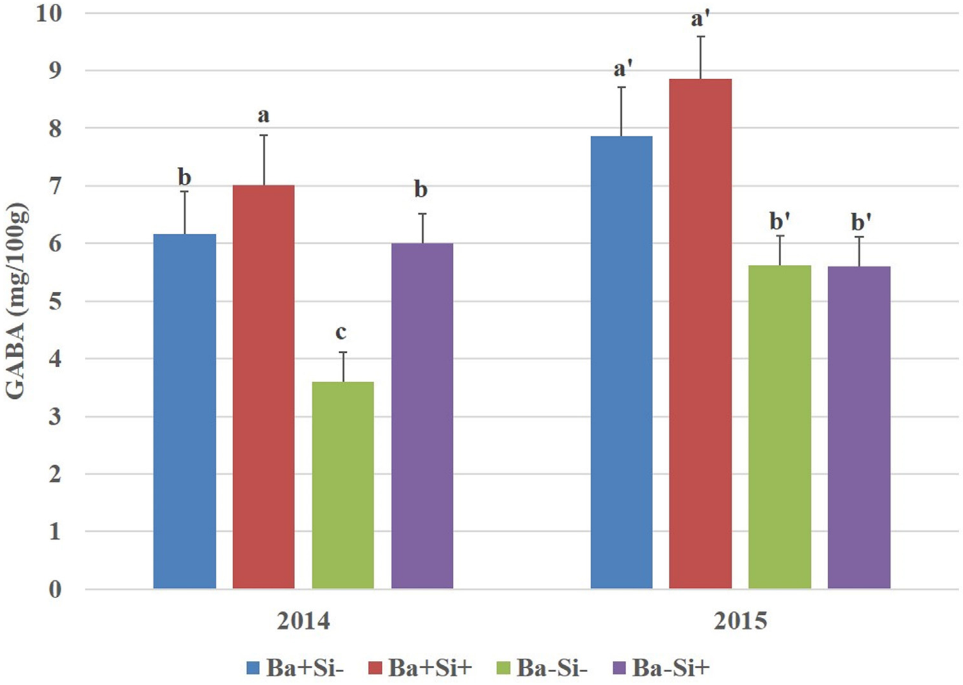

Fig. 3. Content of GABA in the brown rice after harvest. 2014 Junam rice cultivar were harvest from reclaimed salty rice soil, 2015 Hiamyi rice cultivar was harvested in normal rice paddy soil. Ba + Si⁻, only DCY84 dressing on the sprouted rice; Ba + Si+, *Paenibacillus yonginensis* and silica dressing; Ba-Si-, mock without any treatment; Ba-Si+, only silica dressing. The differences are highly significant (a, p <0 .001), (a', p < 0.01).

nel indicated that DCY84^T and/or SiO_2 treatment can alleviate the salt stress interference during the grain filling process. Meanwhile, chalkiness percentage of all treatment seems to be correlated with its total N content in which higher N content reduce the percentage of chalky kernels on yield. Qiao et al. (2011) reported that N fertilizer had a favorable effect in decreasing the ratio of chalky grains. It is well-known that the formation of chalkiness is associated with an insufficient substrate supply during the arrangement, size and shape of starch granule on the developing endosperm (Lisle et al., 2000). Based on amylose content, milled rice is classified in "amylose group" such as: waxy (1-2% amylose), very low amylose content (10-20% amylose), intermediate amylose content (20-25% amylose), high amylose content (25-33% amylose) (IRRI-International Rice Research Institute, 2004). Belong to Japonica, amylose content of Junam cultivar kernels were similar each other (range between 17.08-18.27%) regardless its treatment (Fig. 2B). This result confirms Junam cultivar as very low amylose content group, which commonly consumed in Northeast Asia, where a more cohesive cooked grain is often preferred; they are characteristic of the temperate Japonica variety groups that predominate in this region (Juliano and Villareal 1993). However, DCY84^T plus SiO_2 treatment resulted on highest protein content, which was above 7%. Generally, the taste of rice is determined by protein and amylose content. Rice with a good taste has a protein content under 7% and a water content between 15.5% and 16.5%. When rice is cooked, rice with a higher protein content is more compact, less elastic and less viscous (Yun et al., 2014). It already known that the protein content of milled rice is low in comparison with other cereals, although the whole rice grain protein content ranged from 7.0 to 10.8% of which 70–80% is in the glutelin. Commonly eaten rices generally contain about 7% protein and do not fluctuate widely form this level, protein content is not considered an important indicator of quality (Hamaker and Griffin, 1993). Meanwhile, there were no significant differences in the water content of the rice in any of the treatment groups (15.4–15.7%). Our findings show for the first time that GABA seems to have a protective role in silica and *Paenibacillus* strain DCY84 treated rice in reclaimed salty soil Fig. 3.

5. Conclusions

This field study provides encouraging results and a basis for future research. The overall results show that DCY84^T co-inoculation and/or SiO_2 coating of seeds can be useful methods to promote the growth of rice under saline stress conditions. It increased the seed germination and seedling vigor index during saline stress based on pot test and significantly help the seedlings growth on reclaimed tidal land. Thus, the total yield also significantly increased even though the cultivation was done under saline condition. The seed dressing effect seems not too strong for individual bacteria (DCY84^T) or coating (SiO_2) treatment, but stronger on combination of both treatment. The bacterial treatment might affected most on root system while Silicon treatment might affected on shoot system. It may function by increasing the endogenous levels of GABA to improve root growth and by decreasing the salt stress. Further, transcriptomic with metabolomics approach can be done to reveal the role of bacteria and silicon. Optimization the protocol for the use of DCY84^T (or other PGPB) and SiO_2 seed coating with technical formulation trials can be useful for further improved crop quality. Indeed, the results of this study can be applied to other reclaimed tidal lands of South Korea or other areas in coastal states of South Korea that have similar ecologies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2016.01.005.

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