Irrigation of Secondary Sewage Effluent: Salinity and Nitrogen Effects on Growth and Nitrogen Fixation of Nodulated and Non-nodulated Soybeans.

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Irrigation of Secondary Sewage Effluent: Salinity and Nitrogen Effects on Growth and Nitrogen Fixation of Nodulated and Non-nodulated Soybeans.

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Salinity and nitrogenous components are the most critical water qualities in secondary sewage effluent (SSE) when used as an alternative resource for agricultural irrigation water. In this study a pot experiment was conducted to investigate the effects of salinity and inorganic nitrogen in the irrigation water on the growth and nitrogen fixation of soybean (*Glycine max* (L.) Merrill) isoline T201 and T202. Nitrogen in the irrigation water as the plant nutrient contributed slightly to dry matter production because the total amount of nitrogen applied into a pot was small compared to the plant's demand for nitrogen or to the dosage of applied fertilizer nitrogen. Nitrogen in the irrigation water, however, alleviated the toxic effect of salinity on nitrogen fixation of soybean. Moderate salinity that is similar to the salinity of SSE from the city sewage disposal plant slightly affected nitrogen fixation and reduced dry matter production. Sever salinity water with electroconductivity of 270 mS/m greatly reduced both growth and nitrogen fixation. We should pay more attention to salinity as the water quality of SSE rather than nitrogen components when SSE is reclaimed and used as irrigation water in upland fields.

INTRODUCTION

The scarcity of water resources in some parts of the world is well known. The growth of population in large cities causes increasing demands for water. When there is a scarcity of rain for the most part of a year, those cities are often struck by water famine. Almost all of the tap water consumed by the populace of the city drains into the sewage disposal plant, in which sewage is biologically treated after sedimentation of any solid material and in the end secondary sewage effluent (SSE) is discharged. If the effluent can be reclaimed as a substitute for agricultural irrigation water, then part of the water reserved for the agricultural sectors can be rechanneled to service water for human consumption. The problem is that the effluent may contain high concentrations of nitrogen and phosphorus and sometimes dissolved salts such as sodium chloride, and microbes.

In Japan, the application of SSE is practically hoped in paddy rice cultivation because of a huge demand for irrigation water. There are several systematic studies on the application of SSE to paddy rice (Hidaka 1990). The growth and yields of rice plants are strongly affected by ammonium nitrogen in SSE although nitrate nitrogen has adverse

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effects on rice growth during the reproductive growth period because reproductive rice prefers nitrate nitrogen to ammonium nitrogen.

Recently, agricultural water in the small rivers and channels in the towns neighboring to the large city is usually severely eutrophicated, and some adverse effects sometimes occur to paddy and upland crops when this water is used as irrigation water. Farmers are advised to reduce the amount of fertilizer applied to the farm lands irrigated with eutrophicated water or SSE. In this context we can also save the fossil fuel that is otherwise consumed for the production of chemical fertilizers.

On the other hand, SSE often contains high levels of salts. When SSE is applied to upland crops, salinity problems occur unless the irrigated SSE does leach out. Attention should be paid in use of SSE from a sewage disposal plant that is located along the shore because sewage contains much sodium chloride due to possible entering of brackish water.

In this study we investigated the effects of salts and nirogen contained in the SSE when used as irrigation water under upland conditions on nodulated and non-nodulated soybeans grown in pots. For this purpose we applied irrigation water that was supplemented with salts alone and with salts and nitrogen.

MATERIALS AND METHODS

Plant culture

Gray lowland soil (2.46 kg dry soil) was placed on a sand and gravel layer (5 cm) in a a/5000 pot with a drain hole.

Phosphorus and potassium fertilizers (1.0 g P_2O_5 and 1.0 g K_2O as KH_2PO_4 and K_2HPO_4) were applied with mixing in the upper layer (5 cm) of the pot soil. Three soybean (*Glycine max* (L.) Merrill) seeds, isoline T201 or isoline T202 were placed 1 cm in depth in the centre of the pot, and were inoculated with a suspension (5 mL [10^8 cells] per seed) of *Bradyrhizobium japonicum* USDA 110, and were covered with moistened soil on July 30, 1996. The pot was covered with a plastic blind until the emergence of the cotyledon. When the first leaf appeared (August 5) one healthy and uniform plant in each pot remained while other two plants were thinned, and urea nitrogen (1.0 g N per pot) was applied to half the number of pots (T201 + N) in which non-nodulating isoline T201 seedlings were planted. Additional urea-nitrogen (0.5 g N per pot) was top-dressed to T201 + N plants on August 31. T202 plants did not receive any nitrogen fertilizer.

Application of irrigation water

From August 5, five kinds of irrigation water were applied to both T201 and T202 plants when necessary, but the applied water was not drained. The amount of irrigated water was recorded every time. Tap water (W1) was used as the control irrigation water. Analytical results showed that tap water contained K^+: 0.35, Ca^{2+}: 1.13, Mg^{2+}: 0.42, Na^+:0.72, NO_3^-:0.24, Cl^-:0.61, and $SO4^{2-}$: 0.34 in mM. To prepare the other four kinds of the treatment water, ions of concentrations as shown in Table 1 were added to the tap water. W2 and W4 water were supplemented with salts and inorganic nitrogen whereas W3 and W5 water were supplemented with only salts. The salinity of W2 and W3 water was similar to that of the SSE discharged from the Fukuoka West Sewage Disposal Plant. W4 and W5 water were of severe salinity. From 21st day of irrigation the application of

Table. 1 Composition of supplemental ions and elctroconductivity in treatment water

Treatment water	Concentration of ions (mM)					EC
	Na^+	NH_4^+	NO_3^-	Cl^-	SO_4^{2-}	(mS/m)
W2	5.07	0.8	0.26	4.99	0.31	100
W3	5.61	0	0	4.99	0.31	100
W4	18.87	0.8	0.26	16.19	1.61	270
W5	19.41	0	0	16.19	1.61	270

The electroconductivity (EC) of tap water (W1) was 45 mS/m.

W4 and W5 water was discontinued and was replaced by the application of W1 water. Each irrigation treatment was done in triplicate.

Acetylene reduction activity (ARA) measurements

The soybean plants at the early pod setting stage were used for ARA measurements. After the roots were pulled out with the pot soil from the pots, almost all the soil was removed carefully to avoid release of nodules. The roots were washed with tap water to clean up the surface of roots and nodules. The roots with the intact nodules were cut at the cotyledonary node and were individually placed in 500-mL conical flasks for ARA measurements. The flask was sealed with a rubber stopper with a serum septum, and 10% of the air inside the flask was replaced with acetylene gas. The nodulated roots were incubated at room temperatures. Incubation was carried out in triplicate. One mL of subsamples taken at 5 and 35 minutes after incubation was analyzed for ethylene production with a GC-14A gas chromatograph (Shimazu, Kyoto) fitted with a stainless steel column (3 mm in diameter, 0.5 m in length). The column was filled with Porapak R (80–100 mesh). Column, injector and detector temperatures were 45, 170, and 170 °C, respectively. Carrier gas was nitrogen and the flow rate was 30 mL per minute.

Harvest of plants and determination of mineral content

Leaves of isoline T201 with no nitrogen fertilizer fell on September 16, and those plants were harvested on September 18. Isoline T201 with nitrogen fertilizer and isoline T202 were harvested on October 9 and September 26 , respectively. The harvested plants were separated into the various parts, which were dried at 70 to 80 °C and weighed for the determination of dry weight. Powdered leaf samples were incinerated at 500 °C for analyses of mineral nutrients, and the residues were dissolved in dilute HCl. Potassium and Na were determined by flame-emission spectrometry, and Ca and Mg by atomic absorption spectrometry (Hitachi 170–30 spectrometer).

RESULTS

Plant growth

Until August 26, 2.8 liters of water were applied to each pot. The height of the plants was measured and the number of trifoliolate leaves was counted every week. Growth as

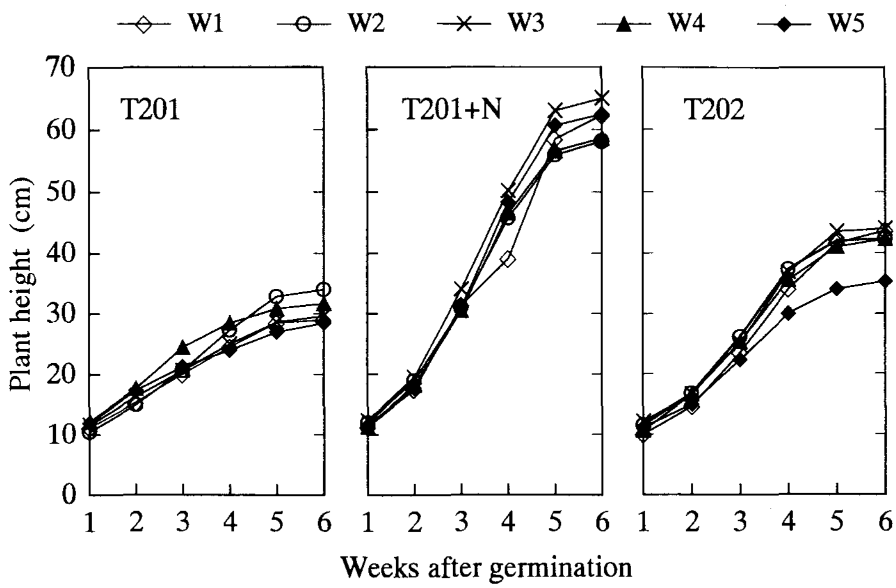

Fig. 1 Growth of soybean isoline T201 and T202 as affected by salinity and nitrogen in irrigation water. T201+N indicates isoline T201 that was applied with nitrogen fertilizer.

indicated by plant height was best in isoline T201 with nitrogen fertilizer followed by isoline T202 and isoline T201 with no nitrogen fertilizer (Fig. 1). However irrigation water without any nitrogen fertilizer had no effect on plant height even in both isolines. This result suggests that nitrogen contained in the irrigation water was scarcely effective on plant growth. The development of plants as indicated by the number of trifoliolate leaves showed a similar trend to plant growth in each irrigation treatment (data not shown).

Nodulation and acetylene reduction activity

Because the nodulating isoline T202 did not receive nitrogen fertilizer the plant height of isoline T202 was similar to that of isoline T201 without any nitrogen fertilizer two weeks after treatment. But a week later the increment of the plant height of isoline T202 for the week was larger than that of isoline T201 without any nitrogen fertilizer. It seems that nitrogen fixation in isoline T202 was initiated by effective nodulation during this week. Measurements of ARA were done for the roots with intact nodules separated from the shoot of plants treated for four weeks. The weather was fine on the day of measurements. Table 2 shows nodulation and ARA of detached intact roots in isoline T202. Nodulation in both the upper and lower part of roots as indicated by nodule number was greatly suppressed by the application of W4 and W5 water. Although the application of W2 and W3 water did not suppress nodulation in the upper part of the roots, it resulted in approximately 35% less nodulation in the lower part of the roots

Table. 2 Nodulation and acetylene reduction activity (ARA) of isoline T202 of soybean as affected by salinity and nitrogen in irrigation water.

Water	Nodule number (/plant)		Nodule dry weight	Root dry weight	Total ARA	Specific ARA
	Upper	Lower	(g)	(g)	(μmol/h/plant)	(μmol/h/g dry nodule)
W1	59	103	0.58	1.34	36.9	63.4
W2	64	61	0.54	1.62	43.4	80.6
W3	62	65	0.49	1.23	32.0	67.0
W4	37	34	0.28	1.19	16.0	58.6
W5	23	34	0.21	0.75	7.9	38.4

compared to the application of W1 water. Nodule weight appears to show a similar trend to nodulation.

The total ARA was clearly low when W4 and W5 water were applied. On the other hand plants that received W2 and W3 water had values of total ARA similar to those of plants applied with W1 water although the latter plants had more nodules. Among the various kinds of treatments of the prepared water the supplement of nitrogen seems to give a positive effect on ARA even though this nitrogen affected little plant growth (Fig. 1).

The specific ARA was the highest in W2 treatment and the lowest in W5 treatment, but almost the same among W1, W3 and W4 treatments. The root mass of W5-treated plants was smaller than that of W4-treated plants, which seems to be related to the lowest specific ARA in W5-treated plants.

Dry matter production

The lowest dry matter production was recorded in non-nodulating isoline T201 that did not receive any nitrogen fertilizer although the values of their dry weight did not include root weight because of decay of the root tissues at harvest (Fig. 2). In these plants nitrogen is the limiting factor for growth, thus the salinity effect on dry matter production was not clear. Between the plants irrigated with water of the same salinity (i.e. W2 vs W3 and W4 vs W5), the dry matter was produced more when a low concentration of nitrogen was supplemented. Dry matter production was larger in isoline T201 that was applied with nitrogen fertilizer than that in nodulating isoline T202 regardless of the quality of the irrigation water. Low salinity water (W2 and W3) caused 12 to 18% less dry matter production, and the irrigation of high salinity water (W4 and W5) resulted in severe reduction in dry matter production. In isoline T201 supplied with nitrogen fertilizer, supplemented nitrogen in the irrigation water did not contribute to an increase in the dry weight. In nodulating isoline T202, dry matter production was greatly decreased by the irrigation of saline water, especially by the irrigation of high salinity water without any nitrogen supplement.

Foliar mineral content

Irrigation of high salinity water did not always decrease K, Ca, and Mg contents in both the lower and upper leaves (Table 3). On the other hand, Na content in the leaves was very low. It was not affected in isoline T202 plants by the salinity of the irrigation water, but it was increased in isoline T201 plants when high salinity water was applied.

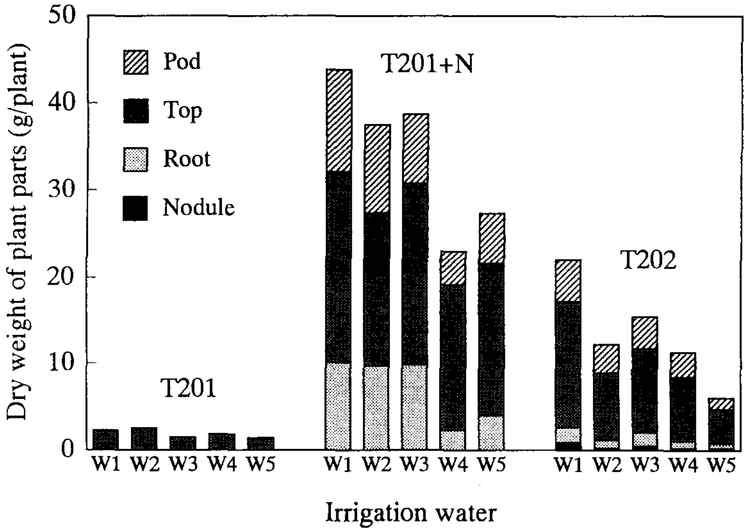

Fig. 2 Effect of salinity and nitrogen in irrigation water on dry matter production in soybean isoline T201 and T202. T201+N indicates isoline T201 that was applied with nitrogen fertilizer.

Table. 3 Effect of salinity and nitrogen in irrigation water on mineral contents in leaves of two soybean isolines.

Isoline	Water	Lower leaves				Upper leaves			
		K	Ca	Mg	Na	K	Ca	Mg	Na
		g/kg dry matter							
T201*									
	W1	10.3	18.5	4.04	0.21	6.6	16.1	2.73	0.20
	W2	10.0	23.0	5.56	0.04	5.2	19.5	4.02	0.12
	W3	10.3	22.7	5.92	0.07	5.3	20.7	4.26	0.21
	W4	16.3	29.7	6.98	0.45	8.0	18.1	3.78	0.42
	W5	17.8	23.8	6.67	0.41	11.3	13.5	3.52	0.85
T202									
	W1	12.3	14.3	2.80	0.27	14.0	7.6	1.86	0.51
	W2	18.9	21.9	5.52	0.49	13.7	13.5	3.11	0.35
	W3	13.6	17.7	4.09	0.38	17.6	9.2	2.52	0.50
	W4	24.7	18.5	5.78	0.14	15.2	12.5	3.19	0.33
	W5	30.6	22.1	5.80	0.28	12.2	11.9	2.76	0.50

*Nitrogen fertilizer was applied to only isoline T201 plants.

DISCUSSION

Irrigation water that contains more than 5 mg/L of nitrate-N or ammonium-N may affect the growth of susceptible crops (Ayres and Westcot 1976). In the present study 14 mg/L of inorganic nitrogen was supplemented in some irrigation water. In non-nodulating isoline T201 that was not supplied with nitrogen fertilizer, supplemental nitrogen was beneficial for dry matter production, but the amounts of nitrogen from the soil and irrigation water were not sufficient for the complete performance of isoline T201 when no nitrogen fertilizer was applied. On the other hand, in isoline T201 that received nitrogen fertilizer (1.5 g N per pot) an additional effect of nitrogen from the water was not found. Since nitrogen fertilizer was not applied to nodulating isoline T202, nitrogen in the irrigation water alleviated suppression of nitrogen fixation (total and specific ARA) due to salinity. It is reported that in faba bean (*Vicia faba* L.) an exogenous supply of nitrate-N moderated the suppressive effects of salinity and would improve the vegetative growth of *V. faba* plants (Cordovilla *et al.* 1995). Yoshida and Ishii (1988) reported that irrigation of SSE containing nitrogen of 20 mg/L did not affect the growth of soybean (cv. Tamahomare) under field conditions. Conclusively, such levels of nitrogen in the irrigation water as supplemented in the present study would not affect soybean growth.

Ayres and Westcot (1976) indicated in their guidelines that irrigation water with electroconductivity of more than 3.3 mmho/cm (330 mS/m) begins to affect the growth of soybeans under field conditions. In soybean isoline T201 and isoline T202, even moderate salinity (100 mS/m) resulted in reduced dry matter production. For isoline T201 supplied with nitrogen fertilizer, salinity affected the growth of plants themselves while nodulation and nitrogen fixation activity were also influenced by salinity in isoline T202. In this study treatment was initiated a few days after inoculation, thus irrigation of water with moderate salinity did not affect nodulation at the upper part of the roots but reduced late nodulation at the lower part of roots, probably resulting from salt accumulation during a longer period of irrigation. Even severe salinity of water did not completely inhibit nodulation. Singleton and Bohlool (1984) reported that the early process involved in nodule formation by soybean was sensitive to NaCl and nodule function was relatively less sensitive to salts than nodule initiation. Hence it is thought that the early process of nodulation already terminated at the time of emergence of soybean cotyledon and nodule growth continued under severe salinity conditions. Soybean was classified as one of the legumes with high tolerance to salinity (Delgado *et al.* 1994). However, severe salinity caused decreased number of nodules and smaller root mass, resulting in reduced nitrogen fixation activity in isoline T202. Because foliar Na content was not increased by the irrigation of saline water, an adverse effect of salinity on soybean could be related to the osmotic conditions of the rhizosphere, but not to foliar toxicity of Na in soybean. In conclusion, when drainage is insufficient in the fields, there is a possibility that even irrigation of water with moderate salinity, i.e. electroconductivity of 100 mS/m, will affect adversely soybean growth and yields.

It should be kept in mind that the application of SSE requires careful management of issues related to public health and public acceptance (Ayers and Westcot 1976).

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