Effect of Coating on Plant Seeds for Rehabilitating Deteriorated Highland

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Abstract

Seeds of two grass species, Italian ryegrass and Chinese milk vetch, were coated with plant-derived hygroscopic polysaccharide gums and powdered dry alga. The coated seeds were then inoculated with the spores of microorganisms that had been isolated as decomposers of the gums and the alga.

Under the controlled conditions (low precipitation and low temperatures), simulating the spring climate of the Qinghai-Tibetan Plateau, in a growth chamber, the germination percentage of the uncoated seeds was remarkably lower than those under the moderate full-moisture conditions (control), while the seedling emergence percentage of the coated seeds showed similar levels to the control. Coated seeds started germination earlier than the uncoated ones. The seedling emergence roughly reversely paralleled to the rates of water loss from the gels of the coating mixtures (water conserving capacities), which suggests that moisture conserved by the coating mixtures was responsible for enhancement of seedling emergence under the controlled conditions.

Effect of the alga in the coating materials and the spores of the microorganisms which had been isolated as the decomposers of the coating materials was examined on the growth of two plants. The results showed that the dry weight of both plants (photosynthesis production) were remarkably increased only when both the alga and the spores were present in the coat. The number of the nodules on Chinese milk vetch varied in the same tendency as the dry weight. The apparent O_2 consumption of the soil after the plants were harvested as an indicator of respiring soil microbes was found to be also in parallel with the dry weight of plants on the soil. It is strongly

suggested that the coat-dependent improvement of plant growth was largely due to the increase in the amount/activity of soil microbes and/or in favorable production in the soil which directly or indirectly promoted the growth of plants and soil microbes.

After the plants from the coated seeds were harvested (the first harvest plants), uncoated seeds were sown on the post-harvest soil in the original pots. The dry weight of the plants from uncoated seeds (second harvest plants), the number of nodules and the apparent O_2 consumption of the soil after the second harvest plants showed the same tendency as obtained with the first harvest plants. It is suggested that soil conditions favorable to plant growth have improved and sustained even after the plants grew from the seeds that were coated with both the alga and the spores.

It is demonstrated that seed coating with hygroscopic nutrient-source materials and inoculation with microorganisms/spores as their decomposers can be a promising method for re-vegetation of deteriorated rangeland through constructing own rhizospheres by conserving moisture around seeds and propagating the soil microbes.

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1 Introduction

Worldwide problem of land deterioration is exemplified seriously on the rangeland of the Qinghai-Tibetan Plateau in China. Deterioration of the rangeland on the plateaus is generally characterized by reduction of plant diversity and density, and loss of soil fertility, which eventually lead to destruction of ecological environment. Because the rangeland carries relatively stable ecosystems due to its special inheritance with a certain level of productivity over thousands of years, it is very difficult to restore these ecosystems of the rangeland once destroyed. At present, re-vegetation has been considered to be an effective option to rehabilitate the deteriorated land, in which large amount of investment has been poured (Zhang, Y., 2003).

Soil moisture and microbes are two important factors for establishment of plant communities. However, drought is predominant characteristics of the climate on the plateau; there are less than 300 mm annual rainfall over 65% of the rangeland and around 500 mm over the rest (Long, R. 2003). The population of soil microbes on the rangeland (3200 m above sea level) varies seasonally; reaches the highest in between mid-July to mid-September, depending on plant density and types, while the activity and diversity of the soil microbes on the rangeland are extremely poor in the rest of the year (Zhu et al. 1982). Organic matters in soil is not effectively mineralized and converted to available nutrients to plants due to low population and activity of soil microbes. Application of chemical fertilizers ensures certain plant production only temporarily but gives little effect on soil microbes, so that soil quality can not be improved and deteriorated land can not be remedied for long-term plant production. Therefore, even though re-vegetation programs have been implemented on the

plateau for decades, they have not yet been very successful. (Ma, Y. et al, 1999).

Applications of algae and microorganisms on the soil of the rangeland have been reported. Spray of an alga (unicellular marine alga *Phaeocystis* sp. necolon-1) suspension directly on the deteriorated soil resulted in overall increase in the activity of soil microbes as well as the capacity of preventing silt from flowing away (N. Liang, 2006). Application of effective microorganisms (so-called EM) on soil was also reported to be favorable to root growth, photosynthesis efficiency and grain yield, and has been widely used to improve soil conditions and crop production (Kobayashi, M. 1980; Nandi, A.S. et at., 1981). Nutrition in soil became more available through increased activities of soil microbes when some microorganisms were applied on the peat soil (Perner, H., et al., 2006). Phosphate content in the volcanic soil was improved through application of certain microbes (Morales, A. et al., 2007). These reports suggested that favorable effects algae and microorganisms can be expected in improvement of the deteriorated soil.

Attempts have been made under a concept that when a sound micro-cosmos of rhizosphere is created for individual plants, it may initiate a process of land rehabilitation. In an experiment, holes were made on the wood piles, which were stuffed with the powder of dried alga, newspaper waste and spores of microorganisms which digest the stuffing. A tree seed was mounted in the stuffing in the wood pile, which was then punched into land. It was found that seedling emergence and growth of the tree seeds were enhanced especially on barren land (Kobayashi, A. 2006). The results may be extended to re-vegetation of the deteriorated rangeland by coating plant seeds (hereafter noted as grass seeds) with similar ingredients in order to tackle the problems of poor soil moisture and microbes.

Seed coating technique has been utilized since 1930's mostly as a carrier of agricultural inputs such as fertilizers, plant growth regulators, fungicides etc., or as a method to uniform seed size and to control seeding rate. Seed coating with certain polymers, such as agglomerating vermiculite, CF Clear® can regulate the moisture around the seeds. When being coated with these polymer, high germination percentage of seeds was found in carrots (Medeiros, E. et al, 2006.), rice (Arsego, O. et al 2006), corn (Gesch, R. W. at al 2005) and bush plants for post-mining restoration (Turner, S. R et al. 2006). Although the results showed promising effect of seed coating with polymer, negative effects of acrylamide copolymer hydrogel coating were also reported in emergence of wheatgrass (Mangold, J. et al., 2007).

In this study, effect of coating seeds of rangeland plants were examined, with polysaccharides for retaining moisture, the alga for supplying nutrients when digested, and the spores for digesting the coating material after germinated. Gramineal Italian ryegrass (IRG) and legume Chinese milk vetch (CMV) were chosen which are the most dominant plants families on the rangeland of the plateau. The seeds were coated with plant-derived polysaccharide gums and the powder of dried marine alga *Phaeocystis* sp. necolon-1 (hereafter noted and the alga). Conidoispore of microorganisms, *Aspergillus* sp*. and Streptomyces* sp. (hereafter noted as the spores) were inoculated on the coat as decomposers of the coat.

The effects of seed coating on germination and seedling emergence were studied in a growth chamber under the conditions simulating the spring climate on the Qinghai-Tibetan Plateau. In the growth chamber, the outdoor roofed pots and open beach land, the effects of the spores and the alga in the coat on plant growth and on soil conditions were examined. The results showed that the coated seeds had much

better rates in seedling emergence than the uncoated control, and that plant growth was remarkably improved when both the alga and the spores were involved in the coat. The results also indicated that improved soil conditions were sustained even after the plants of the coated seeds were harvested.

2 Materials and methods

2.1 Materials

2.1.1 Plant species

Plant species used were monocotyls gramineal Italian ryegrass (*Lolium multiflorum*, IRG) and dicotyls legume Chinese milk vetch (*Astragalus sinicus*, CMV), which were obtained commercially.

2.1.2 Coating materials

- *Polysaccharide gums*

Gum Xanthan (Xan), carboxymethyl cellulose sodium (CMC), carrageneen (Car), and wheat flour (WF) were chosen as coating materials and obtained commercially.

- *Alga; Phaeocystis necolon-1 sp. powder*

Phaeocystis sp.necolon-1 was collected and isolated in Okinawa, Japan. The alga was cultured for 4 weeks in artificial seawater enriched with Eppley's medium in 100L tanks at room temperature with aeration and illumination at 1600 lux. The alga was collected by centrifugation, washed with water and oven-dried at 65℃, then milled to powder (denoted as the alga). About one half of the dry weight of the alga corresponds to polysaccharide encapsulating the algal body. The components of the alga powder were reported to be 60% organic materials, and 37% of inorganic materials (Kurachi, T. 2003)

2.1.3 Spores of *Aspergillus* sp. and *Streptomyces* sp.

Aspergillus sp., a fungus, and *Streptomyces* sp., an actinobaterium, were isolated as the decomposers of newspaper waste/alga mixed sheets. Conidiospores (hereafter noted as the spores) of the two microorganism species were inoculated and incubated on potato dextrose agar plates at 25℃. After 14 days, the spores produced by newly

grown hypha were washed out from the agar surface with NaCl solution (0.85%) containing 0.01% Triton X-100, collected by centrifugation and stocked at –20℃. The suspension made as 1:1 mixture of the two microorganisms contained $1.7x10^6$ spores/ml (denoted hereafter x1 spores) or, after 10 times dilution, $1.7x10^5$ spores/ml (denoted x0.1 spores). Germination percentage of the spores was found to be 89-96% at the first thaw of the frozen spore suspension on mixture of above gums as the sole carbon sources (data not shown).

2.1.4 Soil

Commercially available gardening soil and soil mixtures consisting of mostly sand and clay were used. The soil used was in the category of sandy loam or loamy sand in the triangle of soil texture Fig. 1 (USDA), with quite low water holding capacity.

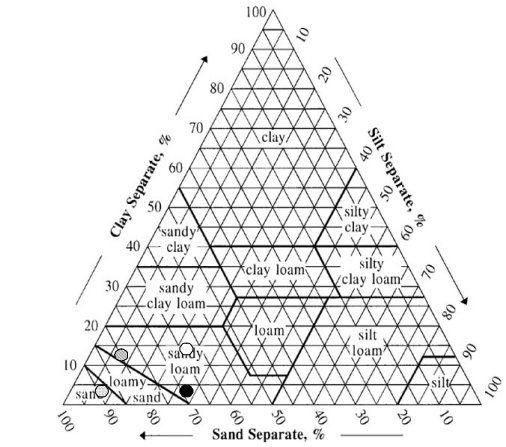

Fig.1 Texture of the soil in the study in the triangle of soil texture

● A soil sample of the rangeland of the plateau (3920 m)

○ Soil in the growth chamber

◎ Soil in the outdoor pots

2.2 Methods

2.2.1 Apparatus for seed coating

The apparatus was a vertical plastic cylinder equipped with an electric blower at the bottom and sealed with a ventilating net on the top. The seeds in cylinder were forced to fly by air blow from the bottom (Fig. 2). Another apparatus was a rolling bowl, where seeds and coating mixtures (gums and the alga) were mixed together (Fig.3).

2.2.2 Seed coating

Seeds were wet with water and mixed with coating mixtures (containing the gums and the alga powder) in the rolling bowl (Fig.3). The slightly coated seeds were blown in the cylinder (Fig. 2) and sprayed with water mist from the side hole on the lateral wall of the cylinder. The misted seeds were again mixed with the same coating mixture

Fig.2 Vertical cylinder of seed blower

Fig. 3 Rolling bowl for seed coating

in the rolling bowl. The process was repeated 2-3 times, until seeds were sufficiently coated, then air-dried at room temperature.

For inoculating the spores on the coated seeds, thirty each coated seeds were dipped in 1 ml each of the stock spore suspension (x1 or x0.1spores) for 30 seconds.

2.2.3 Apparent O_2 consumption of soil

In order to estimate rough population/activity of soil microbes, the apparent O_2 consumption of soil was measured after showering a glucose solution on the soil. The apparatus fundamentally consisted of two tight-sealed glass jars containing 4M KOH solution at the bottom and connected to the two inputs of a micro-pressure a difference transducer (KYOWA, PDV-10GA), which had an electric output to recorder (Fig. 4). Five ml water or glucose solution (700mg/ml) was showered on either one of

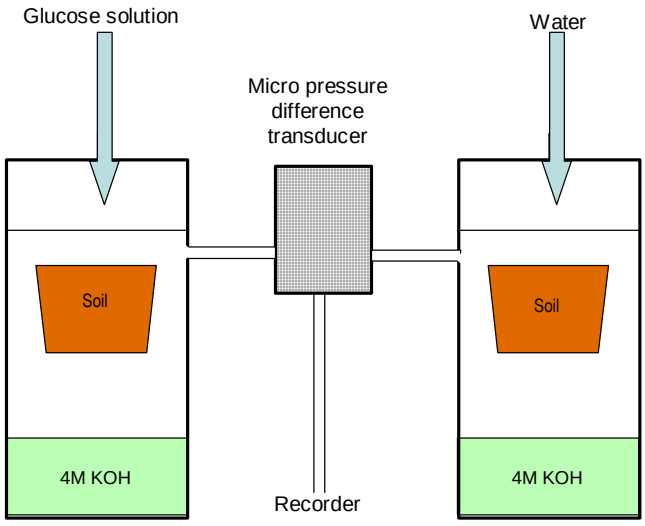

Fig. 4 Schematic diagram of the apparatus for apparent O_2 consumption

the soil sample in the jars, and the rate of the pressure change was recorded. The rate of the pressure decrease due to absorption of the produced CO_2 by the KOH solution 2 min after the showering was listed as the relative population /activity of (respiring) microbes in the soil. The apparatus was tested for its sensitivity, stability and linearity with soil samples containing crop field soil and autoclaved soil (at 120℃ for 40 minutes) at given ratios (Appendix Fig.1).

2.2.4 Experimental conditions

Growth chamber

Laboratory experiments were conducted in a growth chamber (NK System LH 220 SP 22F-A4P), which was programmed to be 16 hrs light (2400 lux) and 8 hrs dark at 25℃ for germination of seeds, and to be 16 hrs light (2400 lux) at 15℃ and 8 hrs dark at 5℃ for seedling emergence. Seeds were saturated with water in the test for the germination. Coated seeds were given 40 ml water to each tray 7X7X2 cm^3 at sowing and 5 ml water every day later on when they were tested for seedling emergence. The spring climate on the rangeland of the plateau was simulated in the conditions for seedling emergence.

Outdoor roofed pots

The outdoor experiments were carried out in the pots, 18X18X20 cm^3, under roof from September 5th to November 23rd, under the temperature ranged between 9 and 31.6℃, and the average sunlight was recorded to be 6.8 hrs/day. Water (300 ml) were given to each pot every 2 days.

Open sandy beach land

Field experiments were carried out in sandy beach land about 100 m away from the Pacific ocean in Nankoku, Kochi, Japan from August 1st to October 20th under the temperatures ranged between 16℃ and 31.6℃, in the average sunlight 6.4 hrs/day and average rainfall 5.8 mm/day. There was no vegetation for years before the experiment. About 3 L water were given to the plants every day.

3 Results

3.1 Effect of the seed coating with different coating mixtures on seedling emergence

3.1.1 Germination of the seeds under different conditions

Under two climate conditions two grass species, IRG and CMV, were tested for germination. The one condition was set to be 16 hrs light (2400 lux) and 8 hrs dark at 25℃ with water saturation in Petri dishes throughout, and to be moderate conditions for germination. The other was controlled to be 16 hrs light at 15℃ and 8 hrs dark at 5℃ with 40 ml water to soil at sowing and 5 ml water to each tray every morning later on, simulating the spring climate on the plateau. Seeds were sown on soil in a plastic tray 7X7X2 cm^3. Germination was counted when radicals became at least 1 mm high, counting was done every morning and germinated seeds were removed from the Petri dishes. The averaged results of 3 repeats are shown in Table 1.

Table 1. Germination characteristics of grass seeds under two watering conditions in the growth chamber*[1]

		Grass species	
Watering conditions		Italian ryegrass (IRG) *Lolium ultiflorum*	Chinese milk vetch (CMV) *Astragalus sinicus*
Saturated*[2] at 25℃*[3]	Seeds germinated/100 sown	86±3.3	88±1.7
	Germination (%)	86	88
Controlled*[4] at 5 -15℃*[3]	Seedlings emerged/50 sown	19±1.3	12±0.7
	Seedling emergence (%)	38	24

*[1] Averaged data from 3 repeated experiments

*[2] the seeds were always kept wet

*[3] 16 hrs light (2400 lux) and 8 hrs dark, common to both temperature conditions

*[4] 40 ml/50 cm^2 water at sowing and 5 ml day later on to 50 cm^2X2cm soil

Germination percentages of the randomly chosen seeds of IRG and CMV were 86%

and 88%, respectively, within 5 days under moderate conditions. Under the controlled growth conditions, it was found to be 38% and 24% for IRG and CMV, respectively, even after 45 days of culture.

3.1.2 Effect of seed coating with different coating mixtures on seedling germination

Effect of seed coating and coating mixtures on the seedling emergence was tested under conditions of 16 hrs light at 15℃ and 8 hrs dark at 5℃. Fifty seeds coated with 4 different coating mixtures, #A, #B, #C, #D (Table 2), were sown on soil in a plastic tray with soil size of 7X7X2 cm^3. Uncoated seeds were control. Each tray was given 40 ml (equivalent to 8.2 mm rainfall) water at sowing and 5 ml/day (1 mm rainfall) water every morning. For the coated seeds, no radicals but only coleoptiles were visible. Therefore, seedling emergence was tested for the coated seeds. Seedling emergence was counted when coleoptiles became at least 1 mm high and emerged seedlings were removed from the tray. Seedling emergence was counted every morning until no more new seedling emerged. Each experiment was repeated 3 times. The seedling emergence percentage and the emergence rates (midpoint increment) are listed in Table 2. The seedling emergence processes are shown in Fig. 5 and Fig. 6. It was clearly shown that under the controlled growth conditions, regardless of plant species, the seedling emergence percentages of any coated seeds became similar to the germination under the moderate conditions. Furthermore, the initial date of emergence, the emergence rate and final number of plants were found to be different, depending on the compositions of the coating materials.

Table 2. Seedling emergence of the seeds coated with coating 4 mixtures in the growth chamber*[1]

		IRG					CMV				
Labels of the coating mixtures		#A	#B	#C	#D	Uncoated	#A	#B	#C	#D	Uncoated
Coating materials (%)	Algal powder	20	20	20	20		20	20	20	20	
	Xan		20	40				20	40		
	CMC	40	40		30		40	40		30	
	Car	40	20		20		40	20		20	
	Wheat flour			40	30				40	30	
coating material / seed (mg)		4.6±0.4	4.2±1.1	4.9±2.3	4.4±1.7		5±0.7	4.3±0.9	5.2±1.3	4.5±0.4	
Seedlings emerged / 50 sown		40±7	38±3.3	31±2.7	30±1.7	19±1.3	41±4.7	37±5.3	27±2.7	24±4	12±0.7
Seedling emergence (%)*[2]		80	76	62	60	38	82	74	54	48	24
Emergence rate (%)*[3]		15.6	11.1	4.4	4	2.2	17.8	15.6	6.7	6.7	4.4

*[1]16 hrs light (2400lux) at 15℃ and 8 hrs dark at 5℃; 40 ml/50 cm^2 water at sowing and 5 ml/50 cm^2·day later on; averaged data from 3 repeated experiments

*[2] Final percentage of seedling emergence after 45 days of culture

*[3] Mid point increment of seedling emergence/day (Fig. 5 – 6)

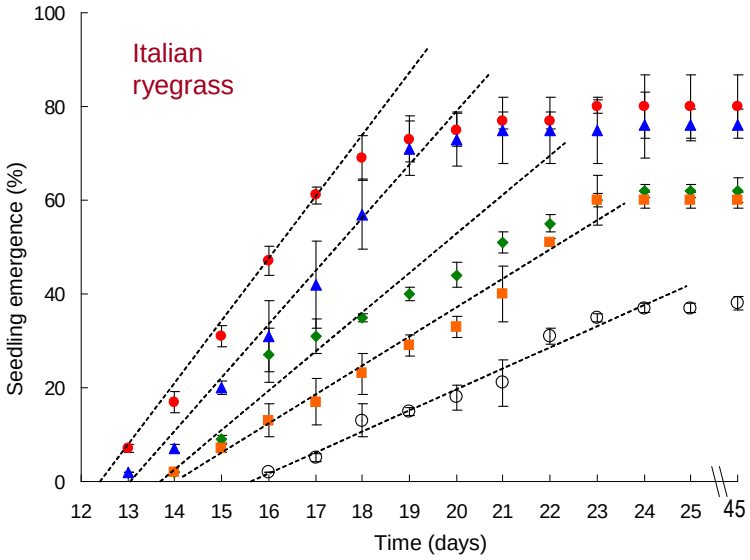

Fig. 5 Seedling emergence of IRG seeds coated with 4 different mixtures in the growth chamber

#A(●), #B (▲), #C (◆), #D (■), Uncoated seeds (○).

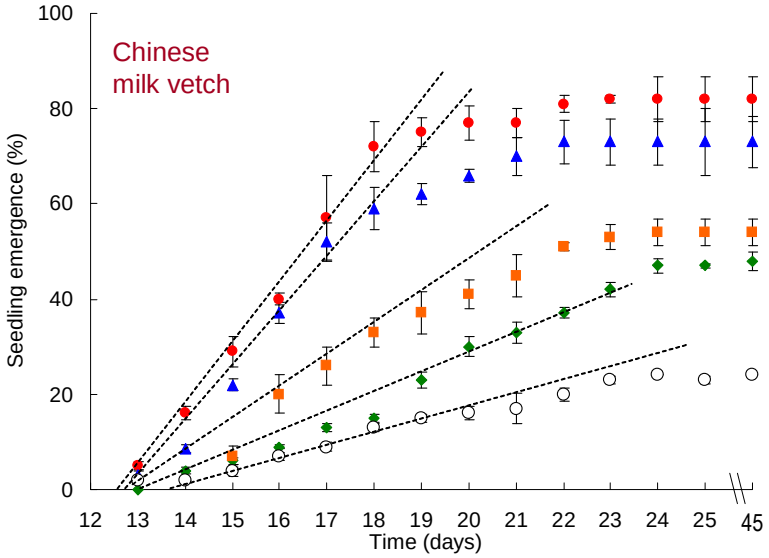

Fig. 6 Seedling emergence of CMV seeds coated with 4 different mixtures in the growth chamber

#A(●), #B (▲), #C (◆), #D (■), uncoated seeds (○).

3.1.3 Rate of drying of the gels of the coating mixtures

Water conserving capacities of the coating mixtures were estimated by the water loss from the gels made of the four mixtures, #A, #B, #C and #D (Table 2). One half gram powder of each of the mixtures was dissolved in 1 ml water and the gels were oven-dried at 40℃. Soil (0.5 g) was mixed with 1 ml water as the control. Weight of the gels and the soil with water was measured every 0.5 hr. The experiment was repeated 3 times and averaged. As shown in Fig. 7, the gels of the mixtures lose water in different time processes.

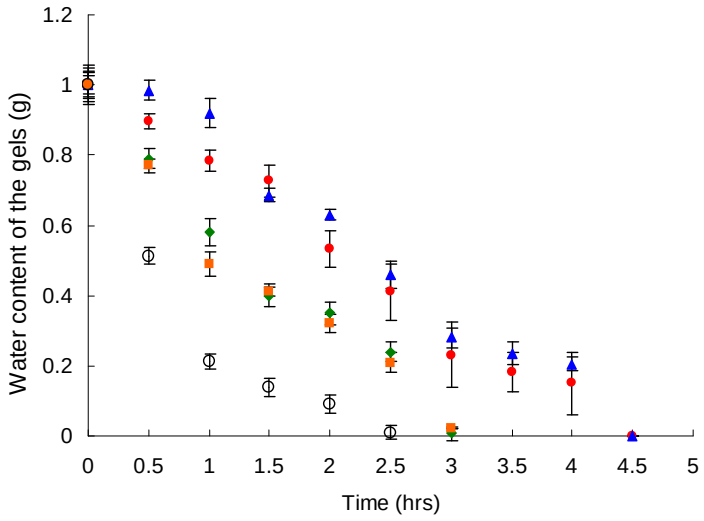

Fig. 7 Water loss of the gels made by the coating mixtures #A(●), #B (▲), #C (◆), #D (■), #S soil+water (○).

3.2 Effect of the alga and the spores in the coated seeds on plant growth

3.2.1 Effect of the alga and the spores on plant growth in the roofed outdoor pots

Coating mixture #B was chosen to coat the seeds of the two plant species. To test

the effect of the alga, the algal powder was replaced with Car in the mixture #B (20% Xan, 40% CMC, 40% Car, Table 2). To test the effect of the spores/microorganism, the coated seeds were inoculated with x1 or x0.1 spore suspension. Coated seeds without the spores and uncoated seeds were also tested as the control. Seeds were sown in the roofed outdoor pots with soil size 300 cm^2x20 cm, which were under conditions of average 6.8 hrs/day sunlight, from 9℃ to 31℃. Water (300 ml) was given to each pot every 2 days. Seedling emergence was counted every morning. Initial seedlings were kept for measuring the growth parameters on the 80th day, including dry weight of plants, dry weight of leaves, height, number of leaves, dry weight of roots, root length and number of nodules (Table 3 and Table 4). After the plants were harvested, the soil was tested for the apparent O_2 consumption.

The growth parameters were listed in the Table 3 and Table 4. The difference among the plants grown under different conditions in dry weight (photosynthesis production of plants) was further illustrated in Fig. 8 and Fig. 9. For both IRG and CMV, the plants from the coated seeds with the alga (+) had notably larger dry weight than the plants from the coated seeds without the alga (–) and the plants from uncoated seeds. When the alga was present in the coating, the dry weight increased substantially along with increase of the spores from the x0 to x1. The highest dry weight of plants was observed when the alga and x1 spore were simultaneously included in the coat. In addition, for CMV plants, the number of nodules was remarkably large when both the alga and the spores were present in the coat (Fig. 9 and Table 4). Distribution of dry weight of leaves and roots of the individual plants in the outdoor pots were shown in Table 5 and Table 6. The distribution clearly indicated the combined effects of the alga and the spores. Apparent O_2 consumption of the soil

in the outdoor pots (Fig.10) showed that, regardless of the plant species, the alga and the spores cooperatively led to higher O_2 consumption. Apparent O_2 consumption of the soil changed in parallel with the dry weight of plants.

Table 3. Growth parameters of IRG plants in the outdoor pots *[1]

	Coated				Uncoated
		Spore dosages			
Parameters*[2]*[3]	The alga	X1 spores	X0.1 spore	X0 spore	control
Seedling emergence %	+	77	87	90	57
	-	83	80	87	
Dry weight of plants (mg/plant)	+	184.2±61	109.0±57	45.4±19	17.7±4.9
	-	45.1±17.3	32.9±15.8	21.9±4.7	
Dry weight of leaves (mg/plant)	+	133.7±46.2	76.0±4.2	35.3±19.2	11.5±3.0
	-	33.4±14.3	23.3±12.2	15.5±4.1	
Height (cm)	+	21.5±5.5	18.4±5.9	13.8±4.6	10.1±2.2
	-	14.7±5.2	10.9±4.6	10.3±2.6	
Number of leaves	+	9.6±2.5	7.9±2.1	5.2±1.5	3.2±0.4
	-	5.6±1.5	4.3±1.1	3.2±0.8	
Number of shoots	+	2.7±0.9	2.4±0.8	0.8±0.8	1
	-	1.7±0.7	1.3±0.3	1	
Dry weight of roots (mg/plant)	+	50.5±19.4	33.0±18.2	10.1±3.1	6.2±2.4
	-	10.9±4.2	9.1±4.0	6.4±2.5	
Root length (cm)	+	25.0±4.4	21.7±3.5	15.8±2.4	7.7±2.2
	-	14.9±2.8	13.3±2.6	7.3±2.4	

*[1] 9 - 31.6℃, 6.8 hrs sunlight/day, 300 ml water every 2 days from Sept.1st to Nov. 23rd

*[2] Growth parameters in this table are from all plants, heights are from all the shoots; leaves/plant include leaves of main shoots and tillers

*[3] Averaged data of 3 repeated experiments for seed emergences

Table 4. Growth parameters of CMV plants in the outdoor pots *[1]

Parameters*[2]*[3]	Coated				Uncoated control
		Spore dosages			
	The alga	X1 spores	X0.1 spore	X0 spore	
Seedling emergence %	+	77	73	75	57
	-	86	86	86	
Dry weight of plants (mg/plant)	+	156.4±91.1	117.7±53.9	28.2±10.1	10.8±5.3
	-	24.3±11.5	20.3±8.6	15.9±2.9	
Dry weight of leaves (mg/plant)	+	119.7±72.9	86.7±47.1	15.2±6.2	5.3±2.5
	-	14.4±7.5	12.1±5.2	9.1±1.9	
Height (cm)	+	7.7±1.0	6.9±1.6	2.7±0.7	1.9±0.4
	-	3.4±1.3	2.6±0.8	2.0±0.6	
Number of leaves	+	14.4±5.2	14.1±5.9	4.2±1.1	1.5±0.7
	-	4.2±0.9	3.8±0.9	3.2±0.8	
Dry weight of roots (mg/plant)	+	36.8±20.7	30.0±11.1	13.0±6.0	5.6±3.1
	-	9.9±5.3	8.2±3.5	7.7±1.6	
Root length (cm)	+	16.0±2.3	16.2±3.2	12.0±2.2	6.8±2.3
	-	12.7±3.3	11.2±2.7	11.1±2.3	
Number of nodules	+	24.5±10.3	20.9±8.8	0.1±0.2	0
	-	1.9±1.9	1.5±1.6	0	

*[1] 9 - 31.6℃, 6.8 hrs sunlight/day, 300 ml water every 2 days from Sept.1st to Nov. 23rd

*[2] Growth parameters in this table are from all plants, averaged data of 3 repeated experiments for seed emergences

*[3] Averaged data of 3 repeated experiments for seed emergences

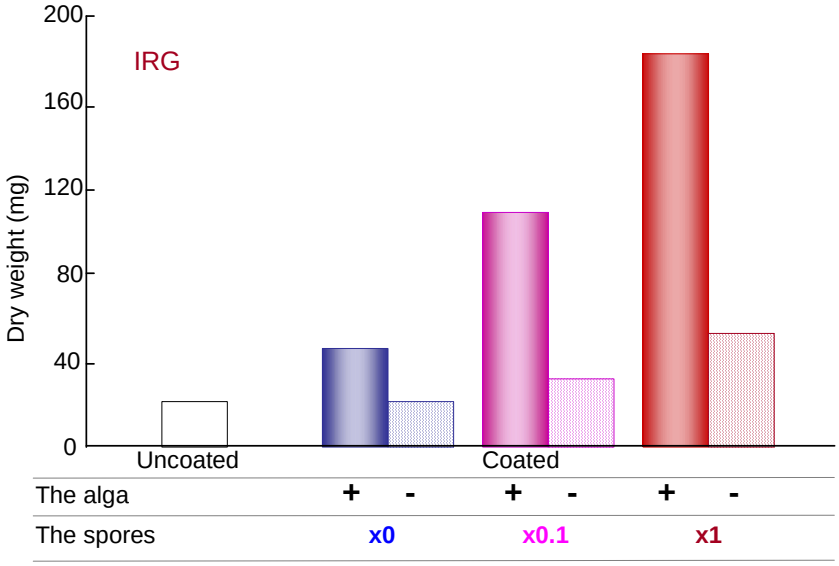

Fig. 8 Dry weight of IRG plants in the outdoor pots

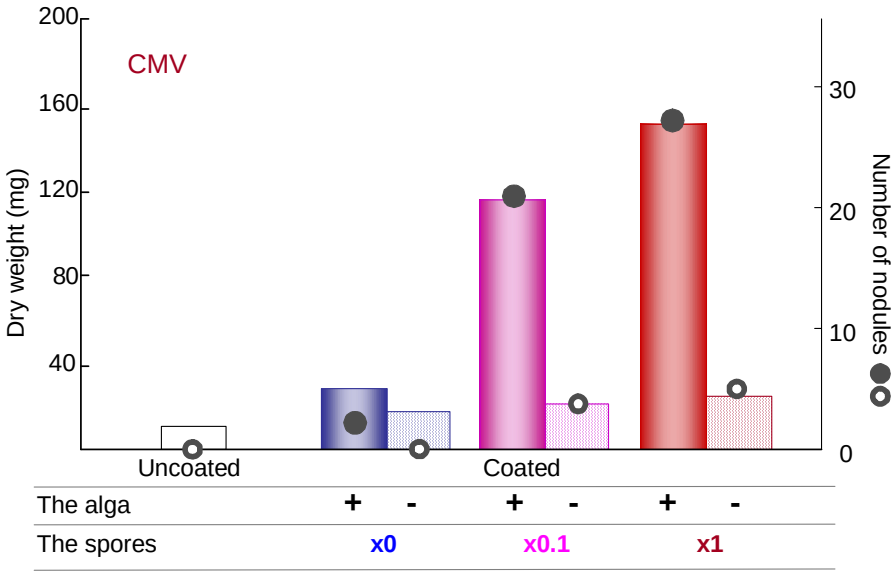

Fig. 9 Dry weight and number of nodules of CMV plants in the outdoor pots

Table 5. Distribution of dry weight of IRG plants in the outdoor pots[*1]

			Range of dry weight (mg)					
	The alga	The spore	0-50	51-100	101-150	151-200	201-250	>300
	+	X1		9	11	6	2	2
	+	X0.1	12	10	5	2	1	
Leaf	+	X0	25	5				
	-	X1	20	10				
	-	X0.1	26	4				
	-	X0	30					
	Uncoated		30					
	+	X1	18	12				
	+	X0.1	25	5				
Root	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					

[*1]Plant numbers under each range of the dry weight were listed in the table

Table 6. Distribution of dry weight and number of nodules of CMV plants in the outdoor pots[*1]

			Range of dry weight (mg)					
	The alga	The spore	0-50	51-100	101-150	151-200	201-250	>300
	+	X1	7	11	4	2	4	2
	+	X0.1	13	6	5	5	1	
Leaf	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					
	+	X1	25	5				
	+	X0.1	29	1				
Root	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					
			Range of number of nodules					
			0	1-10	11-20	21-30	31-40	>40
	+	X1		4	10	8	4	4
	+	X0.1		6	14	7	3	
Nodule	+	X0	28	2				
number	-	X1	14	16				
	-	X0.1	15	15				
	-	X0	30					
	Uncoated		30					

[*1]Plant numbers under each range of the dry weight were listed in the table

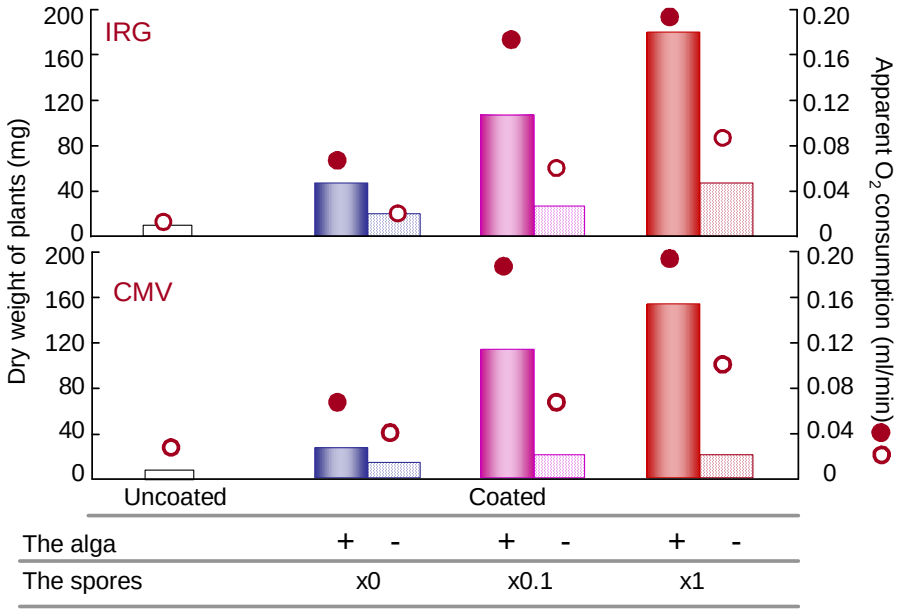

Fig. 10 Apparent O_2 consumption of the soil and dry weight of plants in the outdoor pots

3.2.2 Effect of the spores on plant growth in the growth chamber and beach land

IRG seeds coated with the mixture #B were inoculated with the x1 spores or x0.1 spores. Coated seeds without spores and uncoated seeds as the control were also included in the experiment. Seeds were sown in a pot with soil size 13 cm in diameter and 13 cm in depth. The pots were incubated in the growth chamber with 16 hrs light at 15℃ and 8 hrs dark at 5℃, and were watered with 100 ml/pot every 2 days. Initial seedlings were kept for measuring for the growth parameters on the 80th day. The experiment was repeated 2 times. Meanwhile, three hundred IRG seeds coated with #B and inoculated with the spores and without the x1 spores were sown on the beach land in pair with 300 uncoated seeds as the control in a plot of 40x80 cm^2. The seeds in the plots were covered with 1-2 mm sand. Water (3 L) was given to each plot every day; there was average 5.8 mm/cm^2· day natural rainfall and 6.4 hrs sunlight/day from

August 31 to November 19. Seedling emergence was counted every 2 days and plants in all the plots were harvested on the 80th day and measured for the growth parameters. Seedling emergences in the experiments under the 3 conditions were listed in Table 7. The dry weight of plants in the growth chamber, the outdoor pots and the beach land were compared in Table 8, and other growth parameters were shown in Appendix table 1 and 2.

In Table 8, under 3 different conditions/locations but within the same growth durations, dry weight of the IRG plants from the coated seeds was different, depending on the presence of the alga and the spores in the coat. The dry weight of plants with the same set of the alga and the spores under different growth conditions were also different. However, dry weight of plants still increased along with increase of the spores. The seedling emergence percentages of the coated seeds were similar, and were not affected by the alga and the spores in the coated seeds. Coated seeds had higher seedling emergence than the uncoated seeds.

3.3 Conditions of the soil after harvest of the first plants

3.3.1 Growth of the uncoated seeds secondly sown on the soil after the first harvest

After the plants in the growth chamber and the outdoor pots were harvested (hereafter denoted as the 1st harvest), the soil was left in the original pots, where uncoated seeds were sown, IRG in the growth chamber pots and CMV in the outdoor roofed pots (denoted as the 2nd harvest).

Table 7. Seedling emergence of the coated IRG seeds*[1]

		Coated			Uncoated control
		Spore dosages			
	The alga	X1 spores	X0.1 spore	0 spore	
Outdoor pots*[2] (%)	+	77	87	90	
	-	83	80	87	57
Growth chamber*[3] (%)	+	ND	ND	76	38
Beach land*[4] (%)	+	75	ND	75	25

*[1] Averaged data from 3 repeated experiments

*[2] From 9℃ to 31.6℃, average 6.8 hrs sunlight/day, 300 ml water to pots 13(diameter)13(depth) every 2 day

*[3]16 hrs light (2400lux) at 15℃ and 8 hrs dark at 5℃; 100 ml water to pots 13(diameter)13(depth) every 2 days

*[4] From 16℃ to 31.6℃, average 6.4 hrs sunlight/day, 5.8 mm/day average precipitation and artificial watering 3L/3200 cm^2 every day

Table 8. Dry weight of IRG plants under different growth conditions*[1]

		Coated			Uncoated
	Culture duration	X1 spore	X0.1 spore	0 spore	
Growth chamber*[2] (mg)	80	103±20.5	56±7.5	25±5	23±3.2
Outdoor pots*[3] (mg)	80	184.2±61	109.0±57	45.4±19	17.7±4.9
Beach land*[4] (mg)	80	106±51	ND	60.5±22	34.4±21.7

*[1] Averaged data from 3 repeated experiments

*[2] From 9℃ to 31.6℃, average 6.8 hrs sunlight/day, 300 ml water to pots 13(diameter)13(depth) every 2 day

*[3]16 hrs light (2400lux) at 15℃ and 8 hrs dark at 5℃; 100 ml water to pots 13(diameter)13(depth) every 2 days

*[4] From 16℃ to 31.6℃, average 6.4 hrs sunlight/day, 5.8 mm/day average precipitation and artificial watering 3L/3200 cm^2 every day

The plants growing on new soil were to be the control. Water (300 ml) was given to each pot every 3 days (the light and the temperature condition have been otherwise noted). Plant were harvested on the 60th day and measured for the growth parameters (Appendix table 4 and 5). The experiment was repeated 3 times. Dry weight of the second harvest plants was presented in Fig. 11.

For both IRG and CMV, the effect of the alga and the spores on plant growth of the 1st harvest plants was reflected on that of the 2nd harvest plants as well. Similarly, the effect of the alga and the spores on the number of nodules of the 1st harvest CMV was also observed on the number of nodules of the 2nd harvest plants (Fig. 11). Distribution of dry weight of leaves and roots of the 2nd harvest plants also showed same tendency as in the 1st harvest plants (Table 9 and 10).

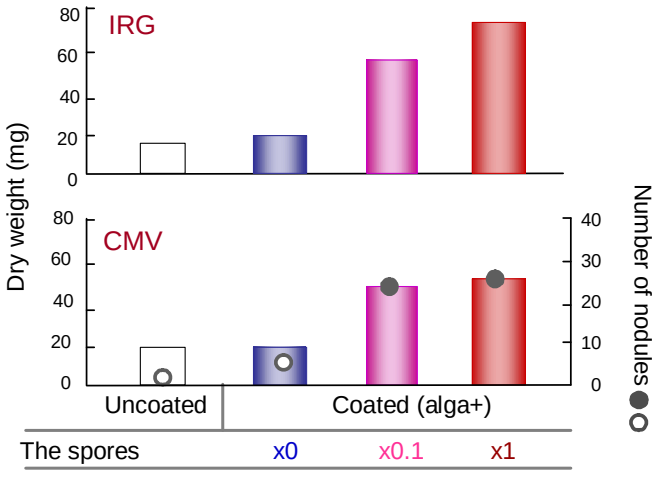

Fig. 11 Dry weight and number of nodules of 2nd harvest plants on the post-harvested soil

Table 9. Distribution of dry weight of IRG plants in the growth chamber[*1]

			Range of dry weight (mg)									
	The alga	The spore	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	>90
Leaf	+	X1			2	4	3	6	2	4	5	4
	+	X0.1		3	5	6	6	5	3	2		
	+	X0	16	10	3	1						
	Uncoated twice		30									
	Uncoated Once		30									
Root	+	X1	1	13	10	5	1					
	+	X0.1	7	15	7	1						
	+	X0	30									
	Uncoated twice		30									
	Uncoated Once		30									

[*1]Plant numbers under each range of the dry weight were listed in the table

Table 10. Distribution of dry weight and number of nodule of CMV plants in the growth chamber[*1]

			Range of dry weight (mg)						
	The alga	The spore	0-10	11-20	21-30	31-40	41-50	51-60	>60
Leaf	+	X1	1	2	8	16	2	1	
	+	X0.1	1	16	13				
	+	X0	13	16	1				
	Uncoated twice		30						
	Uncoated Once		30						
Root	+	X1	5	25					
	+	X0.1	2	28					
	+	X0	30						
	Uncoated twice		30						
	Uncoated Once		30						
			Range of nodule number						
			0	1-10	11-20	21-30	31-40	41-50	>50
Nodule number	+	X1			14	7	4	3	2
	+	X0.1		10	7	6	3	2	2
	+	X0	7	20	3				
	Uncoated twice		8	21	1				
	Uncoated Once		30						

[*1]Plant numbers under each range of the dry weight were listed in the table

3.3.2 Apparent O_2 consumption of the soil

After 2nd harvest plants were removed, the soil was tested for the apparent O_2 consumption. The apparent O_2 consumption of the soil after the 1st harvest and the

2nd harvest were shown in Table 11. After the 1st harvest, the soil where the coated seeds with the spores were sown showed the higher O_2 consumption than the soil where the coated seeds without the spores or uncoated seeds were sown. This tendency in both IRG and CMV was observed in the soil after 2nd harvest. The coated seeds but without spores or uncoated seeds in the 1st harvest led increase in the O_2 consumption of soil after 2nd harvest, from 0.07 to 0.13 ml/min etc. Furthermore, O_2 consumption of soil after the 2nd harvest of CMV was slightly higher than that in the 1st harvest IRG.

Table 11. Apparent O_2 consumption of the soil after second harvest[*1]

		Coated			Uncoated	Uncoated seeds on the control soil
		X1 spore	X0.1 spore	x0 spore		
IRG	1st harvest	0.19	0.17	0.07	0.01	
	2nd harvest	0.17	0.16	0.13	0.11	0.03
CMV	1st harvest	0.2	0.19	0.07	0.03	
	2nd harvest	0.22	0.22	0.19	0.15	0.05

[*1]Apparent O_2 consumptions of the soil in the table are expressed in ml/min.

4. Discussion

Nowadays, the re-vegetation is being widely carried out to rehabilitate the deteriorated rangeland on the plateau. However, poor soil moisture and nutrition on the deteriorated rangeland adversely affect plant growth and establishment of plant community, and have not admitted the re-vegetation programs. It is necessary to find the ways to take advantage of existing rainfall and soil moisture for plant growth. Besides moistures, even though organic matters and total nitrogen contents in top soil of the rangeland is comparatively high, they are not able to be mineralized to be available nutrients for plant growth due to poor soil microbes (Xiong, Y et al. 1987). Therefore, for re-vegetation of the deteriorated rangeland, at least these two key words, moisture and soil microbes, have to be concerned. In addition, instead of quick but temporary effect of chemical fertilizers, long-lasting, slow but steady fertilization have to be surveyed. Application of the alga and the spores *via* the coat on seeds reported in this study is expected to be a starting point, which is followed by sustainable improvement of soil environment and plant growth.

4.1 Seed coating and its effect on seedling emergence

Gramineal (IRG) and legume (CMV) plants chosen in this study are two most dominant species on the rangeland of the plateau. Additionally, *rhizobium* nodules in the legume CMV may be connected with microbe communities in soil and will be quite important as nitrogen fixer for sustainable rangeland.

Percentages of germination of IRG and CMV were 86% and 88%, respectively, at 25℃ with water saturation within 5 days, while they decreased to 38% and 24%, respectively, under the controlled temperature (5-15℃) and watering (Table 1).

Watering of 5 ml/50 cm^2 could be compared to the dawn precipitation, and temperature and illumination were set to simulate a spring climate on the plateau, where (3900 m above sea level) seedling emergence of a perennial grass was reported to be 42% (Liu, Y et al. 2002). Under the same controlled conditions, when the seeds were coated, the seedling emergence reached to 80% for IRG and 82% for CMV (Table 1) which were close to the values obtained under the conditions of water saturation and 25℃ (Table 2). In addition, the seedling emergence processes (Fig. 5 and Fig. 6) showed that the final (maximum) numbers of emerged plants 45 days after seeding, the rates of seedling emergence (the midpoint increments in emergence /day) and the date of the first emergence differed from the uncoated control. Depression of seed germination by the moisture stress under the controlled conditions seemed to be relieved by coating seeds. Under limited watering conditions, the coat of the seeds seems to be favorable for the seeds to conserve moisture, to germinate, then grow and cover the land.

In a preliminary experiment (not shown), the seedling emergence of IRG coated with the #D was 80% under 6.5ml/50cm^2・day watering (uncoated 38%), while it became 36% under 6.5ml/50cm^2・2days with negligible germination of the uncoated seeds. This result also suggests that the coated seeds have much lower threshold of moisture for germination than the uncoated seeds.

These seedling emergence characteristics differed with different coating mixtures, #A, #B, #C and #D (Table 2). Because the difference has to be reduced to the nature of the coating mixtures, the water conserving capacity of individual coating mixtures was estimated by the processes of water loss of the gels made by mixing the mixtures with water (Fig. 7). The processes may be described in an equation W = a *exp* (-bt),

where W is the weight of water in the gels, a is a constant, b is the rate of water loss from the gels, t is the time in the drying oven. The water conserving capacities of 4 coating mixtures were thus expressed in the numerical values (-b) from the slopes in Fig. 12. The –b values were plotted against the final seedling emergence over the uncoated control (Fig. 13). The roughly linear correlation between the water conserving capacity of the coating mixtures and the seedling emergence of the coated seeds of two grass species (Fig. 13) suggests that the moisture supplied from the coat is essential for the seeds to germinate, although inhibitory or promoting effect of the component(s) of the coat on germination may not be excluded. The results show that the seedling emergence under conditions simulating spring climate on the plateau can be improved by seed coating with good water conserving capacity.

Although applications of hygroscopic polymers on seed coating have been reported to improve the seed germination or seedling emergence successfully (Diniz, K. A. et al., 2006; Arsego, O. et al., 2006; Korpal, W. 1999), the coating materials used in this study were not only for their hygroscopic nature but for their availability as precursors of fertilizers for plants and soil microbes, as shown below.

4.2 The alga and the spores in the coat

In the above mentioned wooden pile experiment (Kobayashi, A. 2006), tree seeds in the stuffings (waste pulp, the alga and the spores) in the hollowed piles germinated and grew nicely even in sand as well as in farmland. When re-vegetation of deteriorated rangeland was planned, the grass seeds were designed to be coated with digestible hygroscopic materials and to be inoculated with the spores which, after germination, digest the coating materials and may supply nutrients.

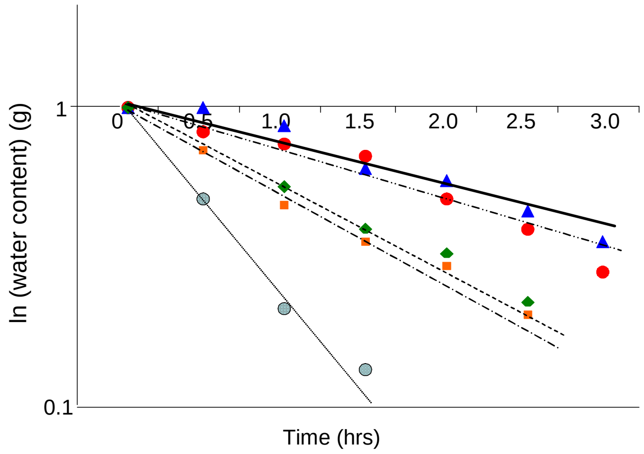

Fig. 12 Logarithmic expression of the water loss of the gels

#A(● ————), #B (▲ -·-·-·-·-), #C (◆ ------------),
#D (■ -·-·-·-·-), #S (○ ············).

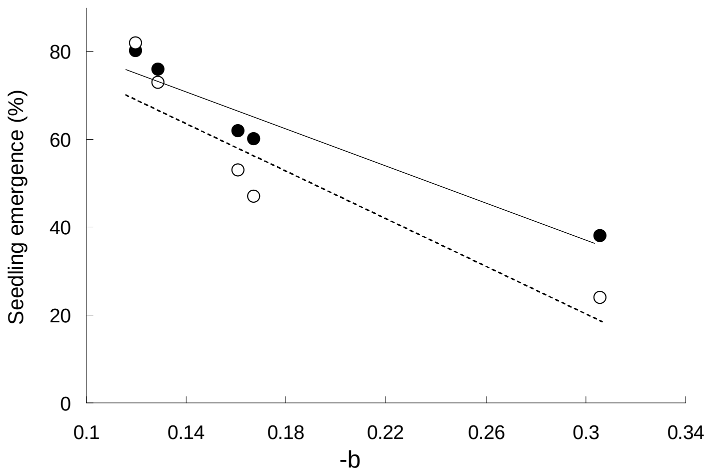

Fig. 13 Relationship between water conserving capacity and seedling emergence

IRG(● ———— R^2=0.92), CMV (○ ---------- R^2=0.84)

In the experiments of plant growth, the coating mixtures #B was chosen mostly because Xan, a main component in #B, was highly viscous and better to be involved to organize the coat, although the mixture #A had a little higher values in seedling emergence percentage and the rate of emergence (Fig.5 and Fig. 6). Xan is a well-digestible gum for the two microorganisms (data not shown). Soil was limited to be only sandy loam and loamy sand which has similar soil texture with the soil on rangeland of the plateau, they are all less likely to be fertile soil.

As shown in Table 7 and Table 8, under the watering conditions of 300 ml/300cm^2 pot once in the morning every 2 days, the percentages of seedling emergence showed little difference among the coated seeds and a little lower value for the uncoated control. This is because the experiments were designed to detect possible effects of the alga and the spores without any other stress (i.e. water-stress) on the seeds.

On the other hands, photosynthetic production (dry weight) of the plants was remarkably different; the highest values were obtained in the presence of both the alga and the x1 spores, followed by that in the presence of both the alga and the x0.1 spores. In the absence of either one of the two, the dry weight is much lower than that of the presence of the two, although it is still double of the uncoated control (Fig. 8 and Fig. 9). This tendency is common to both plant species, suggesting that the effect of both the alga and the spores is likely common to other plant species. In other words, the cooperative effect may not be due to the interaction between the specified plant species and set of alga and spores, but may be caused by the production by the set in the soil. The results imply that seed coating can supply moisture to seeds and facilitate seed germination and seedling emergence, while the alga and the spores

support the plant growth in later stages.

The apparent O_2 consumption was measured right after the plants were harvested in order to estimate amounts/activity of microbes respiring in the soil (Fig. 10). The estimated amount of soil microbes (the rate of oxygen consumption after glucose addition) differed with the composition of the coat and changed almost in parallel to the dry weight of the plants grown on the individual soil (Fig. 10).

These results strongly suggest that improvement of plant growth largely due to the increase in the amount/activity of soil microbes and/or in the favorable production by the set of alga and spores in the soil which directly or indirectly promoted the growth of plant and microbes. It may be predicted that the growth of the microorganisms is strengthened by the alga, and that the spores/microorganisms digest the powdered alga and the gums, and obtain necessary nutrition, e.g. N, P and K for their growth, and that increased amount of microorganisms introduces amount/activities of soil microbes, which will construct a rhizosphere favorable to the plant.

The experiment on the beach land has obtained more artificial interference than the field experiments should do. In order to provide basic moisture for germination and seedling emergence, the plots were built in ditches with depth of 15 cm, and 3 L water was given to each plot every day. However, difference in plant growth due to the alga and the spores was still shown. Similar tendencies have been shown on the effects of the alga and the spores under 3 different growth conditions (Table 8). The results in the growth chamber are presumed that same results may be achieved on the rangeland of the plateau.

Table 5 and Table 6 show not only the combined effect of the alga and the spores but the distribution of the dry weight of leaves and roots. The higher dry weight (and

longer length in Appendix table 12 and 13) of roots is of specially importance for preventing soil from erosion.

The number of nodules on the CMV roots was also dependent on the alga and the spores, and they were in parallel with dry weight of CMV plants. *Rhizobium* in soil and nodules on CMV roots are encouraging members of the soil microbe community, which has to be formed even in barren soil. Further studies are necessary to find out soil microbes and interaction between the spores and soil microbes.

The experiments on plant growth were conducted under sufficient watering in order to get the direct effect of the alga and the spores without any other stresses. The effect under the conditions of water stress has to be examined.

4.3 For sustainable rangeland

In the study, the dry weight of the 1st harvest plants was dependent on the alga and spores, and the same tendency has been found in the 2nd harvest plants grown on the post-harvest soil (Fig. 11). In addition, the number of nodules of the 1st harvest CMV plants was also determined by the alga and the spores, and the same tendency was observed in the 2nd harvest CMV plants.

It is suggested that the soil conditions might have been improved by the set of the alga and the spores in the coat of the 1st harvest plant seeds. The tendency of apparent O_2 consumption in the 1st harvest soil was observed again in the soil after the 2nd harvest (Table 11). These results suggest that the improvements of plant growth of the 2nd harvest plants from the uncoated seeds was promoted by something which had been left in the soil after the 1st coated seeds were sown, grown, and harvested. This is presumed to be soil microbes.

On the rangeland with sound ecological environment, the plants, soil (moisture) and soil microbes interact and depend on and support with each other (Fig. 14). When this relationship/balance is broken, the rangeland will be deteriorated. Then, soil microbes are the one that can give a clue to restore the rangeland.

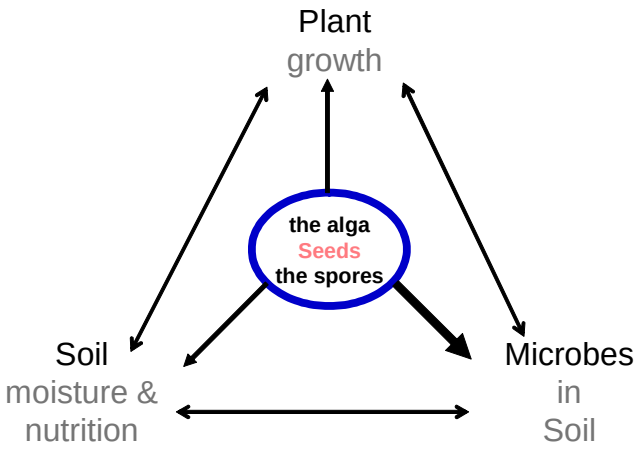

Fig. 14 The set of the alga and the spores in the coat initiates a positive and sustainable ecological circle on rangeland

Based on the above mentioned wooden pile experiment (Kobayashi, A. 2006), it was expected that the alga and the spores not only promote current plant growth, but also create a sound environment in soil for soil microbes. The present results obtained by the seeds coated with the set of the alga and the spores reveal that the set performed the expected function and can be used for sustainable plant production.

It was reported that inoculation with rhizobial fungi and bacteria not only enhanced the establishment of key plant species, but also increased soil N content, organic matter and hydrostable soil aggregate (Requena, N. 2001). Sustainable improvement

of soil conditions requires persistent supply of necessary N, P and K, which may come from organic matter in soil on the plateau and further decomposed by the microbes. This is same logic as the application of organic fertilizers together with effective microorganisms (Wu, S. C. et al., 2005; Xu, H., 2000).

Other researches have been successful in improving plant growth through inoculating native critical microorganisms for plant growth back to soil, e.g. bush fungus in re-vegetation of limestone mine sites (Rao,. A. V. et al 2002), nitrogen fixing or phosphate solubilizing bacterium back to their natural habitat soil to improve maize production (Mehnaz,. S. et al., 2006).

In this study, the *Aspergillus* sp and *Streptomyces* sp were isolated for digesting the mixture sheet of the *Phaeocystis* sp necolon-1 and waste pulp. The spores of the microorganisms germinate, digest the mixture, and will support the soil microbes, then promote the plant growth. The seed coating with the hygroscopic nutrient-source materials together with the microorganisms/spores that can utilize the materials, in general, is unique and will be one of the promising techniques for rehabilitation of deteriorated rangeland.

4.4 Further studies

This is just the start of long-lasting trial (and error) on rejuvenation of the barren land. Further experiments are expected to confirm the present results and idea, particularly on the rangeland of the plateau.

The microorganisms/spores for digesting the coating materials should have little disturbance on the rangeland ecology. Although many alternatives can be found for the alga used in this study, unicellular microalgae may be one choice, because they

can be collected readily by filtration, dried and powdered. In fact, there are so many isolated salt lakes on the Qinghai-Tibetan plateau that may supply the culture milieu for culturing marine algae. Since marine algae can not survive in the rangeland of fresh water territory, marine algae seem to be less pollutant than fresh water algae, even if live algae are contaminated in the algae powder in the coat.

The apparatus for seed coating should fit to mass production of coated seeds, and the machine for spraying the coated seeds on the rangeland should be constructed.

5. Conclusive remarks

An attempt was made to solve the problems in rehabilitation of the deteriorated rangeland on the Qinghai-Tibetan Plateau. To cope with shortage of soil moisture and microbes, and to create a rhizosphere micro-cosmos with a favorable moisture and nutrition for growth of plants and soil microbes, the grass seeds were coated with the mixture of polysaccharide gums and the powdered dry alga, and inoculated with the spores of the microorganisms, which had been collected as the decomposer of the coating mixtures.

Under the controlled conditions simulating the spring climate on the plateau, the germination percentage of the seeds of two grass species was much lower than that under the moderate conditions. While the seedling emergence of the coated seeds revived close to the germination under the moderate conditions, and initial date for seedling emergence of the coated seeds was also earlier than that of the uncoated seeds. Improvements in seedling emergence and initial date were caused by the moisture supplied from the coat. It is suggested that coated seeds germinate normally on the drought rangeland of the plateau, and seedlings quickly dominate land.

When both the alga and the spores were present in the coat, dry weight of plants and number of nodules remarkably increased. The amount/activity of soil microbes were in parallel with the dry weight of plants. The results suggest that the improvement of plant growth was due to increase in the amount/activity of soil microbes, which were activated through digesting the alga and the coat. These processes directly or indirectly promoted the growth of plants and soil microbes. The experiments under three growth conditions (growth chamber, outdoor pots and beach land) showed same results.

After the first plants were harvested, growth of the second plants from uncoated seeds on the post-harvested soil and the soil microbes improved with same tendency as the 1st plants. The result suggests that soil conditions have been improved due to continuing activity of soil microbes after first harvest. This improvement may be sustained.

The problems of poor moisture of soil and low population of soil microbes in rehabilitating the deteriorated rangeland of the plateau may be effectively addressed through seed coating with hygroscopic nutrient-source materials and inoculating microorganisms/spores that digest the materials. Further studies are necessary to confirm the present results and idea, particularly on the plateau. Local sources of coating materials, the microorganisms/spores and nutrients source for the microorganisms in place of the marine alga have to be surveyed and utilized.

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8. List of Appendix tables

Appendix table 1. Growth parameters of IRG in the growth chamber *[1]

Parameters*[2]	Coated seeds and the alga presence			Uncoated seeds(control)
	X10 spore	X1 spore	0 spore	
Dry weight of plants (mg/plant)	103±20.5	56±7.5	25±5	23±3.2
Dry weight of leaves (mg/plant)	66±6.6	30±0.9	13±0.5	11±0.4
Height (cm)	20.7±5.3	14±6	12.5±3.1	11.5±2.6
Shoots/plant	2.2±1.3	2±0.7	1.1±0.03	1
Root weight	37±0	26±0.5	12±0.1	12±0.5

*[1] 16 hrs light (2400lux) at 15℃ and 8 hrs dark at 5℃; 7.6 mm water every 2 days

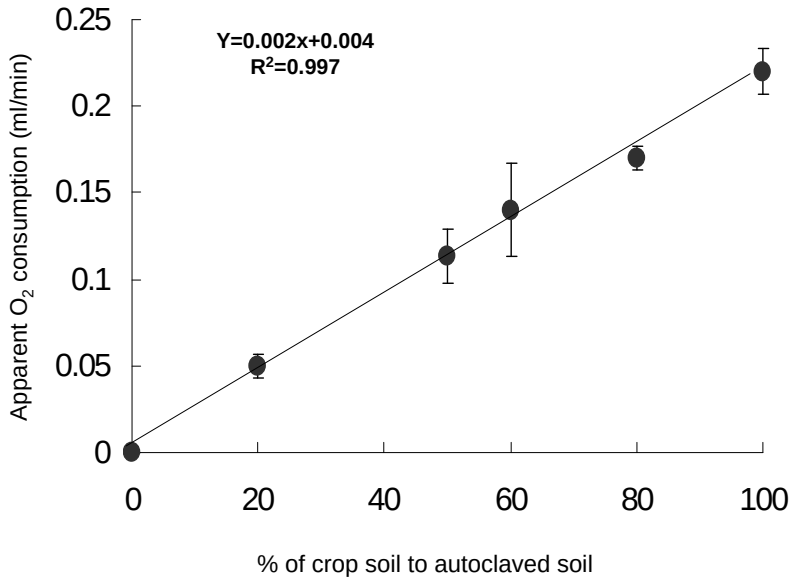
*[2] Averaged data of 20 plants

Appendix table 2. Growth parameters of IRG in the beach land*[1]

Parameters*[2]	Coated seeds		Uncoated Seeds (control)
	X1 spore	0 spore	
Seedling emergence (%)	25	25	15
Dry weight (mg/plant)	106±51	60.5±21.6	34.4±21.7
Dry leaf weight (mg/plant)	54±47	36±22	23±22
Height (cm)	10.2±4.5	9.6±3.2	8.9±2.8
Shoots/plant	3.2±0.8	0.6±0.3	0.4±0.7
Leaves/ plant	10.1±1.5	5.8±1.4	4.6±1.2
Dry root weight (mg/plant)	53±34	25±25	11±10
Root length (cm)	13.5±3.7	12.5±2.8	9.6±2.6

*[1] 16 – 31.6℃, 6.4 hrs sunlight/day, 5.8 mm/day average precipitation and 3 L/day· plot watering, from Aug. 1st to Oct. 20th

*[2]Averaged data of 75 plants for the coated seeds and of 45 plants for the uncoated.



Appendix figure.1 Calibration between the amount of microbes and apparent O_2 consumption of soil

Appendix table 3. Apparent O_2 consumption of the soil in the growth chamber and the beach farm *[1]

	Coated seeds and the alga presence			Uncoated seeds	Control soil
	X1 spore	X0.1 spore	X0 spore		
Growth chamber	1±0.04	0.8±0.03	0.67±0.1	0.28±0.02	0
Beach farm	1.2±0.04	ND	0.53±0.09	0.27±0.04	0.13±0.04

*[1]Apparent O_2 consumption (ml) is from 3 soil samples

Appendix table 4. Growth parameters of 2nd harvest IRG in the growth chamber *1

Parameters*2	Coated seeds			Uncoated seeds	Control soil*3
	X1 spore	X0.1 spore	X0 spore		
Seedling emergence (%)	73	70	75	80	78
Dry weight (mg/plant)	77.3±27	58.2±22	19.3±6.1	10.4±4.2	10.1±0.9
Dry leaf weight (mg/plant)	54.9±19	44.7±15.6	13.7±4.4	7.4±2.7	6.9±3.1
Height (cm)	15.3±3.4	13.4±2.5	10.1±2.2	5.2±0.9	5.0±1.1
Leaves/plant	4.4±0.6	3.6±0.6	2.8±0.6	2.3±0.4	2.2±0.3
Shoots/plant	1.4±0.5	1.1±0.2	1	1	1
Dry root weight (mg/plant)	22.4±7	16.5±6.3	5.6±1.7	3.1±1.2	3.2±1.8
Root length (cm)	21.0±3.3	17.0±3.1	12.2±3.4	14.1±1.7	13.4±0.6

*1 16 hrs light (2400lux) at 15℃ and 8 hrs dark at 5℃; 100 ml water every 2 days, from Sept. 23 to Nov. 23.

*2 Averaged data of 30 plants

*3 The soil same as the first growth soil

Appendix table 5. Growth parameters of 2nd harvest CMV plants in the outdoor pots *1

Parameters*2	Coated seeds			Uncoated seeds	Control soil*3
	X1 spore	X0.1 spore	X 0 spore		
Seedling emergence (%)	72	68	65	70	68
Dry weight (mg/plant)	45.7±7.6	43.9±5.8	16.3±3.7	14.7±2.8	14.2±1.4
Dry leaf weight (mg/plant)	32.6±5.6	31.3±4.2	11.4±3	10.4±2	9.8±2.1
Height (cm)	6.4±1.2	6.4±0.7	3.7±0.6	3.7±0.6	3.7±1.1
Leaves/plant	6.3±1.4	6.5±1.8	3.7±0.7	3.2±0.6	3.1±0.6
Dry root weight (mg/plant)	12.9±2.2	12.5±1.7	4.9±1	4.3±0.8	4.4±3.2
Nodules/plant	25.3±11.7	22.7±13.3	3.7±3	3.5±2.9	0
Root length (cm)	23±2.3	21.6±2.4	14.9±2.5	15.1±2.7	14.9±3

*1 9-31.6℃, 6. 8 hrs sunlight/day, 300 ml / 300 cm^2 water was given every 3 days, from Sept. 23 to Nov. 23rd

*2 Averaged data of 30 plants

*3 The soil same as the first growth soil

Appendix table 6. Distribution of dry weight of IRG plants in the outdoor pots[*1]

The alga	The spore	Range of dry weight (mg) 0-50	51-100	101-150	151-200	201-250	251-300	301-350	>350
+	X1	1	2	11	8	3	2	2	1
+	X0.1	6	11	5	6		1	1	
+	X0	19	11						
-	X1	19	11						
-	X0.1	26	4						
-	X0	30							
Uncoated		30							

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 7. Distribution of dry weight of CMV plants in the outdoor pots[*1]

The alga	The spore	Range of dry weight (mg) 0-50	51-100	101-150	151-200	201-250	251-300	301-350	>350
+	X1	6	7	6	3	3	2	1	2
+	X0.1	6	9	7	6	1		1	
+	X0	30							
-	X1	29	1						
-	X0.1	29	1						
-	X0	30							
Uncoated		30							

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 8. Distribution of dry weight of leaves and roots of IRG plants in the outdoor pots[*1]

			Range of dry weight (mg)					
	The alga	The spore	0-50	51-100	101-150	151-200	201-250	>300
Leaf	+	X1		9	11	6	2	2
	+	X0.1	12	10	5	2	1	
	+	X0	25	5				
	-	X1	20	10				
	-	X0.1	26	4				
	-	X0	30					
	Uncoated		30					
Root	+	X1	18	12				
	+	X0.1	25	5				
	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					

[*1]Plant numbers under each range of the dry weight were listed in the table

Appendix table 9. Distribution of dry weight of leave and roots and number of nodules of CMV plants in the outdoor pots[*1]

			Range of dry weight (mg)					
	The alga	The spore	0-50	51-100	101-150	151-200	201-250	>250
Leaf	+	X1	7	11	4	2	4	2
	+	X0.1	13	6	5	5	1	
	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					
Root	+	X1	25	5				
	+	X0.1	29	1				
	+	X0	30					
	-	X1	30					
	-	X0.1	30					
	-	X0	30					
	Uncoated		30					
			Range of number of nodules					
			0	1-10	11-20	21-30	31-40	>40
Nodule number	+	X1		4	10	8	4	4
	+	X0.1		6	14	7	3	
	+	X0	28	2				
	-	X1	14	16				
	-	X0.1	15	15				
	-	X0	30					
	Uncoated		30					

[*1]Plant numbers under each range of the dry weight were listed in the table

Appendix table 10. Distribution of height of IRG plants in the outdoor pots[*1*2]

The alga	The spore	Range of height (cm) 1-5	6-10	11-15	16-20	21-25	26-30	31-35	>36	Total shoot
+	X1		9	4	22	18	19	6	2	80
+	X0.1	3	12	8	17	19	12	1		72
+	X0	1	15	14	14	4				48
-	X1	6	7	14	14	9				50
-	X0.1	2	7	13	12	4				38
-	X0	1	11	16	4					30
Uncoated		3	13	14						30

[*1]Number of the individual plants in the given range of dry weight in mg is shown

[*2] Plant height of IRG are from more than 30 shoots

Appendix table 11. Distribution of height of CMV plants in the outdoor pots[*1]

The alga	The spore	Range of height (cm) 1	2	3	4	5	6	7	8	9	10
+	X1					1	4	7	11	4	3
+	X0.1				5	6	8	6	4	1	
+	X0	2	11	13	4						
-	X1	2	8	7	7	1	2	3			
-	X0.1	2	13	10	4	1					
-	X0	10	14	5	1						
Uncoated		8	20	2							

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 12. Distribution of root length of IRG plants in the outdoor pots[*1]

The alga	The spore	Range of root length (cm) 1-5	6-10	11-15	16-20	21-25	26-30	31-35	>35
+	X1				7	9	9	4	1
+	X0.1			1	13	10	5	1	
+	X0		2	12	13	3			
-	X1		4	17	8	1			
-	X0.1		8	14	8				
-	X0		14	12	4				
Uncoated		6	20	4					

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix Table 13. Distribution of root length of CMV plants in the outdoor pots[*1]

The alga	The spore	Range of root length (cm) 1-5	6-10	11-15	16-20	21-25
+	X1		1	10	16	
+	X0.1		5	10	9	6
+	X0	1	16	11	2	
-	X1		2	12	12	4
-	X0.1	2	22	2	4	
-	X0	3	19	6	2	
Uncoated		8	18	3	1	

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 14. Distribution of leave number of IRG plants in the outdoor pots[*1]

The alga	The spore	Range of leave number 1-2	3-4	5-6	7-8	9-10	11-12	12-13	>13
+	X1		2	3	7	7	5	4	2
+	X0.1		3	7	7	9	1	3	
+	X0		16	6	7	1			
-	X1		11	9	7	3			
-	X0.1	3	16	9	2				
-	X0		24	5	1				
Uncoated		3	27	5	1				

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 15. Distribution of leave number of CMV plants in the outdoor pots[*1]

The alga	The spore	Range of leave number 1-5	6-10	11-15	16-20	21-25	26-30	>30
+	X1		10	11	2	5	1	1
+	X0.1	3	8	7	6	3	3	
+	X0	26	4					
-	X1	26	4					
-	X0.1	27	3					
-	X0	29	1					
Uncoated		30						

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 16. Distribution of dry weight of IRG plants in the beach land[*1*2]

			Range of dry weight (mg)						
	The alga	The spore	0-50	51-100	101-150	151-200	201-250	251-300	>300
Plant	+	X1	33	10	9	12	5	3	3
	+	X0	39	25	7	4			
	Uncoated		38	2	4		1		
Leave	+	X1	30	16	17	10	2		
	+	X0	53	20	2				
	Uncoated	35	10						
Root	+	X1	52	23					
	+	X0	67	8					
	Uncoated		43	2					

[*1]Number of the individual plants in the given range of dry weight in mg is shown

[*2] Number of plants of the coated seeds was 75; number of plants of uncoated seeds was 45

Appendix table 17. Distribution of height, root length and leaf number of IRG plants in the beach land[*1*2]

			Range of height (cm)									
	The alga	The spore	0-5	6-10	11-15	15-20	21-25	Total shoot number				
	+	X1	51	91	54	45		241				
Height	+	X0	21	50	40	10		121				
	Uncoated		8	39	11	4		62				
			Range of root length (cm)									
	+	X1	7	9	33	25	1					
Leave	+	X0	2	21	36	16						
	Uncoated	35	3	29	9	4						
			Range of leaf number									
			1	2	3	4	5	6	7	8	9	Total shoot
	+	X1	51	85	33	22	21	15	6	6	2	241
Root	+	X0	18	16	21	19	34	11	2			121
	Uncoated		2	20	19	8	6	5	1	1		62

[*1]Number of the individual plants in the given range of dry weight in mg is shown

[*2] Number of plants of the coated seeds was 75; number of plants of uncoated seeds was 45

[*3] Plant height, and leaf number of IRG are from more than 75 shoots

Appendix table 18. Distribution of dry weight of 2nd harvest IRG plants in the growth chamber[*1]

The alga	The spore	Range of dry weight (mg) 0-20	21-40	41-60	61-80	81-100	101-120	>120
+	X1		3	9	4	7	4	3
+	X0.1	2	8	6	6	6	2	
+	X0	18	12					
2 nd uncoated		27	3					
1st uncoated	Control	29	1					

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 18. Distribution of dry weight of 2nd harvest CMV plants in the outdoor pots[*1]

The alga	The spore	Range of dry weight (mg) 0-20	21-40	41-60	61-80	81-100	101-120	>120
+	X1		6	23	1			
+	X0.1		9	21				
+	X0	27	3					
2 nd uncoated		30						
1st uncoated	Control	30						

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 20. Distribution of dry weight of leaves and roots of 2nd harvest IRG plants in the growth chamber[*1]

	The alga	The spore	Range of dry weight (mg) 0-10	11-10	21-30	31-40	41-50	51-60	61-70	71-80	81-90	>90
Leaves	+	X1			2	4	3	6	2	4	5	4
	+	X0.1		3	5	6	6	5	3	2		
	+	X0	16	10	3	1						
	2 nd uncoated		30									
	1st uncoated	Control	30									
Roots	+	X1	1	13	10	5	1					
	+	X0.1	7	15	7	1						
	+	X0	30									
	2 nd uncoated		30									
	1st uncoated	Control	30									

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 21. Distribution of dry weight of leaves and roots and number of nodules of 2nd harvest CMV plants in the outdoor pots[*1]

	The alga	The spore	Range of dry weight (mg) 0-10	11-20	21-30	31-40	41-50	51-60
	+	X1	1	2	8	16	2	1
Leaves	+	X0.1	1	16	13			
	+	X0	13	16	1			
	2 nd uncoated		30					
	1st uncoated	Control	30					
	+	X1	5	25				
Roots	+	X0.1	2	28				
	+	X0	30					
	2 nd uncoated		30					
	1st uncoated	Control	30					

			Range of number of nodules 1	1-10	11-20	21-30	31-40	41-50	51-6-
	+	X1			14	7	4	3	2
Nodule	+	X0.1		10	7	6	3	2	2
	+	X0	7	20	3				
	2 nd uncoated		8	21	1				
	1st uncoated	Control	30						

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 22. Distribution of plant height and root length of 2nd harvest IRG plants in the growth chamber[*1]

			Range of height and length (cm)					
	The alga	The spore	1-5	6-10	11-15	16-20	21-25	26-30
Leaves	+	X1	1	3	13	9	4	
	+	X0.1		6	18	5	1	
	+	X0		17	13			
	2 nd uncoated		18	12				
	1st uncoated	Control	22	8				
Roots	+	X1			2	11	14	3
	+	X0.1		2	9	13	6	
	+	X0	1	13	10	4	2	
	2 nd uncoated			2	19	9		
	1st uncoated	Control		6	20	4		

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 23. Distribution of plant height and root length of 2nd harvest CMV plants in the outdoor pots[*1]

			Range of height and length (cm)					
	The alga	The spore	1-5	6-10	11-15	16-20	21-25	26-30
Leaves	+	X1	8	12				
	+	X0.1	4	26				
	+	X0	30					
	2 nd uncoated		30					
	1st uncoated	Control	30					
Roots	+	X1				6	16	8
	+	X0.1				13	16	1
	+	X0		1	18	10	1	
	2 nd uncoated		3	16	9	2		
	1st uncoated	Control	12	10	8			

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 24. Distribution of leaf number of 2nd harvest IRG plants in the growth chamber[*1]

		Range of leaf number			
The alga	The spore	0-20	21-40	41-60	61-80
+	X1		16	14	
+	X0.1		28	2	
+	X0	11	19		
2 nd uncoated		21	9		
1st uncoated	Control	26	4		

[*1]Number of the individual plants in the given range of dry weight in mg is shown

Appendix table 25. Distribution of leaf number of 2nd harvest CMV plants in the outdoor pots[*1]

		Range of leaf number			
The alga	The spore	0-20	21-40	41-60	61-80
+	X1	3	4	10	3
+	X0.1	7	11	8	4
+	X0	25	5		
2 nd uncoated		4	26		
1st uncoated	Control	30			

[*1]Number of the individual plants in the given range of dry weight in mg is shown