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Effect of Geothermal Water on Germination, Seedling Growth and Development of Vascular Bundle in Rice

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温泉水가 벼種子의 發芽, 幼苗生長 및 維管束發達에 미치는 影響

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ABSTRACT : Geothermal water contains toxic quantities of sulfur, potassium, sodium, boron, and other toxic elements. These toxic elements can substantially restrict germination and seedling growth in rice plant. Germination percentage, average days required for germination and germination velocity were drastically affected by geothermal water. Binato cultivar has higher germination rate compared to IR58 and Unbong 7. Plant height, root length, leaf number and total dry weight decreased with increased geothermal water concentration. Binato and IR58 showed higher total dry weight than Unbong 7 at 25 percent geothermal water at 15 days after treatment (DAT). Binato and IR58 were relatively more tolerant than Unbong 7 in terms of percentage of leaf damage at 25, 50 and 75% concentration of geothermal water at 10 DAT. The development of large and small vascular bundles decreased with increasing concentration of geothermal water from control to 50% in three rice cultivars.

Key words : Rice, Geothermal water, Germination, Seedling growth, Vascular bundle.

Water is an active reagent and can dissolve some of the minerals in the host rocks. Hence, the mineralized water usually differs in composition from the ordinary water in the area. The origin of some waters is difficult to determine as they come from great depths and pass only through crystalline rocks. They are hot (termed thermal springs) and contain dissolved carbonic acid and various mineral elements. They are often found in volcanic regions or regions of recent volcanic activity (Furon, 1967). The Philippines is located along the earth's volcanic belt. According to the record of the commission on volcanology, there are 44 volcanoes in the Philippines, thirteen of which are active (Juinio, 1980).

In the Philippines, the use of geothermal energy as an alternate source of electricity has resulted in several exploratory diggings for geothermal wells. Environmental scientists are alarmed at the possible pollution that these geothermal wells may bring to the sur-

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rounding population and agricultural centers.

Ellis and Mahon (1964) reported the presence of high concentration of elements such as ammonia, boron, fluoride, lithium and chloride in thermal waters. The Philippine National Oil Company's study (Lopez, 1989) revealed high levels of toxic arsenic and boron at the center of rice fields irrigated by Marbol and Matinao Rivers in Mindanao where the water from the diggings flow.

Geothermal water has high electrical conductivity because of the excessive amounts of minerals. Some rice cultivars which germinated and sprouted at high electrical conductivity failed to resume growth at 4 mmho/cm. On the other hand, three and six week old seedlings survived even at 9 and 14 mmho/cm. The grain yield was not appreciably affected by salinity at 4 mmho/cm but was reduced by 50% at 8 mmho/cm (Pearson, 1961).

Mengel and Kirkb (1978) reported that high levels of fluoride are usually toxic to plants. This competitively inhibits the enzyme aconitase responsible for the conversion of citrate to isocitrate in TCA cycle.

Toxic effects of boron result in leaf tip yellowing followed by progressive necrosis. This begins at the tip and margins and finally spreads between the lateral veins towards the midrib (Lantin et al., 1980). Rice growth was hampered when the boron content of irrigation water was greater than 2 ppm (De Datta, 1981; FAO-UNESCO, 1973; Kanwar and Randhawa, 1974; Ponnamperuma, 1979). At maturity, tocicity symptom appeared at 100 ppm (Tanaka and Yoshida, 1970).

The critical sulfur content in straw from maximum dry weight varied from 160 ppm at tillering stage, 70 ppm at flowering and 60 ppm at maturity (Yoshida, 1981).

The effect of excess chloride in the plant is a more serious problem. Crops growing on salt affected soils often show symptoms of Cl toxicity. These include burning of leaf tips or margins, bronzing, premature yellowing and abscission of leaves.

Geothermal water containing materials in toxic concentrations can substantially restrict plant growth and development. However, little is known about the effect of geothermal water on germination and growth of rice seedlings.

This information would be potentially helpful in future crop management in agricultural areas near geothermal plants. The objective of this study is to determine the effects of geothermal water on rice germination, seedling growth and development of vascular bundles.

MATERIALS AND METHODS

The experiment was conducted in 1989~1990 at the Department of Agronomy, University of the Philippines at Los Banos (UPLB). Binato, a traditional philippine cultivar, IR58, a modern high yielding cultivar, and Unbong 7, an advanced breeding line from Korea, were used as test materials in the experiment.

Geothermal water was taken from the Philippines geothermal plant. The analysis of the geothermal as compared with tap water in the area is shown in Table 1. Geothermal water has much higher electrical conducivity, sulfur, potassium, sodium and boron content than those of tap water. pH was measured with a glass electrode with reference calomel electrode connected to a Beckman pH meter. Na and K were analyzed

Table 1. Chemical analysis of geothermal and tap waters

	Geothermal water	Tap water
PH	6.80	8.00
EC(dS /m)	8.25	0.52
SO_4^{-2} (ppm)	27.00	ND
K (ppm)	354.00	13.00
Na (ppm)	1,690.00	56.00
B (ppm)	75.00	0.53

[1] ND= Not detectable

by atomic absorption spectrophotometry. Elelctrical conductivity (EC) was determined with a Philips Model No.DW 9501 /01 direct reading conductivity bridge. Boron was determined with an autoanalyzer (azomethine-H method). Sulfur was determined by ICP (inducing coupled plasma).

Germination tests were conducted on the three cultivars using full strength geothermal water and distilled water as the control. Fifty seeds were used in each treatment with three replications. The seeds were placed in petri dishes lined with filter paper wetted with just enough distilled and geothermal water. They were incubated at 30℃ for 6 days. Germinated seeds were counted daily.

Experiments on seedling growth was carried out with five levels of control, 25, 50, 75 and 100% geothermal water. The germinated seeds were sown in 5 × 5 × 5 cm plastic pots containing Maahas clay soil and watered with the different concentration of the test solutions. Ten seedling per replication from each cultivars were measured for leaf number, plant height and dry weight and so on. At 5, 10 and 15 DAT, the number of leaves per seedling was counted. Shoot and root lengths were measured with a ruler. The shoot and roots were separated and dried at 70℃ drying oven for two days before weighting. Geothermal toxicity in leaf blade was measured in the proportion of leaf area developing symptom of leaf tip yellowing and drying. With five leaf blades per replication, the widest part of the middle region were collected to observed development of vascular bundles. The number and cross sectional area of vascular bundle, phloem and xylem were observed from the leaf blades using a microscope.

The materials were fixed in FAA solution for 24 to 48 hours. The air from the fixing fluid was drawn out with a vacuum pump. After fixing, they were washed in running tap water for 5 to 6 minutes. For dehydration, the ethyl alcohol series was used. After the dehydration process, the dehydrated tissues were transferred to a solvent (xylene). The tissues were infiltrated and embedded in paraffin and allowed to solidify. Rotary microtome sections of the materials were dried on a hot plate at 50~55℃ for 24 hours to make sure that the sections will not be detached from the slide while passing through the down grade of the staining series. Hematoxylin and safranin were used for staining and finally were mounted with canada balsam. The experiment was conducted in factorial design(cultivars and geothermal water concentrations) in CRD with 3 replications.

RESULTS AND DISCUSSION

1. Effect of geothermal water on germination

Geothermal water treatment resulted in significantly lower percent germination than distilled water (Table 2). In geothermal water treatment, germinations were 85.3 percent in Binato and 69.3 and 69.7 percent in

Table 2. Effect of geothermal water on the germination of rice cultivars

Cultivars	Treatment	Germination (%)	Average days required for germination	Germination[2] velocity
Binato	G.W.[1]	85.3 a[3]	1.9 a	49.8 b
	D.W.	93.3 a	1.5 b	69.4 a
IR 58	G.W.	69.3 b	4.7 a	21.2 b
	D.W.	96.0 a	2.7 b	36.9 a
Unbong 7	G.W.	69.7 b	2.9 a	37.0 b
	D.W.	92.7 a	2.1 b	48.0 a

[1] G.W. =Geothermal Water, D.W. = Distilled Water

[2] Germination velocity $= \frac{\sum Gn}{\sum (Gn \times Dn)} \times 100$

where Gn = number of seeds germinated on day

Dn = day from initial sowing

[3] Means followed by common letter in a column are not significantly different at the 5 % level by DMRT

IR58 and Unbong 7, respectively. This result showed that Binato cultivar has greater adaptability than IR58 and Unbong 7 under geothermal water treatment.

The average days required for germination was longer in geothermal water than distilled water indicating that it has inhibitory effect on germination in the three cultivars. Germination velocity was also slower in geothermal water than distilled water in all cultivars.

This result generally agree with the findings of Palwal and Mehta (1973) who found that germination of paddy varieties was significantly decreased with increasing levels of boron (0~100 ppm). Boron up 40 ppm had no adverse effect on the germination. None of the boron treatments has reduced the germination percentage below 50% that of the control. In this experiment, the boron content of the geothermal water was 75.0 ppm. Treated G.W. contains higher concentration of sodium, potassium and sulfur elements. It is well known that excessive amounts of salt in the irrigated water limit seed germination in several crops. When seed are sown in an environment with a high sodium and potassium concentration, both the rate and percentage of germination decrease (Reddy and Vora, 1983). The process of early germination depends upon the balance between the expansive force of the protoplast of the embryo and the restrictive force of the cell wall. Prisco and O'Leary(1970) reported that reduction in germination under saline conditions could be attributed to increased osmotic pressure of the soil solutions, which diminish the water absorption rate, leading to moisture stress in the seeds.

2. Effect of different levels of geothermal water on seedling growth

1) Plant height

Plant height was highest in the control and gradually decreased with increasing concentration of geothermal water in all the three intervals of observation (Table 3). Shoot growth was severely affected at the highest concentration of geothermal water.

Statistical analysis shows that plant height decreased with increase of geothermal water concentration significantly at all stages.

2) Root length

Table 4 shows that root length of control

Table 3. Plant height at five days interval as affected by dlfferent concentration of geothermal water

Cultivars	Days after treatment	Plant height(cm)				
		control	25%	50%	75%	100%
	5	16.7	12.7	11.2	7.6	4.4
Binato	10	19.5	16.7	14.8	11.1	5.1
	15	30.5	21.2	17.2	13.2	6.3
	5	8.8	7.8	5.0	3.3	2.7
IR 58	10	16.6	12.4	9.5	5.6	3.6
	15	21.7	16.5	11.8	7.1	4.6
	5	8.5	7.1	6.0	3.7	2.7
Unbong 7	10	16.6	10.3	9.1	7.4	4.3
	15	22.7	15.1	11.5	7.3	4.3

was longer than geothermal water treatments. The root length decreased significantly with increased geothermal water concentration (25~100%) at 5 DAT to 15 DAT.

The root length of Binato was reduced by 1% compared to control at 25% G.W. treatment at 15DAT, while IR58 and Unbong 7 were reduced by 14%, and 16%, respectively at 15 DAT. In the other treatments (50, 75 and 100%), there was no significant difference among the three cultivars. At 100% G. W., root length was two to three times shorter than that of control.

3) Leaf number

The number of leaves are affected by genotype and environment. The time interval between the appearance of successive leaf primordia is termed as the plastochron. The position of a leaf on the plant is designated as the plastochron number which is controlled by genotype and have shown pronounced effect on leaf growth rate (Gardner et al., 1985).

The number of leaves decreased with increase in geothermal water concentration (Table 5). For Binato, the number of leaves in control increased from 2.9 to 4.0 between 5 to 15 DAT, but that of 100% G.W. treatment increased from 2.1 to 2.3 only.

Table 4. Root length at five days interval as affected by different concentration of geothermal water

Cultivars	Days after treatment	Root length(cm)				
		control	25%	50%	75%	100%
	5	8.3	7.7	6.9	5.5	3.3
Binato	10	9.2	8.7	7.0	5.3	3.7
	15	10.2	10.1	7.6	5.6	4.1
	5	9.3	8.6	8.0	5.2	4.7
IR 58	10	11.3	8.9	8.1	6.2	5.1
	15	11.5	9.9	8.7	6.5	5.4
	5	9.5	7.7	6.9	5.0	4.0
Unbong 7	10	10.0	8.2	8.1	4.9	4.8
	15	11.5	9.7	8.5	5.4	5.4

Table 5. Number of leaves at five days interval as affected by different concentration of geothermal water

Cultivars	Days after treatment	Number of leaves				
		control	25%	50%	75%	100%
Binato	5	2.9	2.7	2.5	2.3	2.1
	10	3.7	3.4	3.2	3.0	2.3
	15	4.0	3.8	3.5	3.0	2.3
IR 58	5	2.8	2.6	2.3	2.1	2.0
	10	3.5	3.4	3.2	3.0	2.3
	15	4.0	3.9	3.6	3.0	2.4
Unbong 7	5	2.8	2.7	2.5	2.3	2.1
	10	3.8	3.7	3.3	3.1	2.3
	15	4.1	4.0	3.8	3.2	2.3

4) Dry weight

Dry weight was significantly reduced by G. W. treatment than that of control in three cultivars (Table 6). Reduction in dry weight from 25 to 100% G.W. concentration in Binato cultivar were 27, 48, 62 and 73 percent at 15 DAT. The corresponding reduction in IR58 were 19, 53, 73 and 83 percent, and for Unbong 7, 34, 52, 72 and 80percent, respectively at 15DAT. Geothermal water contains several toxic substances and it inhibit mineral and water uptake. Binato had generally smaller reduction in dry weight than IR58 and Unbong 7. This results showed that Binato was more adaptable to G.W. condition than Unbong 7.

5) Geothermal toxicity in leaf blades

Boron toxicity in rice plants start with a yellowish discoloration of the tips of older leaves (Lantin et al., 1980). As the symptoms progress the tips and margin turn yellow somewhat like what is seen in potassium deficiency.

One of the most prominent symptom observed in plant watered with geothermal water was the yellowing and subsequent dry-

Table 6. Total weight at five days interval as affected by different concentration of geothermal water

Cultivars	Days after treatment	Total dry weight(mg /plant)				
		control	25%	50%	75%	100%
Binato	5	14.0	11.3	10.4	8.3	4.3
	10	23.1	16.6	15.4	10.2	7.0
	15	36.8	26.8	19.1	13.9	8.4
IR 58	5	9.8	9.1	6.4	4.0	3.4
	10	21.2	16.4	11.8	7.3	4.5
	15	36.5	29.7	17.3	9.9	6.1
Unbong 7	5	9.4	8.1	7.6	5.6	4.1
	10	16.3	12.6	11.8	10.4	6.3
	15	30.5	20.1	14.5	8.4	6.2

ing of the leaf tips. The degree of toxicity was measured in the 2nd and 3rd leaf blades and are shown in Table 7. Percentage of damage in leaf blade was siginificantly increased with increasing G.W. concentration from 25% to 100%. For the varietal differences, IR58 showed more tolerance in 25% G.W. at 10 DAT than Binato and Unbong 7. In 50 and 75 % concentration of G.W., IR58 and Binato cultivars should be stronger than Unbong 7 at 10 DAT. However, leaf damage of three cultivars were not significantly different at 15 DAT. The cause of the eventual death of the leaf tips in the treated seedlings is not known. However, earlier obervations in geothermal sites by Maccarter (1985) indicated the possible role of arsenic, boron and iron toxicity. Mercardo(1983) speculated that high cl^- and Na^+ levels (i.e. 1300 ppm Na) in Tongonan BRIS(Bao River Irrigation system) soils might themselves be drying up the rice levels.

3. Effect of geothemal water on development of vascular bundle

Aside from the parallel system of longitudinal vascular bundle(VB), there are fine transversely oriented VB connecting them in the rice leaf (Hoshikawa, 1989). The vertical VB in the leaf blade are both large and small in cross sectional diameter, and several small vascular bundle(SVB) occur between the large vascular bundle(LVB). The number of LVB at 5th leaf significantly decreased with increasing concentration of G.W. from control to 50%. The number of SVB follow the same trend with the number of LVB(Table 8).

On the other hand, cross sectional area of LVB decrease significantly with increasing G.W. concentration. Cross sectional area of phloem, and xylem of LVB.

This result showed that G.W. contains toxic elements inhibiting the development of VB(number and size) in leaf blade. The poor seedling growth may result in less or smaller VB which in turn may result in poor growth.

In conclusion, the geothermal power plant is simple but massive. One simply digs down to tap the hot water and steam near a dead valcano and converts this into electric energy. After electricity, geothermal water erupted nearly stream and use for irrigated water in rice field. This water contains several toxic materials which restricted germination, seedling growth and VB development in rice. The tolerance at germination early seedling growth is important because the initial stand determines the final production to large extent. However, an attempt must be

Table 7. Percentage of damage in leaf blade of three cultivars to different concentration of geothermal water(GW) at 10 and 15 days after treatment

Cultivars	Percentage of leaf damage									
	10 DAT[1]					15 DAT				
	con.	25%	50%	75%	100%	con.	25%	50%	75%	100%
Binato	0	26.8b[2]	62.6b	70.3b	100	0	73.4	90.7	100	100
IR 58	0	22.2b	56.0b	69.4b	100	0	74.6	99.3	100	100
Unbong 7	0	36.3a	71.1a	100.0a	100	0	76.7	97.8	100	100

[1] Days after treatment

[2] Means followed by common letter in a column are not significantly different at the 5 % level by DMRT

Table 8. Number and cross sectional area of vascular bundles in 5th leaf blade at different concentration of geothermal water(GW)

Cultivars	Conc. of GW (%)	Large vascular buncle				Small vascular bundle	
		No.	Area ($10^{-3}mm^2$)	Phloem area ($10^{-3}mm^2$)	Xylem area ($10^{-3}mm^2$)	No.	Area ($10^{-3}mm^2$)
Binato	0	7.0a[1)]	2.78a	0.25a	0.28a	20.7a	0.45a
	25	6.0b	1.89b	0.22a	0.19b	17.7b	0.36b
	50	5.3c	1.66b	0.18b	0.15b	12.7c	0.32b
IR 58	0	7.0a	2.81a	0.29a	0.21a	25.0a	0.44a
	25	6.3b	2.25b	0.29a	0.23a	20.3b	0.36b
	50	5.0c	2.02b	0.25b	0.16b	16.3c	0.31b
Unbong 7	0	6.0a	2.76a	0.26a	0.29a	21.3a	0.44a
	25	5.3b	2.43b	0.26a	0.24ab	20.3a	0.34b
	50	5.0c	2.36b	0.24a	0.22a	16.0b	0.34b

[1)] Means followed by common letter in a column are not significantly different at the 5% level by DMRT

made to identify the cultivars that produce high yields.

摘 要

필리핀은 火山地帶에 位置해 있으며 44개 火山중 13개가 活火山으로 되어있는 狀態이다. 電氣發電을 위해 地下에 있는 高溫의 溫泉水를 利用하고 있으며 그 후 시냇가에 放流한다. 이 溫泉水에는 毒性이 强한 物質들이 많이 包含되어 있어 灌漑水로 利用하였을 경우 農作物에 至大한 惡影響을 주고 있는 實情이다. 본 實驗에서는 溫泉水를 利用하여 벼品種의 發芽, 幼苗生長 및 維管束發達에 미치는 影響을 究明하여 作物 栽培管理를 위한 基礎資料를 얻고자 遂行하였던 바 그 結果를 要約하면 다음과 같다.

1. 溫泉水內에는 황, 칼리, 나트륨, 및 붕소 含量이 灌漑水에 비해 현저하게 높았다.
2. 最終發芽率, 發芽所要日數 및 發芽速度는 溫泉水의 處理에 의해 크게 減少하였으며, 필리핀 在來種인 Binato品種은 IR58 및 雲峰7號보다 높은 發芽率을 보였다.
3. 草長, 根長, 葉數 및 總乾物重은 溫泉水의 濃度가 增加될수록 뚜렷하게 減少하였다. Binato와 IR58品種은 處理 後 15日, 25% 溫泉水 濃度에서 雲峰 7號보다 乾物重이 다소 높았다.
4. 幼苗期 葉身의 維管束發達은 無處理에 비하여 溫泉水는 50%까지 濃度를 높일수록 維管束數 및 크기 등 發達을 低下시켰다.
5. 溫泉水 處理에 따른 葉의 損傷度는 濃度가 높아질수록 크게 나타났으며, Bitano와 IR58號는 雲峰 7號보다 溫泉水에 대한 損傷度가 작아서 多少 強한 것으로 判斷되었다.

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