Effects of soil water stress on nodulation, leaf nitrogen accumulation and grain yield at three different growth stages of soybean

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Abstract: An experiment was conducted in a vinyl house at Gifu University, Japan, from June to November 2008 to assess the effect of water stress on nodulation of uninoculated soybean and leaf N accumulation to grain yield at three different growth stages of soybean. The experimental design was a randomized complete block of five treatments with nine replications. The deficit irrigation treatments imposed were D_1 (0 – 20 %), D_2 (20 – 40 %), D_3 (40 – 60 %), D_4 (60 – 80 %) and D_5 (80 – 100 %) of water deficit total available water (TAW). The three growth stages were flowering (49 DAS: days after sowing), seed growth (77 DAS), and maturity (140 DAS). The highest leaf N accumulation was in the D_2 treatment at the flowering and seed growth stage. The soybean grain yield had positive significant correlation ($p < 0.01$) with leaf nitrogen at seed growth stage. Total nodule numbers at $\geq$ 4.75 mm diameter size had non-significant effect on leaf N accumulation, but had positive significant effect ($p < 0.05$) on grain yield of soybean at seed growth and maturity stage. On the other hand, total nodule numbers at $<$ 4.75 mm size had positive significant effect ($p < 0.01$) on leaf N accumulation and grain yield of soybean at seed growth stage. Total nodule fresh and dry weight at $\geq$ 4.75 mm size had non-significant effect on leaf N accumulation and grain yield of soybean, but nodules at $<$ 4.75 mm size had a positive significant effect ($p < 0.01$) at seed growth stage. Individual nodule fresh and dry weight at $\geq$ 4.75 mm size showed negative significant correlation ($p < 0.01$) with leaf N accumulation and grain yield, but nodules at $<$ 4.75 mm size showed positive significant correlation ($p < 0.01$) at seed growth stage. Our studies demonstrated that the water deficit level D_2 (20 – 40 % of TAW) was the best for an efficient *Rhizobium*-host association and subsequent nodule development. Based on our results, it can be concluded that successful root infection of uninoculated soybean was more pronounced in $<$ 4.75 mm diameter size class nodule than the larger ones ($\geq$ 4.75 mm) under different water deficit levels.

Key Words : deficit irrigation, leaf N accumulation, nodulation, soybean

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1. Introduction

Nodulation and leaf nitrogen (N) accumulation in soybean [*Glycine max* (L.) Merrill] are sensitive to water deficit conditions, and can have significant effects on yield. There have been numerous studies on the relationship between soil moisture and activities of soil microorganisms as well as nodulation (Hill et al., 2000). Soil moisture is known to affect various physiological processes in plants (Gan et al., 2008). A disturbed water metabolism of the macrosymbiont may cause an impairment of the soil-plant-water balance, which may lead to reduce N_2 fixation and uptake (Upreti and Murti, 1999). The soil moisture that is adequate for seed germination is also adequate for bacterial activity and nodules formation. The soil moisture condition changes with time and may not be sufficient for subsequent nodulation and their potential activities (Ramos et al., 1999). The response of N_2 fixation rates to drought is related in part to nodule formation and growth (Serraj et al., 1999 ; King and Purcell, 2001).

The ability of legumes to derive N through symbiotic N_2 fixation reduces their dependence on soil N for growth. However, several factors can affect N_2 fixation in legumes. Kirda et al. (1989) demonstrated that N_2 fixation was the most sensitive parameter to drought, followed by plant growth, and the least sensitive by soil N uptake. The N_2 fixing effectiveness of the legume-*Rhizobium* symbiosis has been estimated in various ways. Little is known about the effect of deficit irrigation scheduling on nodulation and leaf N accumulation at different growth stages of uninoculated soybean.

The present study was, therefore, designed to investigate the impacts of soil water deficit at different growth stages of soybean on uninoculated nodulation in two size classes ($\geq$ 4.75 mm and $<$ 4.75 mm diameter), and the subsequent leaf N accumulation in the plant. The factors contributing to grain yield were also examined.

Table 1 The soil physical, moisture and chemical properties.

Physical properties	Texture (g g^{-1})	sand: 0.40 silt: 0.27 clay: 0.33
	Textural class	clay loam
	Particle density (g cm^{-3})	2.49
	Bulk density (g cm^{-3})	1.07
	Total porosity (m^3 m^{-3})	0.57
Moisture properties	Field capacity, θ_{FC} (31 kPa) (m^3 m^{-3})	0.516
	Wilting point, θ_{PWP} (1553 kPa) ($m^3 m^{-3}$)	0.296
	Total available moisture, $\theta_{FC} - \theta_{PWP}$	0.220
Chemical properties	pH (H_20)	6.41
	Organic matter (g g^{-1})	0.065
	Total carbon (g g^{-1})	0.038
	Total nitrogen (g g^{-1})	0.0026
	C/N	13.3
	Available phosphorus (g kg^{-1})	0.164
	Exchangeable potassium (mg $100g^{-1}$)	13.2

2. Materials and methods

2.1 Area description

This research was conducted in a vinyl house (surrounding sides were open) located in the experimental farm of Gifu University (35°27' N. and 136°44' E.), Japan, from June to November 2008. The average temperature was 22.4 °C and the relative humidity was 67.5 % during experiment duration. The soil physical, moisture, and chemical properties are shown in Table 1.

2.2 Treatments and experimental design

Five water deficit treatments namely; D_1 (0 – 20 %), D_2 (20 – 40 %), D_3 (40 – 60 %), D_4 (60 – 80 %) and D_5 (80 – 100 %) water deficit of total available water (TAW) were arranged in a completely randomized block design with nine replications. The water deficit level of D_2 (20 – 40 %), for example, meant that the available water deficit was maintained between 20 % and 40 % of TAW throughout the growing season. When the maximum allowable depletion of available water came close to 40 % of TAW, water was applied to restore the available water to the deficit level of 20 % of TAW. The TAW is defined as the water content between field capacity (θ_{FC}) and permanent wilting point (θ_{PWP}).

Plastic pots (10 liters volume and 23.8 cm diameter) with no drainage holes were filled with 7 kg air-dried Inceptisol (clay loam in texture). Five soybean seeds [*Glycine max* (L.) Merrill] were sown in each pot. Prior to planting, water was applied to all the pots to bring them to field capacity (θ_{FC}) for uniform germination. The soil moisture for all pots was maintained at field capacity (θ_{FC}) until 14 days after sowing (DAS) and the deficit irrigation treatments were initiated. The growing period of soybean was 20 weeks from June 16 to November 3. The plants were thinned to one per pot at the 2 to 3 leaf stage. Three replicate pots of each water deficit level were sampled at 49 DAS (flowering stage), 77 DAS (seed growth stage), and 140 DAS (maturity stage) during the experiment. Three pots per treatment were used for final yield analyses.

2.3 Sampling method

Plants were harvested in a laboratory so that nodule fresh weights (NFW) could be recorded immediately. Soil was removed from plant roots, and nodules were separated from the roots. Nodules were sorted using 4.75-mm wire-mesh sieves resulting in two nodule diameter size classes ($\geq$ 4.75 mm and < 4.75 mm). The NFW and the number of nodules per plant were recorded according to the two diameter size classes. All plant parts (leaves, stem, root, and nodules by size class) were dried at 65 °C for 96 h and dry weights recorded.

Ground samples of dried soybean leaves were screened through 1 mm sieve. The leaf N status was determined with an automatic high sensitive NC analyzer (Sumigraph NC 95 A, Shimadzu Co. Ltd., Japan). Available soil phosphorus for plants was determined by Bray and Kurtz method.

3. Results

3.1 Grain yield and leaf nitrogen accumulation under different water deficit levels

The grain yield decreased with increasing water deficit levels (Fig. 1). Significant differences were observed in decreasing grain yield from D_3 to D_5, but not in D_1 and D_2. The percentage reduction in grain yield compared to D_1, was 1 % for D_2, 12 % for D_3, 21 % for D_4, and 47 % for D_5.

Leaf N accumulation was the highest in D_2 treatment, but decreased up to the D_5 at both flowering and seed growth stages. At the maturity stage, leaf N accumulation increased up to the D_3, and then decreased from D_3 to D_5. Irrespective of the water regime treatment, leaf N accumulation was the highest at the flowering stage and the lowest at the maturity stage (Fig. 2). The soybean grain yield has positive significant correlation with leaf nitrogen at seed growth stage (Fig. 3).

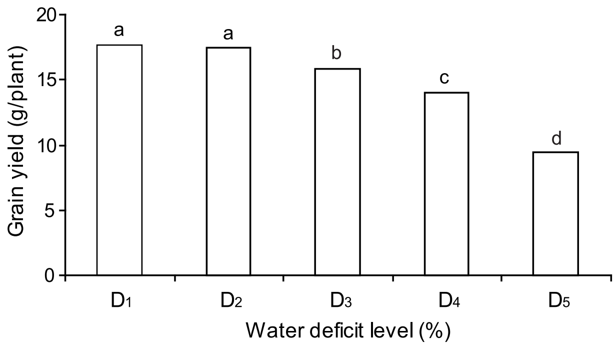

Fig. 1 The effect of water deficit levels on grain yield of soybean. Means followed by different small letters (a–d) in the column under different water deficit levels are significantly different according to Tukey's multiple comparison test ($p < 0.05$).

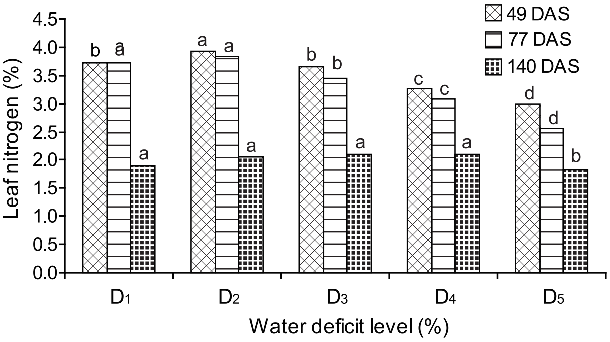

Fig. 2 The effect of water deficit levels on leaf nitrogen at different growth stages of soybean. Means followed by different small letters (a–d) at each growth stage under different water deficit levels are significantly different according to Tukey's multiple comparison test ($p < 0.05$).

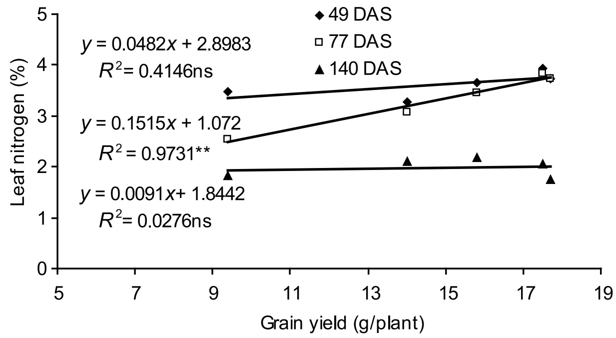

Fig. 3 Plot of leaf nitrogen at different growth stages against grain yield under different water deficit levels. ns: non significant, and ** significant at $p < 0.01$.

Table 2 The effect of water stress on nodule development parameters at different growth stages of soybean.

Growth Stage (DAS)	Treatment	TNN (mg/plant)		TNFW (mg/plant)		TNDW (mg/plant)		INFW (mg/plant)		INDW (mg/plant)	
		Nodule size class		Nodule size class		Nodule size class		Nodule size class		Nodule size class	
		≥ 4.75 mm	< 4.75 mm	≥ 4.75 mm	< 4.75 mm	≥ 4.75 mm	< 4.75 mm	≥ 4.75 mm	< 4.75 mm	≥ 4.75 mm	< 4.75 mm
		①	②	③	④	⑤	⑥	⑦ = (③ / ①)	⑧ = (④ / ②)	⑨ = (⑤ / ①)	⑩ = (⑥ / ②)
49	D_1	24.0 a	39.5 a	2800 a	1580 a	870 a	530 a	117 a	40 a	36 b	13 a
	D_2	9.0 b	47.7 a	850 b	1200 ab	260 b	350 ab	94 ab	25 a	29 bc	7 a
	D_3	5.0 c	29.7 b	370 c	690 ab	90 c	220 b	74 ab	23 ab	18 bc	7 a
	D_4	4.0 c	20.0 bc	500 b	490 bc	300 b	130 c	125 a	25 ab	75 a	7 a
	D_5	7.3 b	12.3 c	830 b	430 bc	270 b	120 c	113 a	35 a	37 b	10 a
77	D_1	43.7 a	48.0 a	6000 ab	1700 a	2400 b	670 a	138 bc	36 a	55 bc	14 a
	D_2	46.0 a	55.7 a	5610 ab	1870 a	2480 ab	840 a	122 bc	34 a	54 bc	15 a
	D_3	55.0 a	42.0 ab	7980 a	1470 ab	3660 a	510 ab	145 b	35 a	67 bc	12 a
	D_4	36.3 a	33.3 bc	8020 a	680 ab	2980 a	210 b	221 ab	20 bc	82 ab	6 a
	D_5	9.3 b	20.0 c	2910 c	150 c	1060 c	30 c	312 a	8 c	113 a	1 b
140	D_1	68.7 a	51.0 a	13010 ab	2310 a	3970 a	700 a	190 ab	45 ab	58 a	14 a
	D_2	76.7 a	58.0 a	14310 ab	1630 a	4020 a	470 b	187 ab	28 bc	52 ab	8 a
	D_3	58.7 a	48.3 a	16700 a	940 b	4730 a	240 c	285 a	19 c	81 a	5 b
	D_4	39.0 bc	41.0 b	8320 c	1570 a	2610 b	510 b	213 ab	38 bc	67 a	13 a
	D_5	31.3 c	38.7 b	6150 c	2300 a	170 c	680 a	196 ab	59 a	54 ab	18 a

TNN：total nodule number, TNFW: total nodule fresh weight, TNDW: total nodule oven dry weight, INFW: individual nodule fresh weight, INDW: individual nodule oven dry weight at ≥ 4.75 mm diameter and < 4.75 mm diameter size classes. Means followed by different small letters (a–d) in the same column in each growth stage under different water deficit levels are significantly different according to Tukey's multiple comparison test ($p <$ 0.05).

Table 3 Correlation coefficient of nodulation at ≥ 4.75mm and < 4.75mm diameter size class with leaf nitrogen accumulation at different growth stages of soybean.

Nodule parameter	Nodule size class	Growth stage (DAS)					
		49		77		140	
		r	*p*	*r*	*p*	*r*	*p*
TNN	≥ 4.75mm	0.423	ns	0.737	ns	0.002	ns
	< 4.75mm	0.868	ns	0.982	0.01	0.011	ns
TNFW	≥ 4.75mm	0.323	ns	0.444	ns	0.398	ns
	< 4.75mm	0.731	ns	0.992	0.01	−0.954	0.05
TNDW	≥ 4.75mm	0.155	ns	0.553	ns	0.365	ns
	< 4.75mm	0.702	ns	0.981	0.01	−0.918	0.05
INFW	≥ 4.75mm	−0.556	ns	−0.984	0.01	0.677	ns
	< 4.75mm	0.040	ns	0.960	0.01	−0.810	ns
INDW	≥ 4.75mm	−0.770	ns	−0.992	0.01	0.660	ns
	< 4.75mm	0.106	ns	0.995	0.01	−0.768	ns

r = correlation coefficient, *p* = probability of significance level, ns = non significant

Table 4 Correlation coefficient of nodulation at ≥ 4.75mm and < 4.75mm diameter size class with grain yield at different growth stages of soybean.

Nodule parameter	Nodule size class	Growth stage (DAS)					
		49		77		140	
		r	*p*	*r*	*p*	*r*	*p*
TNN	≥ 4.75mm	0.470	ns	0.891	0.05	0.923	0.05
	< 4.75mm	0.917	0.05	0.964	0.01	0.868	ns
TNFW	≥ 4.75mm	0.405	ns	0.558	ns	0.818	ns
	< 4.75mm	0.805	ns	0.964	0.01	−0.296	ns
TNDW	≥ 4.75mm	0.359	ns	0.632	ns	0.867	ns
	< 4.75mm	0.788	ns	0.956	0.01	−0.289	ns
INFW	≥ 4.75mm	−0.303	ns	−0.982	0.01	0.047	ns
	< 4.75mm	−0.141	ns	0.964	0.01	−0.661	ns
INDW	≥ 4.75mm	−0.255	ns	−0.999	0.01	0.120	ns
	< 4.75mm	0.013	ns	0.976	0.01	−0.644	ns

r = correlation coefficient, *p* = probability of significance level, ns = non significant

3.2 Nodulation

The nodule numbers at ≥ 4.75 mm and < 4.75 mm diameter size as well as total and individual fresh and dry weights of nodule are shown in Table 2.

At flowering stage (49 DAS), the highest nodule number at ≥ 4.75 mm diameter size was recorded in D_1 treatment, while the D_2 treatment recorded the highest nodule number at < 4.75 mm diameter. In addition, nodule number of smaller size (< 4.75 mm) was more than the larger size (≥ 4.75 mm). Fresh and dry nodule weights of both sizes were higher in full irrigation treatment (D_1) than the other treatments. The highest individual nodule fresh and dry weights of larger size class were recorded in D_4 treatment, but on the contrary, the highest for the smaller size diameter class in full irrigation treatment (D_1).

At seed growth stage (77 DAS), the highest nodule number at ≥ 4.75 mm size was recorded in D_3 treatment, but the highest nodule number at < 4.75 mm size was in the D_2 treatment. The highest fresh and dry weights of nodules ≥ 4.75 mm size were in D_4 and D_3 treatment, respectively. However, D_2 treatment recorded the highest fresh and dry weights of nodules < 4.75 mm size. The D_5 treatment recorded the highest Individual fresh and dry nodule weights for nodules ≥ 4.75 mm size. On the other hand, the highest individual fresh and dry nodule weights at < 4.75 mm size were in D_1 and D_2 treatments, respectively.

At maturity stage (140 DAS), the highest nodule number for both sizes was recorded in D_2 treatment. Total and individual nodule fresh and dry weights at larger size (≥ 4.75 mm) were the highest in D_3 treatment. On the other hand, total nodule fresh and dry weights at smaller size were the highest in full irrigation treatment (D_1). Individual nodule fresh and dry weights at smaller size (< 4.75 mm) were the highest in sever water stress conditions (D_5).

3.3 Relationship among the nodulation, leaf N accumulation, and grain yield at different growth stages

Correlation coefficients of each nodule parameter with leaf N accumulation and grain yield are shown in Table 3 and Table 4.

There was a weak correlation and non-significant relationships among the nodulation, leaf N accumulation, and grain yield at flowering stage, except total nodule number at <4.75 mm size that showed significant positive correlation (< 0.05) with yield. Leaf nitrogen and grain yield showed similar trend of relationships with fresh and dry weights of total and individual nodule weight at both nodule size classes.

On the contrary, at seed growth stage (77 DAS), total nodule numbers at ≥ 4.75 mm size had non-significant effect on leaf N accumulation, but positive significant effect ($p < 0.05$) on grain yield of soybean. On the other hand, total nodule numbers at < 4.75 mm size had positive significant effect ($p < 0.01$) on leaf N accumulation and grain yield of soybean. Total nodule fresh and dry weight at ≥ 4.75 mm size had non-significant effect on leaf N accumulation and grain yield of soybean, but nodules at < 4.75 mm size had a positive significant effect on leaf N accumulation ($p < 0.01$) and grain yield ($p < 0.05$). Individual nodule fresh and dry weight at ≥ 4.75 mm size had negative significant effect, but nodules at < 4.75 mm size had a positive significant effect ($p < 0.01$) on leaf N accumulation and grain yield at seed growth stage.

The leaf N accumulation and grain yield at maturity stage (140 DAS) showed non-significant correlation with nodulation parameters, except total nodule fresh and dry weights at < 4.75 mm size showed significant negative correlation ($p < 0.05$) with leaf N accumulation, and total nodule number at ≥ 4.75 mm size showed significant positive correlation ($p < 0.05$) with grain yield.

4. Discussion

The highest leaf N accumulation in D_2 treatment at flowering and seed growth stages indicated that irrigation scheduling of 20 – 40 % water deficit of TAW might have provided an adequate soil moisture condition that is required for establishing an efficient *Rhizobium*-host association and subsequent nodule development. This result agrees with Pahalwan and Tripath (1984) who demonstrated that under uninoculated soybean plant, more leaf N accumulation were recorded under mild water stress condition. The importance of adequate soil moisture for efficient interaction of *Rhizobium* and host was also pointed out by Gallacher and Sprent (1995).

Our results indicated that water stress conditions did not always inhibit nodulation but rather sometimes enhance nodulation. In saturated soil, microbial activity is depressed by poor aeration and the limited availability of O_2 (Jinfeng et al., 2008). In our full irrigation treatment (D_1), excessive water might have resulted in poor aeration, and thus reduced the number of aerobic soil microorganisms as well as nodulation. On the other hand, under mild water stress conditions (D_2), facultative anaerobic soil microorganisms might have dominated nodule production. Under D_5 treatment (which is nearer to wilting point), the severe water stress resulted in an unfavorable growth environment for the microbes, and this led to the lower nodulation. This result agrees with Sinclair et al. (1987) that nodulation responds to drought only when the stress was extremely severe, and that the sensitivity was distinctly different from the sensitivity of N_2 fixation to drought. Clein and Schmel (1994) also found that lower moisture contents inhibited soil microbial activity.

Water deficit had significant effect on relationships among the nodulation, leaf N accumulation, and grain yield at seed growth stage, because physiological maturity might reach maximum at that time. Sridhara et al. (1995) found the same phenomenon that critically important period for fixation and assimilation of nitrogen in soybean production is during the interval between initial seed formation and the end of the linear seed-filling phase.

Significant positive relationships among the nodulation, leaf N accumulation, and grain yield at < 4.75 mm size indicates that more successful root infection at < 4.75 mm size class nodules than the ≥ 4.75 mm size class nodules.

5. Conclusions

Our studies demonstrated that the water deficit level D_2 (20 – 40% of TAW) was the best for an efficient *Rhizobium*-host association and subsequent nodule development. Water deficit had significant effect on relationships among the nodulation, leaf N accumulation, and grain yield at seed growth stage, because physiological maturity might reach maximum at that time. Based on our results, it can be concluded that successful root infection of uninoculated soybean was more pronounced in < 4.75 mm diameter size class nodule than the larger ones (≥ 4.75 mm) under different water deficit levels.

Given the relationship of nodulation and leaf N accumulation with grain yield, it is obvious that no one single character was important for grain yield. Yield is a complex terminal outcome of growth to which there are diverse and interrelated development tracks. However, based on our results, it appears that nodulation, leaf N accumulation, and grain yield are important characters to consider during soybean cultivation under deficit irrigation practices.

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References

Clein, J.S. and Schmel, J.P. (1994): Reduction in microbial activity in birch litter due to drying and rewetting events. Soil Biol. Biochem., 26: 403–406.

Gallacher, A.H. and Sprent, J.I. (1995): The effect of different water regimes on growth and nodule development of green house growth of V. faba. J. Exp. Bot., 28: 413–423.

Gan, Y.T., Jayakumar, P., Symons, S. and McDonald, C.L. (2008): Synergic effect of N and moisture on biochemical property of nodules and seed yield in chickpea. Australian J. Crop Sci., 1(1): 11–22.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T. and Nelsona, E.B. (2000): Methods for assessing the composition and diversity of soil microbial communities. Appl. Soil Ecol., 15: 25–36.

Jinfeng, W., Shaozhong, K., Fusheng, L., Fucang, Z., Zhijun, L. and Jianhua, Z. (2008): Effects of alternate partial root-zone irrigation on soil microorganism and maize growth. Plant Soil, 302: 45–52.

King, C.A. and Purcell, C. (2001): Soybean nodule size and relationship to nitrogen fixation response to water deficit. Crop Sci., 41: 1099–1107.

Kirda, C., Danso, S.K.A. and Zapata, F. (1989): Temporal water stress effects on nodulation, nitrogen accumulation, and growth of soybean. Plant and Soil, 120: 49–55.

Pahalwan, D.K. and Tripath, R.S. (1984): Nodulation, accumulation and redistribution of nitrogen in soybean (*Glycine max* L.) as influenced by seed inoculation and scheduling of irrigation. Plant and Soil, 81: 235–246.

Ramos, M.L.G., Gordon, A.J., Minchin, F.R., Sprent J.I. and Parsons, R. (1999): Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). Ann. Bot., 83: 57–63.

Serraj, R., Sinclair, T.R. and Purcell, L.C. (1999): Symbiotic N_2 fixation response to drought. J. Exp. Bot., 50: 143–155.

Sinclair, T.R., Zimet, A.R. and Muchow, R.C. (1988): Changes in soybean nodule number and dry weight in response to drought. Field Crops Res., 18: 197–202.

Sridhara, S., Thimmegowda, S. and Prasad, T.G. (1995): Effect of water regimes and moisture stress at different growth stages on nodule dynamics, nitrogenase activity and nitrogen fixation in soybean (*Glycine max* (L.) Merrill). J. Agron. Crop Sci., 174: 111–115.

Upreti, K.K. and Murti, G.S.R. (1999): Water stress induced changes in root nodulation and cytokinin levels in French bean. J. Plant Biol., 26: 187–190.

要　旨

水分ストレスがダイズの根粒形成の状況と葉内窒素含有量が収量に及ぼす影響を各生育ステージで調べるために，2008 年 6 月から 11 月にかけて岐阜大学のビニールハウス内で，栽培実験を実施した．実験方法は 1 因子 5 水準 9 反復の無作為の完全型試験である．すなわち，水分ストレスの処理として，土壌水分欠損量が総容易有効水分量（TAW）の 0 ～ 20 % (D_1)，20 ～ 40 % (D_2)，40 ～ 60 % (D_3)，60 ～ 80 % (D_4)，80 ～ 100 % (D_5) の 5 水準の処理区を設けた．開花期 (49 DAS)，結実期（77 DAS），成熟期 (140 DAS) の 3 段階の生育ステージでサンプリングを行った．葉内窒素含有量は開花期および結実期において D_2 試験区で最大となった．ダイズ収穫量は，結実期の葉内窒素含有量と正の有意な相関 ($p <$ 0.01) があった．4.75 mm 径以上の根粒の総数は葉内窒素含有量と相関が見られなかったが，結実期，成熟期における根粒数はダイズの収量と正の有意な相関が見られた ($p < 0.05$)．一方、結実期において 4.75 mm 径未満の根粒の総数は，葉内窒素含有量やダイズ収量と正の有意な相関 ($p < 0.01$) があった．4.75 mm 径以上の根粒の総乾燥重及び湿潤重は葉内窒素含有量やダイズ収量と相関がなかったが，結実期における 4.75 mm 径未満の根粒の総重量は正の有意な相関があった ($p < 0.05$)．4 結実期における 4.75 mm 径以上の一個体団粒の湿潤重および乾燥重は葉内窒素含有量やダイズ収量と負の有意な相関（$p < 0.01$）があったが，4.75 mm 径未満の根粒は正の有意な相関 ($p < 0.01$) がみられた．以上の結果から，圃場容水量に対して TAW の 20 ～ 40 % に相当する水分欠損状態（D_2）が，根粒菌と宿主の有効な共生関係と根粒の形成にとって最も良好な土壌水分状態であること示している．さらに，結実期における 4.75 mm 径以上の根粒よりも 4.75 mm 径未満の根粒の方が、有効に根粒菌感染していることを示唆した．

キーワード：節水灌漑，葉内窒素含有量，根粒形成，ダイズ