Effects of Chemical Form and Nitrogen Concentration of Fertilizer and Water Culture Medium pH on Growth and Root System Development of Alfalfa (Medicago sativa L.)

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Abstract

Effects of differences in nitrogen concentration and water culture medium pH on growth and root system development of alfalfa grown in plots fertilized with ammonium nitrogen or nitrate nitrogen were investigated. Differences in dry weight, total root length, and mean lateral root length were observed between ammonium and nitrate plots at nitrogen concentrations of 20 and 30 ppm and pH 6.5. However, no difference was observed when the pH was shifted in the acid or alkaline direction. Differences in the number of roots between ammonium and nitrate plots were only observed at a nitrogen concentration of 20 ppm and pH 6.5. Moreover, no significant difference was observed when the pH was shifted in the acid or alkaline direction. These results indicated that the high growth-promoting effect of ammonium nitrogen and nitrate nitrogen on growth and root system development observed at nitrogen concentrations of 20 and 30 ppm and pH 6.5 are lost when the medium pH is shifted in either the acid or alkaline direction.

Keywords: Alfalfa, Growth , Nitrogen Fertilizer, Root System , Water Culture Medium pH.

1. Introduction

In studies to date, it had been reported that application of ammonium nitrogen is superior to nitrate nitrogen in improving early growth and the first year yield and quality of alfalfa seedlings (Hirose et al., 1994; Hirose 1997). It was also clarified that these results were partly due to differences in root system development (Hirose 2003, 2005). In addition, during early growth of alfalfa, application of ammonium nitrogen was superior in improving growth and root system development at a nitrogen concentration of 20 ppm and water culture medium pH of 6.5, whereas at nitrogen concentrations higher than 30 ppm, no significant difference in growth was observed. By contrast, application of nitrate nitrogen was superior in improving root system development (Hirose 2006). However, it has been reported that growth and yield of alfalfa are easily affected by water culture medium pH, as both are reduced when acidity of the medium increases (Jo et al.,1980a, 1980b, 1981; Sato et al., 1984; Fageria et al., 1989; Peters et al., 2005) and root length shortens as pH decreases (Fox et al., 1955; Rechcigl et al., 1986). Hence, the result indicating that the chemical form of fertilizer nitrogen that is superior in improving the growth and root system development changes depending on the nitrogen concentration of the water culture medium may not be applicable to cases when water culture medium pH is changed. This research was performed to clarify the influence of differences in water culture medium pH, in addition to chemical form and concentration of fertilizer nitrogen, on growth and root system development of alfalfa by adjusting water culture medium pH using automatic pH adjusters and applying ammonium nitrogen and nitrate nitrogen.

2. Materials and Methods

2.1. Variety and culturing method of alfalfa

This experiment was conducted in a glasshouse using Tachiwakaba variety alfalfa. On April 12, 2010, alfalfa was seeded in containers (27 × 34 × 10 cm) filled with expanded vermiculites and raised with distilled water alone until April 19.

The alfalfa seedlings were subsequently transplanted to containers (54 × 36 × 25 cm), 35 individuals per container, to start water culture, which was conducted in the same manner as reported previously(Hirose 2003, 2000). The water culture medium was completely replaced every five days.

2.2. Setting test plots

Treatments with nitrogen concentrations of 20, 30, or 40 ppm (ingredient content in all cases) in water culture medium pH that was adjusted to 5.5 ± 0.1 (hereinafter referred to as 20 ppm pH 5.5 treatment, 30 ppm pH 5.5 treatment, and 40 ppm pH 5.5 treatment, respectively), 6.5 ± 0.1 (hereinafter referred to as 20 ppm pH 6.5 treatment, 30 ppm pH 6.5 treatment, and 40 ppm pH 6.5 treatment, respectively), or 7.5 ± 0.1 (hereinafter referred to as 20 ppm pH 7.5 treatment, 30 ppm pH 7.5 treatment, and 40 ppm pH 7.5 treatment, respectively) were prepared by applying either ammonium sulfate (ammonium plot) or sodium nitrate (nitrate plot) (total plots = 18). Two containers were used per plot and the pH of each plot was adjusted using an automatic pH adjuster.

2.3. Preparation of automatic pH adjusters

Each automatic pH adjuster was prepared by connecting a pH sensor (IS-GFV-TV: Iwaki Co. LtD., Tokyo, Japan) and an electromagnetic metering pump, for feeding of 2N hydrochloric acid and 2N sodium hydroxide (EH/R-AE: Iwaki Co. LtD., Tokyo, Japan), to an automatic pH controller (PH-70P model: Iwaki Co. LtD., Tokyo, Japan).

2.4.Timing of collection of materials and their separation into organs

Materials were collected from six individuals per plot (three individuals per container) every ten days between days 30 and 60 after sowing (total collections = 4). The collected individuals were separated into leaves, stems, and roots. Immediately after collection, the leaves and stems were air-dried for two days at 80°C. The roots were initially stored in FAA solution (ethanol: distilled water: acetic acid: formalin = 9:9:1:1 [v/v]), and then taken out as required to measure taproot and lateral root lengths and the number of lateral roots. After measurement, the materials were separated into taproots and lateral roots and air-dried in the same manner as for leaves and stems. After drying, dry weight of each organ was measured.

2.5. Measuring methods of taproot and lateral root lengths and the number of lateral roots

Taproot and lateral root lengths and the number of lateral roots were measured using an image analysis method (Kimura et al., 2001; Kimura and Yamasaki, 2003) with 300 dpi resolution.

2.6. Statistical analysis

Analysis of variance was applied to dry weight, total root length, and number of lateral roots to clarify the influence of chemical form and concentration of fertilizer nitrogen and water culture medium pH on these measurements in more detail.

3. Results

Figure 1 shows changes in dry weight of each organ in each plot during the experimental period. At a nitrogen concentration of 20 ppm, ammonium and nitrate plots exhibited no significant difference between pH 5.5 and 7.5 treatments. On the other hand, after pH 6.5 treatment, the dry weights were significantly heavier in the ammonium plots on day 50 after sowing and thereafter, and ammonium plots exhibited approximately 1.2 times higher values than nitrate plots on day 60. In 30 ppm pH 5.5 and 7.5 treatment plots, no significant difference was observed between ammonium and nitrate plots. In pH 6.5 treatment plots, dry weights of the nitrate plots were higher than those of the ammonium plots on and after day 40 and exhibited approximately 1.9 times higher values on day 60. At 40 ppm, ammonium and nitrate plots exhibited no significant differences for all treatments.

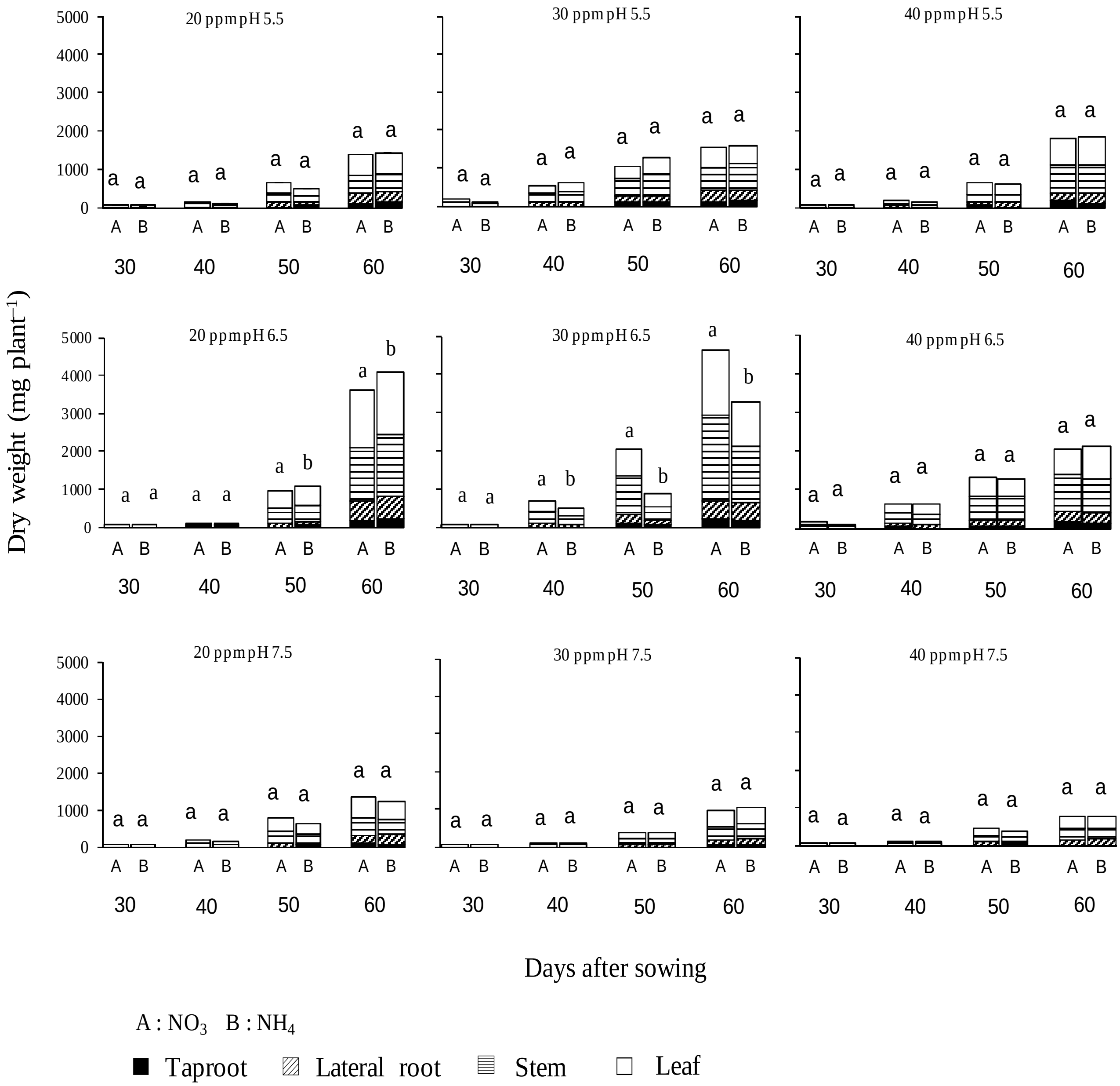

Figure 1: Effects of chemical form and concentration of nitrogen fertilizer and water culture medium pH on dry weights of organs of alfalfa seedlings.Total weights (full column heights) with different letters on the same sampling day in the same nitrogen concentration and water culture pH indicate significant difference at 5% level.

Figure 2 shows changes in taproot lengths in each plot. In 20 ppm pH 5.5 treatment plots, on day 60 after sowing, lengths were significantly higher in ammonium plots than in nitrate plots. On the other hand, neither ammonium nor nitrate plots exhibited significant differences under other treatment conditions.

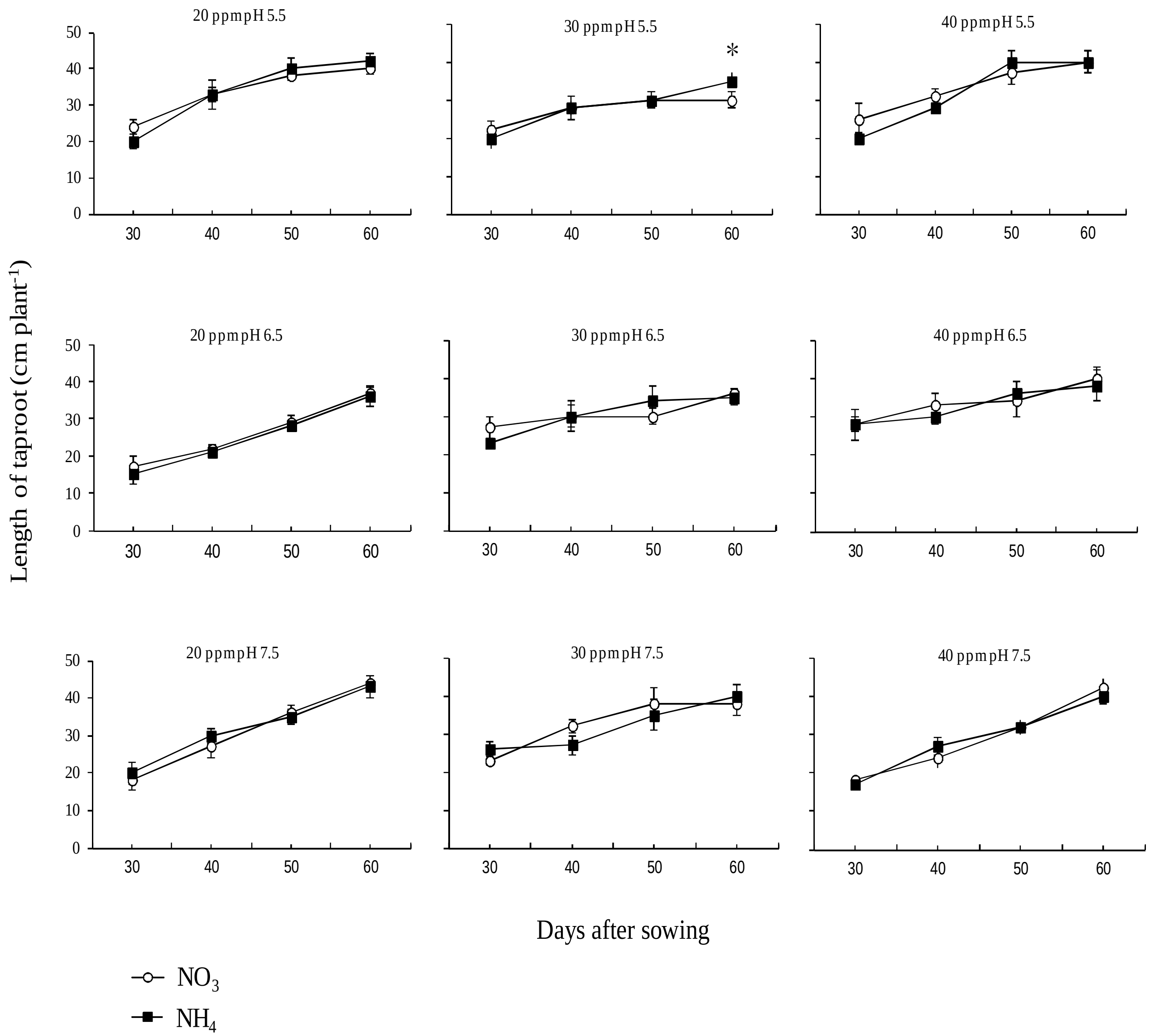

Figure 2: Effects of chemical form and concentration of nitrogen fertilizer and water culture medium pH on the length of taproot of alfalfa seedlings. Vertical bar indicates ± SE. * on the same sampling day in the same nitrogen concentration and water culture pH indicate significant difference at 5**%** level.

Figure 3 shows changes in total root length in each plot. In 20 ppm pH 5.5 and 7.5 treatment plots, nitrate and ammonium plots exhibited no significant differences. In pH 6.5 treatment plots, the total root length in ammonium plots was higher than that in nitrate plots on day 50 after sowing and thereafter, and approximately 1.3 times higher on day 60. At 30 ppm, ammonium and nitrate plots exhibited no significant differences in pH 5.5 and 7.5 treatments, but the value became approximately 1.3 times higher in nitrate plots with pH 6.5 treatment on day 60 after sowing. No significant differences were observed between ammonium and nitrate plots in all pH treatments at 40 ppm.

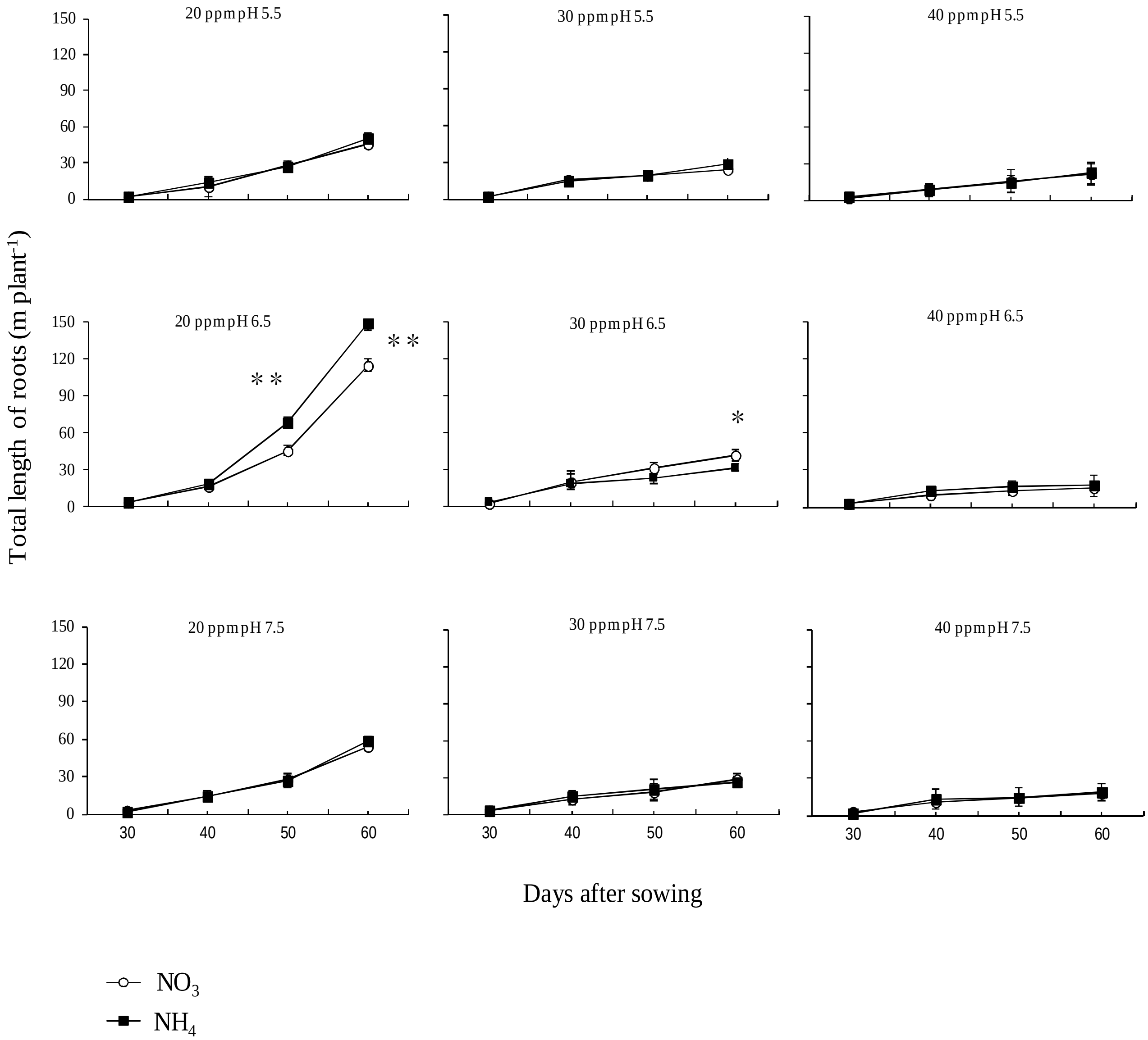

Figure 3: Effects of chemical form and concentration of nitrogen fertilizer and water culture medium pH on the total length of roots of alfalfa seedlings. Vertical bar indicates ±SE. * and ** on the same sampling day in the same nitrogen concentration and water culture pH indicate significant difference at 5 and 1% levels, respectively.

Figure 4 shows changes in the number of lateral roots in each plot. Ammonium and nitrate plots exhibited no significant differences under 20 ppm pH 5.5 and 7.5 treatments. In pH 6.5 treatment plots, the number of lateral roots in ammonium plots was approximately 1.3 times greater than that in nitrate plots on day 60 after sowing. No significant differences were observed between ammonium and nitrate plots in all pH treatments at 30 and 40 ppm. Table 1 shows changes in specific root length of taproot (taproot length/taproot weight). Both nitrate and ammonium plots exhibited the highest values on day 30 after sowing in all treatments, and the values subsequently tended to decrease as the plants grew. As per the results of day 30 after sowing, nitrate plots exhibited higher values than ammonium plots in 20 ppm pH 7.5 treatments, whereas both plots exhibited no significant differences in pH 5.5 and 6.5 treatments. In 30 ppm pH 5.5 and 6.5 treatment plots, nitrate plots exhibited higher values than ammonium plots; however, no significant differences were observed between the two plots in pH 7.5 treatment.

At 40 ppm, ammonium plots and nitrate plots exhibited higher values in pH 5.5 treatment and pH 6.5 treatment, respectively, but the two plots exhibited no significant differences in pH 7.5 treatment.

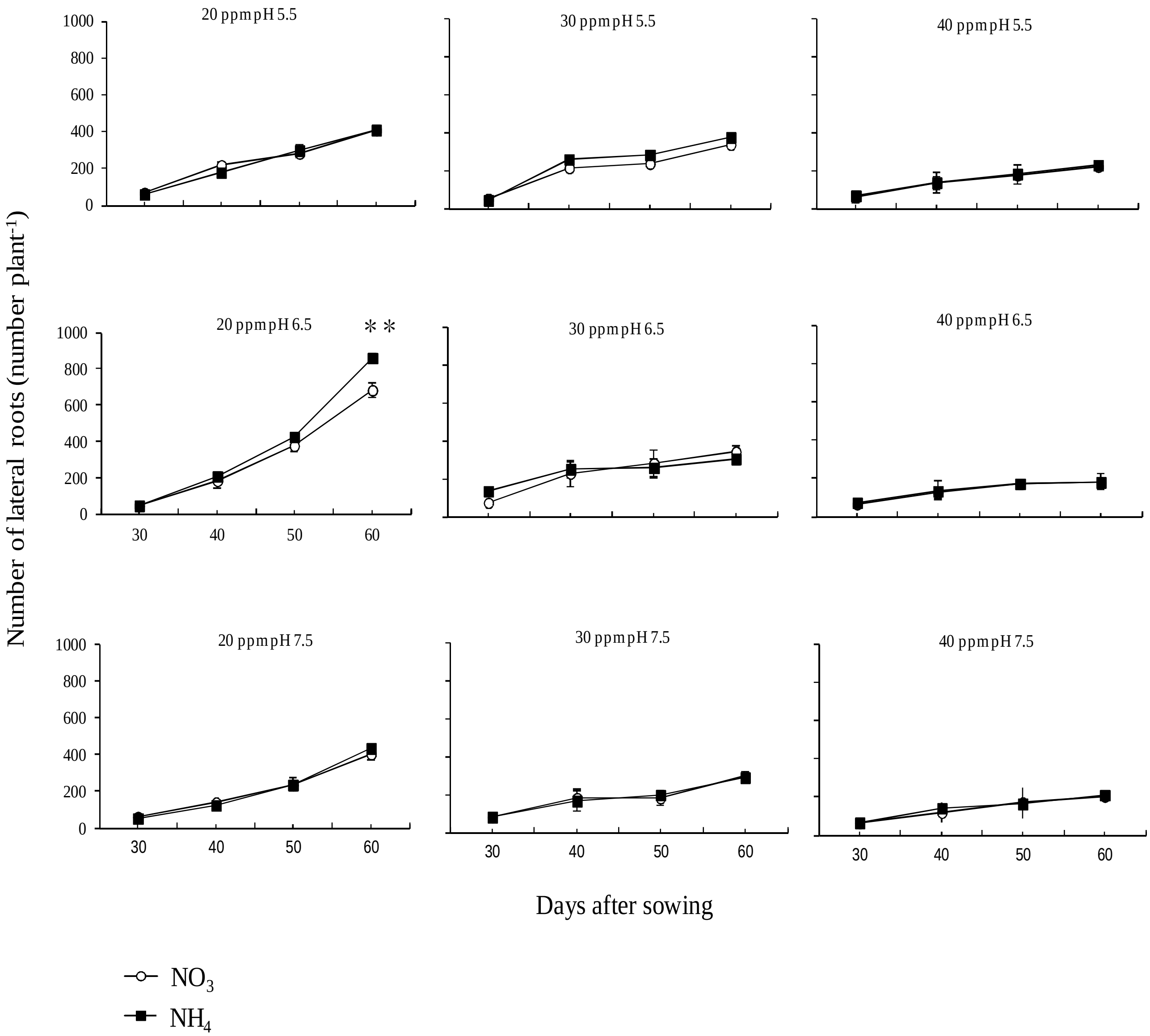

Figure 4 : Effects of chemical form and concentration of nitrogen fertilizer and water culture medium pH on number of lateral roots of alfalfa seedlings. Vertical bar indicates ± SE. ** on the same sampling day in the same nitrogen concentration and water culture pH indicate significant difference at 1% level.

Table 1: Effects of chemical form and cocentration of nitrogen fertilizer and water culture pH on specific root length of taproot of alfalfa seedlings.

	Days after sowing	20ppm		30ppm		40ppm	
		NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
				(m g^{-1})			
pH5.5	30	88.9^a	87.0^a	30.6^a	22.5^b	64.9^a	74.1^b
	40	19.4^a	22.0^a	8.4^a	8.3^a	14.4^a	23.9^b
	50	11.1^a	10.3^a	6.5^a	7.6^a	11.2^a	10.1^a
	60	5.7^a	3.2^a	3.6^a	3.7^a	3.0^a	4.5^a
pH6.5	30	87.2^a	81.1^a	75.0^a	53.5^b	71.8^a	63.6^b
	40	28.3^a	25.9^a	8.9^a	9.4^a	8.0^a	8.2^a
	50	5.8^a	3.0^a	3.9^a	5.4^a	3.5^a	3.7^a
	60	2.9^a	2.7^a	2.5^a	2.6^a	2.7^a	2.9^a
pH7.5	30	56.3^a	40.8^a	39.0^a	37.1^a	43.9^a	39.5^a
	40	23.5^a	25.2^a	21.3^a	15.0^b	11.1^a	11.5^a
	50	18.7^a	11.0^b	6.3^a	4.0^a	10.7^a	11.9^a
	60	6.6^a	5.1^a	2.5^a	2.5^a	11.7^a	16.3^a

Means with different superscripts (a-b) within the same day, nitrogen concentration and water culture pH are significantly different at 5% level.

Table 2 shows the mean length of lateral roots. As per the results on day 60 after sowing, ammonium and nitrate plots exhibited no significant differences in pH 5.5 and 7.5 treatments at nitrogen concentrations of 20 and 30 ppm. However, in the pH 6.5 treatment plots, the length in ammonium plots was higher at 20 ppm, whereas conversely at 30 ppm, the length in nitrate plots was higher. Ammonium and nitrate plots exhibited no significant differences in all treatments at 40 ppm.

Table 2: Effects of chemical form and cocentration of nitrogen fertilizer and water culture pH on mean length of lateral root of alfalfa seedlings.

	Days after sowing	20ppm		30ppm		40ppm	
		NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
				(cm $root^{-1}$)			
pH5.5	30	4.3^a	9.1^b	2.1^a	2.7^a	5.2^a	4.4^a
	40	9.7^a	9.7^a	4.1^a	2.6^a	4.5^a	6.8^a
	50	10.1^a	9.8^a	4.6^a	2.9^a	7.3^a	7.9^a
	60	15.0^a	13.0^a	4.2^a	3.8^a	7.6^a	8.3^a
pH6.5	30	7.2^a	11.4^b	5.1^a	4.7^a	5.0^a	5.8^a
	40	6.9^a	12.0^b	4.5^a	4.9^a	4.6^a	6.0^a
	50	11.1^a	14.1^b	8.8^a	5.6^a	8.7^a	5.7^a
	60	12.7^a	15.5^b	9.6^a	5.1^b	8.7^a	7.0^a
pH7.5	30	8.4^a	8.9^a	7.6^a	5.9^a	5.9^a	7.7^a
	40	8.2^a	9.8^a	7.6^a	7.8^a	6.2^a	7.6^a
	50	8.9^a	10.4^a	8.3^a	8.7^a	8.5^a	9.4^a
	60	9.4^a	11.4^a	8.0^a	7.3^a	8.8^a	9.1^a

Means with different superscripts (a-b) within the same day, nitrogen concentration and water culture pH are significantly different at 5% level.

Table 3 shows the results of analysis of variance on dry weight, total root length, and number of lateral roots. The chemical form of nitrogen greatly affected the dry weight only on day 60. Nitrogen concentration and water culture medium pH significantly influenced dry weight on day 30 after sowing and thereafter. In all cases, chemical form of nitrogen and nitrogen concentration of water culture medium affected dry weight on day 50 after sowing; nitrogen concentration and water culture medium pH affected dry weight on day 30 after sowing and thereafter; and chemical form of nitrogen, nitrogen concentration, and water culture medium pH affected dry weight on days 30 and 50 after sowing, in a highly interactive manner.

Table 3: Analysis of variance(ANOVA) of dry weight, total length of roots and number of lateral roots of alfalfa seedlings.

	Days after sowing			
	30	40	50	60
Dry weight				
nitrogen concentration (A)	**	**	**	**
water culture pH (B)	**	**	**	**
nitrogen form (C)	*	*	**	**
A×B	**	**	**	**
B×C	ns	ns	**	**
A×C	ns	**	**	ns
Total length of roots				
nitrogen concentration (A)	**	**	**	**
water culture pH (B)	**	ns	**	**
nitrogen form (C)	*	ns	ns	**
A×B	ns	*	*	**
B×C	ns	ns	ns	**
A×C	ns	ns	ns	**
Number of lateral roots				
nitrogen concentration (A)	**	**	**	**
water culture pH (B)	*	**	**	**
nitrogen form (C)	**	*	**	**
A×B	**	**	**	**
B×C	ns	ns	ns	**
A×C	*	ns	ns	**

ns, * and ** indicate no significant, significant difference at 5% level and 1% level, respectively.

Chemical form of nitrogen and nitrogen concentration of the water culture medium greatly influenced total root length on day 60 after sowing and on day 30 after sowing, respectively. On the other hand, water culture medium pH exhibited great influence at every time point except on day 40 after sowing. In addition, chemical form of nitrogen and nitrogen concentration of the water culture medium affected total root length on day 50 after sowing and thereafter in an interactive manner. Chemical form of nitrogen and water culture medium pH exhibited the interaction only on day 60 after sowing. Nitrogen concentration and water culture medium pH exhibited high interaction on day 40 after sowing and thereafter. Furthermore, chemical form of nitrogen, nitrogen concentration, and water culture medium pH influenced total root length in an interactive manner only on day 60 after sowing. Chemical form of nitrogen exhibited no significant influence on the number of lateral roots at all the significant interactions in affecting time points. Nitrogen concentration of water culture medium greatly influenced lateral root number on day 30 after sowing and thereafter. Water culture medium pH greatly influenced lateral root number on day 40 after sowing and thereafter.

In addition, chemical form of nitrogen and nitrogen concentration of water culture medium, and chemical form of nitrogen and water culture medium pH exhibited no significant interactions in affecting the number of lateral roots at all the time points. On the other hand, nitrogen concentration and water culture medium pH began to show the interaction on day 30 after sowing and thereafter, and high influence was observed especially on day 50 after sowing and thereafter. Furthermore, chemical form of nitrogen, nitrogen concentration, and water culture medium pH interacted at a high level only on day 60 after sowing. No adherences of root nodules were observed in any plots.

4. Discussion

It has been previously reported that water culture medium pH between 6.0 and 6.5 is most appropriate for growth of alfalfa(Harada 1979). In the present experiment as well, dry weights were highest in pH 6.5 treatment at all nitrogen concentrations in both ammonium and nitrate plots (Figure 1), indicating the same trend. In addition, as per the results of pH 6.5 treatment in ammonium and nitrate plots, the weight was highest at 20 ppm in ammonium plots and 30 ppm in nitrate plots (Figure 1). Thus, nitrogen concentration most appropriate for growth differed depending on the chemical form of the fertilizer nitrogen, both in the present experiment and previous report (Hirose 2006). In addition, the weight increased in ammonium and nitrate plots in 20 ppm pH 6.5 and 30 ppm pH 6.5 treatments, respectively (Figure 1). This finding indicates that the chemical form of fertilizer nitrogen appropriate for growth differs depending on nitrogen concentration of the water culture medium.

Total root lengths were highest in 20 ppm pH 6.5 and 30 ppm pH 6.5 treatments in ammonium and nitrate plots, respectively (Figure 3). In addition, as indicated by the total root length on day 60 after sowing, the length decreased when the water culture medium pH was shifted in either the acid or alkaline direction in both nitrate and ammonium plots at all nitrogen concentrations, and differences in length between the two plots disappeared at 20 and 30 ppm (Figure 3). Based on these results, we speculated that shifting pH in either the acid or alkaline direction inhibited extension of total root length at 20 ppm and 30 ppm, resulting in no observation of significant differences in length between the chemical forms of nitrogen.

As indicated by the results of the number of lateral roots on day 60 after sowing at a nitrogen concentration of 20 ppm, the number of roots decreased in both nitrate and ammonium plots when the pH of the water culture medium was shifted in either the acid or alkaline direction from 6.5, with a higher rate of decrease in the ammonium plots (Figure 4). Therefore, we concluded that lack of significant difference in the total root length between ammonium and nitrate plots after pH change was partly due to a decrease in the number of lateral roots. On the other hand, no significant difference was observed in the number of roots between 30 ppm pH 5.5 and 6.5 treatments in both ammonium and nitrate plots (Figure 4). However, mean lengths of lateral roots in 30ppm pH 5.5 treatment were reduced by approximately 25% and 56% in ammonium and nitrate plots, respectively, compared with the values in the 30ppm pH 6.5 treatment (Table 3). Therefore, we concluded that lack of significant difference in total root length between ammonium and nitrate plots in pH 5.5 treatment was partly due to significantly higher inhibition of extension of individual lateral roots in nitrate plots compared with that in ammonium plots. In addition, the number of roots decreased by approximately 6% and 15% in ammonium and nitrate plots, respectively, in the 30ppm pH 7.5 treatment compared with the 30ppm pH 6.5 treatment (Figure 4). Based on this result, we concluded that lack of a significant difference in total root length between nitrate and ammonium plots in the 30ppm pH 7.5 treatment was partly due to higher reduction of the number of lateral roots in nitrate plots compared with ammonium plots.

In 20 ppm pH 6.5 treatment plots, total root length was higher in ammonium plots than in nitrate plots (Figure 3). In addition, mean length and number of lateral roots were also higher in ammonium plots (Figure 4 and Table 2); however, taproot lengths exhibited no significant difference (Figure 2). Thus, we concluded that higher total root lengths in ammonium plots compared with nitrate plots in the 20ppm pH 6.5 treatment were due to differences in lengths of individual lateral roots and number of lateral roots, as reported previously (Hirose 2003). On the other hand, in 30 ppm pH 6.5 treatment plots, total root length was higher in nitrate plots compared with that in ammonium plots (Figure 3). The mean length of lateral roots was also higher in nitrate plots, but the number of lateral roots and taproot length exhibited no significant difference (Figure. 2 and 3, Table 2). Therefore, we concluded that the greater total root lengths in nitrate plots compared with that in ammonium plots in the 30ppm pH 6.5 treatment occurred because of the difference in length of individual lateral roots.

As shown by the results of analysis of variance, nitrogen concentration and water culture medium pH greatly influenced dry weights on day 30 after sowing and thereafter, whereas chemical form of nitrogen exhibited less influence than nitrogen concentration and water culture medium pH until day 50 after sowing (Table 3). In addition, nitrogen concentration and water culture medium pH affected dry weight in a highly interactive manner on day 30 after sowing and thereafter (Table 3).

Based on these results, we concluded that application amounts of nitrogen fertilizer and soil pH are more important than the type of nitrogen fertilizer used in grassland establishment of alfalfa.

Ammonium sulfate and sodium nitrate used in the present study as nitrogen sources contained SO_4 and Na ions as coexisting ions, respectively, and the concentration of these ions differed among the plots. However, these co-existing ions exhibited no significant growth inhibition in any of the plots. Therefore, we concluded that the influence of these co-existing ions on the results of our experiments was negligible.

Because alfalfa is a leguminous crop, differences in adherence of root nodule bacteria and their nitrogen fixation are considered to significantly influence the yield and quality in actual cultivation. Because no adherences of root nodule bacteria were observed in any plots in the present experiments, it is still unclear how the chemical form and concentration of fertilizer nitrogen and water culture medium pH influence root nodule bacteria. However, adherence and nitrogen fixing activity of root nodule bacteria are affected by soil pH (Jo et al., 1980) and nitrate nitrogen content in soil (Kawahara 1986). Therefore, results different from those reported here may be obtained depending on differences in adherence and nitrogen fixing activity of root nodule bacteria, which will be a subject for future study.

As mentioned above, growth and total root length of alfalfa were not significantly different between ammonium and nitrate plots when water culture medium pH was shifted in either the acid or alkaline direction from 6.5 at nitrogen concentrations of 20 and 30ppm in the water culture medium. The number of roots was not significantly different between ammonium and nitrate plots except in the 20ppm pH 6.5 treatment. In general, water culture medium pH shifts in the acid or alkaline direction when plants absorb ammonium nitrogen and nitrate nitrogen, respectively, and differences in sensitivity to this water culture medium pH shift partly determines which application, ammonium or nitrate nitrogen, is more appropriate for plant growth (Ohyama 1998). In addition, the absorption rate of nitric acid into plants is accelerated at low pH, whereas that of ammonium is decelerated (Ohyama 1998). Taking these results together, we conclude that differences in root system development caused by water culture medium pH shift may be partly due to differences in absorption characteristics for the two forms of nitrogen. It will be necessary to investigate these points as well as the influence on root nodule bacteria in the future to clarify the relationship between chemical form of fertilizer nitrogen and water culture medium pH in more detail.

5. Conclusion

In the present study, we aimed to investigate the effect of culture medium pH and nitrogen concentration on the growth of alfalfa. For the same, we analyzed the development of its root system after treatment with ammonium nitrogen or nitrate-nitrogen using image analysis method for measuring root length and the number of lateral roots. In terms of dry weight, total root length, and length per individual lateral root, a difference between individuals treated with ammonium nitrogen and those treated with nitrate-nitrogen was observed at pH 6.5 for 20ppm and 30ppm nitrogen concentrations. In terms of the number of lateral roots, the difference between individuals treated with both ammonium- and nitrate-nitrogen was observed at pH 6.5 for 20ppm nitrogen concentration. These results indicated that the high growth-promoting effect of ammonium nitrogen and nitrate nitrogen on growth and root system development observed at nitrogen concentrations of 20 and 30 ppm and pH 6.5 are lost when the medium pH is shifted in either the acid or alkaline direction. Furthermore, adjustments in application amounts of nitrogen fertilizer and soil pH are more important than the type of nitrogen fertilizer used in grassland establishment of alfalfa.

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