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ORIGINAL ARTICLE/SHORT PAPER

Impact of plant genotype and nitrogen level on rice growth response to inoculation with *Azospirillum* sp. strain B510 under paddy field conditions

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Abstract

Twenty rice cultivars, including three genetically-distinct groups (*japonica*, *indica-1*, and *indica-2*), were evaluated for their response to inoculation with *Azospirillum* sp. strain B510 in paddy fields with standard nitrogen (SN) and low nitrogen (LN) fertilization. In the SN field, the tiller numbers in most *indica-2* cultivars, 37 days after transplanting (DAT), were significantly increased by the B510 inoculation, whereas those in 4 *japonica* cultivars were significantly decreased. A similar growth response was observed in the LN field, although the impacts of the B510 inoculation were more varied than in the SN field. At 58 DAT, the tiller numbers in most cultivars were lower or unaffected by the B510 inoculation under both SN and LN conditions, except that the tiller number of the Nipponbare cultivar, which is classified as *japonica*, was significantly higher in the LN field only. These results suggest that the effects of inoculation with *Azospirillum* sp. strain B510 on the growth of rice plants, especially on tiller numbers at the early growth stage, vary depending on the rice genotype, as well as nitrogen level. Therefore, the plant genotypes, growth stages, and fertilization managements must be considered when a plant-associated bacterium is evaluated for beneficial effects under field conditions.

Key words: *Azospirillum* sp., nitrogen fertilization, *Oryza sativa* L., plant growth promotion, rice plant

INTRODUCTION

A wide range of microorganisms, including fungi and bacteria, has been found in the phytosphere (Mano and Morisaki 2008; Saito *et al.* 2007, 2008). Among these, plant growth-promoting rhizobacteria (PGPR), which have beneficial effects on host plants, such as increased crop yield, have been well characterized from both scientific and practical perspectives.

The genus *Azospirillum*, characterized by spirillum-shaped, N_2-fixing, Gram-negative alphaproteobacteria, contains species often found in the rhizosphere (Hartman and Baldani 2006). Inoculation with *Azospirillum* sp. promotes plant growth, so agronomic applications of this genus have been developed (Okon and Labandera-Gonzalez 1994). Plant growth promotion using various strains of *Azospirillum* has been investigated on several plant species. Wheat plants treated with *Azospirillum brasilense* Sp245 exhibited reduced root length, increased root–hair formation, and increased above-ground biomass and ear numbers (Spaepen *et al.* 2008). In maize, seed inoculation with *Azospirillum lipoferum* CRT1 induced changes in root morphology, such as increased length and biomass (El Zemrany *et al.* 2007). In tomatoes, *Azospirillum brasilense* Sp7 and *Azospirillum* sp. BNM-65 exhibited positive effects, such as increased shoot and root biomass (Romero *et al.* 2003). The total nitrogen content in rice grains was improved by inoculation with *Azospirillum brasilense* REC3 under low nitrogen (LN) fertilization conditions (Pedraza *et al.* 2009). Treatment with a nitro-

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gen-fixing strain of *Azospirillum lipoferum* to the root zone of rice plants promoted the early tillering and reproductive growth of host plants, although the total dry weight and nitrogen content in the rice plants were not markedly increased by the inoculation (Watanabe and Lin 1984).

Azospirillum sp. strain B510 was isolated from a surface-sterilized stem of a rice plant (*Oryza sativa* cv. Nipponbare [NI]) that was grown in an experimental paddy field (Elbeltagy *et al.* 2001). The B510 strain is closely related to *Azospirillum oryzae* COC8 (97.7% identity in their 16S rRNA gene sequences), which was reported as a paddy soil bacterium (Xie and Yokota 2005). In addition to being a diazotroph under free-living conditions, B510 was found to have positive motility and to be capable of degrading plant cell walls (Elbeltagy *et al.* 2001). Inoculation with *Azospirillum* sp. B510 was shown to promote plant growth under both laboratory and field conditions (Isawa *et al.* 2010). Noticeably, the field experiment in Hokkaido, Japan indicated that inoculation with B510 increases tiller number, resulting in an increase in seed yield at commercial levels (Isawa *et al.* 2010). Moreover, the B510 inoculation enhanced disease resistance to fungal and bacterial pathogens (Yasuda *et al.* 2009). A genome analysis of B510 has been completed and revealed that the B510 strain possesses putative plant hormone-related genes, which could promote plant growth (Kaneko *et al.* 2010). Thus, *Azospirillum* sp. strain B510 is a likely beneficial plant-associated bacterium for agronomic applications.

Plant genotype is known to influence the plant growth-promotion effects of inoculation with *Azospirillum* (Bashan and Holguin 1997). For example, significant effects on growth and yield of maize plants were observed in one bacterium–plant combination and absent from another combination, indicating an unknown interaction between plant genotype and bacterial strain (Garcia De Salomone and Dobereiner 1996). Similar phenomena were reported when local Nepalese wheat varieties and mustard were inoculated with local *Azospirillum* spp. (Bhattarai and Hess 1993; Kesava Rao *et al.* 1990).

It is also known that fertilization management can affect the community structures of plant-associated bacteria (Coelho *et al.* 2009; Ikeda *et al.* 2010; Roesti *et al.* 2006). There is debate regarding the impacts of fertilization strategies on the effects of PGPR, such as *Azospirillum* species. The plant growth promotion was observed only under LN conditions in some studies (Dobbelaere *et al.* 2001; Fallik and Okon 1996), whereas other studies showed that the use of nitrogen in combination with *Azospirillum* produced significantly higher green and dry-matter yields (Chela *et al.* 1993; Okon and Labandera-Gonzalez 1994). Furthermore, little is known about the cross-effects of rice genotypes and nitrogen fertilization levels on plant growth promotion by *Azospirillum* sp. under field conditions. Therefore, the aim of the present study was to examine the cross-effects of rice genotype and nitrogen level on the response of rice plants to inoculation with *Azospirillum* sp. B510 under paddy field conditions.

MATERIALS AND METHODS

Plant materials and growth conditions

Plant materials were obtained from the National Institute of Agrobiological Sciences (NIAS) Genebank (Tsukuba, Japan). In total, 20 rice cultivars (10 cultivars each for *japonica* and *indica* types) were used (Table 1). The 10 *indica*-type cultivars were classified into two subgroups (*indica-1* and *indica-2*), based on a principal coordinate analysis using 179 restriction fragment length polymorphism data (Ebana *et al.* 2008; Kanemura *et al.* 2007; Kojima *et al.* 2005; Uga *et al.* 2009).

Seeds from each cultivar were placed on two layers of filter paper (Toyo Roshi Kaisha, Tokyo, Japan) in a Petri dish (6-cm diameter) containing 4 mL tap water. The Petri dishes were placed in an incubator at 30°C. After 2 days (30 April 2009), the germinated seeds were sown in a commercial soil (Mitsui-Toatsu no. 3, Tokyo, Japan) in a 60 × 30 cm cell tray (cell diameter, 1.5 cm; depth, 3 cm) and grown in a greenhouse under natural light conditions for 4 weeks. After 3 weeks in the greenhouse, selected seedlings of each cultivar were inoculated with *Azospirillum* sp. strain B510, as described in the next sec-

Table 1 Rice plant materials used this study

Cultivar name	Origin	Variety/group
Nipponbare	Japan	*Japonica*
Koshihikari	Japan	*Japonica*
Fukkurinko	Japan	*Japonica*
Nanatsuboshi	Japan	*Japonica*
Akage	Japan	*Japonica*
Sekiyama	Japan	*Japonica*
Habataki	Japan	*Japonica*
Dianyu 1	China	*Japonica*
Khao Nam Jen	Laos	*Japonica*
Khau Mac Kho	Vietnam	*Japonica*
Tupa 121–3	Bangladesh	*Indica-1*
Kasalath	India	*Indica-1*
Muha	Indonesia	*Indica-1*
Basilanon	Philippines	*Indica-1*
Bei Khe	Cambodia	*Indica-2*
Ryou Suisan Koumai	China	*Indica-2*
Deng Pao Zhai	China	*Indica-2*
Naba	India	*Indica-2*
Milyang 23	Korea	*Indica-2*
Bleiyo	Tailand	*Indica-2*

Table 2 Characteristics of field soils

Field	pH (H_2O)	Total C content (%)	Total N content (%)	Truog phosphorous content (mg P_2O_5 kg^{-1})
Standard nitrogen	5.2	4.3	0.10	74.0
Low nitrogen	5.5	3.3	0.08	62.5

tion. A total of 100 seedlings of each cultivar were planted in an experimental field (37°28′N, 141°06′E) in a square pattern (5 × 5 plants). Hills were spaced 30 cm apart. The seedlings inoculated with B510 and those that were not inoculated were cultivated nearby in the same field. Rice seedlings were grown in two neighboring fields: one with standard nitrogen (SN) and the other with LN. Basal fertilizer (phosphorus pentoxide [P_2O_5] and potassium oxide [K_2O] with or without nitrogen) was applied to the paddy fields 4 days before transplanting. In the SN paddy field, nitrogen, P_2O_5, and K_2O (Temairazu 666; Co-op Chemical, Tokyo, Japan) were fertilized at 30, 30, and 30 kg ha^{-1}, respectively. In the LN field, only P_2O_5 and K_2O were added at 30 kg ha^{-1} each. The LN paddy field has been used for rice cultivation using the same field management procedure employed in the SN field, except that no nitrogen had been applied to the LN field since 2004. To maintain the uniformity of soil conditions in these paddy fields, we cultivated the same rice cultivar uniformly in all areas in 2008. The field soil was classified as gray lowland soil. The characteristics of the field soils used in the present study are shown in Table 2.

Bacterial inoculation and plant growth measurements

Azospirillum sp. strain B510 was cultured in nutrient broth (Difco Laboratories, Detroit, MI, USA) at 30°C for 16 h, and from this culture, a bacterial suspension was prepared in sterile, distilled water (1×10^8 colony-forming units [CFU] mL^{-1}). Three-week-old rice seedlings were inoculated with 500 mL of the bacterial suspension (final density: 1.5×10^6 CFU mL^{-1} on a tray containing 300 seedlings). After the inoculation, rice seedlings were incubated in a greenhouse for 5 days prior to transplantation to the experimental fields.

The effects of the inoculation on rice growth were evaluated by measuring the shoot length and tiller number of 9 plants for each cultivar with a square pattern (3 × 3 plants) inside the square pattern (5 × 5 plants) with or without inoculation under SN or LN conditions at 37 and 58 days after transplanting (DAT). The statistical difference in plant length or tiller number between non-inoculation and inoculation was determined by two-sided Student's *t*-test. $P < 0.05$ was considered significant. The inoculation effect index (IEI) for the tiller number and shoot length was calculated for each cultivar grown under SN and LN conditions using the following equation:

$$\text{IEI }(\%) = (\text{tiller number or shoot length of rice plants inoculated with B510})/(\text{tiller number or shoot length of rice plants without inoculation}) \times 100. \quad (1)$$

To evaluate the cross-effects between the inoculation and the nitrogen fertilization levels, the nitrogen effect index (NEI) for the tiller number was calculated for each cultivar without inoculation grown under SN and LN conditions using the following equation:

$$\text{NEI }(\%) = (\text{tiller number or shoot length of plants without inoculation in SN field})/(\text{tiller number or shoot length of plants without inoculation in LN field}) \times 100. \quad (2)$$

RESULTS AND DISCUSSION

We compared the effects of inoculation with *Azospirillum* sp. strain B510 on the growth of rice under SN and LN paddy field conditions. At 37 DAT in the SN field, the tiller numbers were significantly higher ($P < 0.05$, *t*-test) in 8 cultivars (Sekiyama [SE], Khau Mac Kho [KM], Muha [MU], Bei Khe [BE], Ryou Suisan Koumai [RY], Deng Pao Zhai, Naba [NB], and Milyang 23 [MI] in Fig. 1) inoculated with B510, whereas tiller numbers of 4 cultivars (NI, Koshihikari [KO], Fukkurinko [FU], and Nanatsuboshi [NN]) were significantly lower (Fig. 1a; Table 3). In the LN field, the tiller numbers of 4 cultivars (KM, MU, RY, and NB) were increased significantly by inoculation with B510 at 37 DAT, whereas the tiller numbers of 3 cultivars (KO, SE, and Tupa [TU]) were significantly decreased (Fig. 1b; Table 3). At 37 DAT, 18 cultivars in the SN field and 10 in the LN field showed noticeable, but not statistically-significant responses (positive or negative) to the B510 inoculation (Fig. 1a,b; Table 3).

Similarly, 11 cultivars in the SN field and 7 in the LN field were noticeably responsive to the inoculation at 58 DAT (Fig. 1c,d; Table 3), with significantly fewer numbers of tiller for 7 cultivars inoculated with B510 in the SN field (NI, FU, Habataki, TU, Kasalath, BE and NB) (Fig. 1c; Table 3). At 58 DAT in the LN field, only one cultivar (NI) inoculated with B510 significantly showed a

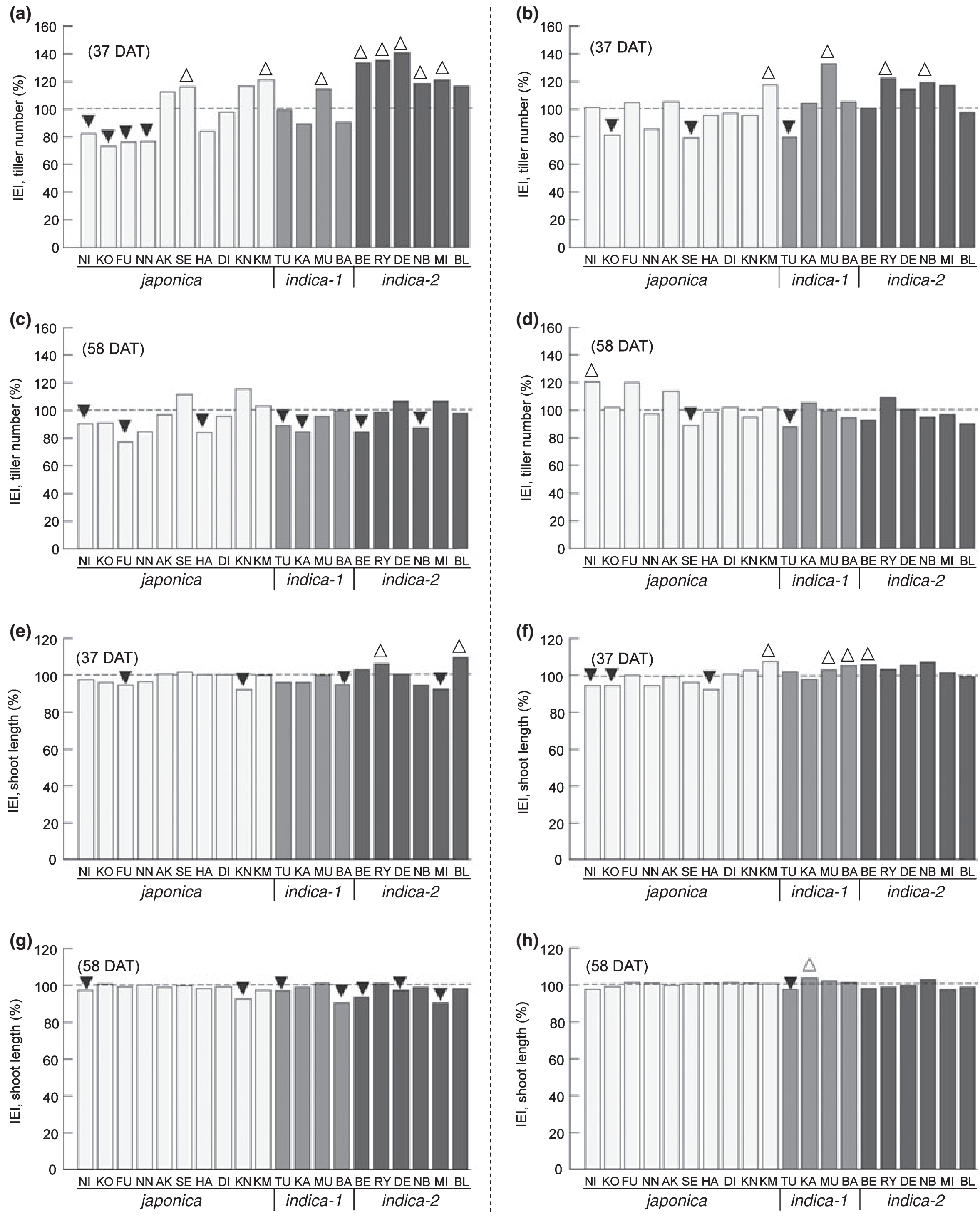

Figure 1 Inoculation effect index (IEI, %) for tiller number (a–d) and shoot length (e–h) of rice plants. IEI = (tiller number or shoot length of rice plants inoculated with B510)/(tiller number or shoot length of rice plants without inoculation) × 100. IEI for tiller number (a) 37 days after transplanting (DAT) under standard nitrogen (SN) conditions; (b) 37 DAT under low nitrogen (LN) conditions; (c) 58 DAT, SN; (d) 58 DAT, LN. IEI for shoot length (e) 37 DAT, SN; (f) 37 DAT, LN; (g) 58 DAT, SN; (h) 58 DAT, LN. White and black arrowheads respectively signify that means (n = 9) of tiller number or shoot length of rice plants inoculated with B510 were significantly higher and lower than those of rice plants without inoculation ($P < 0.05$, t-test). AK, Akage; BA, Basilanon; BE, Bei Khe; BL, Bleiyo; DE, Deng Pao Zhai; DI, Dianyu 1; FU, Fukkurinko; HA, Habataki; KA, Kasalath; KM, Khau Mac Kho; KN, Khao Nam Jen; KO, Koshihikari; MI, Milyang 23; MU, Muha; NB, Naba; NI, Nipponbare; NN, Nanatsuboshi; RY, Ryou Suisan Koumai; SE, Sekiyama; TU, Tupa 121-3.

Table 3 Effects of *Azospirillum* sp. B510 inoculation on tiller number of rice plants

Variety group/ cultivar name	37 DAT				58 DAT			
	Standard nitrogen		Low nitrogen		Standard nitrogen		Low nitrogen	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
Japonica								
Nipponbare	20.9 ± 1.9†	17.2 ± 1.4**	15.2 ± 2.2	15.4 ± 2.4	31.4 ± 2.8	28.4 ± 2.7*	20.9 ± 2.6	25.1 ± 2.7**
Koshihikari	20.6 ± 2.4	15.0 ± 3.0**	11.8 ± 1.6	9.6 ± 1.4**	26.6 ± 2.2	24.1 ± 3.4	18.3 ± 1.2	18.7 ± 1.8
Fukkurinko	23.3 ± 3.1	17.8 ± 1.8**	13.6 ± 3.4	14.2 ± 2.4	29.3 ± 3.0	22.6 ± 1.7**	15.6 ± 3.2	18.7 ± 3.4
Nanatsuboshi	17.9 ± 1.4	13.7 ± 3.3**	12.9 ± 2.8	11.0 ± 2.2	21.8 ± 2.8	18.4 ± 4.2	15.3 ± 2.4	14.9 ± 0.8
Akage	11.9 ± 2.3	13.3 ± 1.7	11.4 ± 2.3	12.0 ± 1.7	16.1 ± 2.0	15.6 ± 1.7	12.3 ± 2.2	14.0 ± 2.1
Sekiyama	9.8 ± 1.3	11.3 ± 1.3*	11.6 ± 1.5	9.2 ± 1.2**	12.8 ± 1.6	14.2 ± 1.1	11.8 ± 1.7	10.4 ± 0.7*
Habataki	9.0 ± 1.9	7.6 ± 1.9	10.5 ± 2.5	10.0 ± 1.7	23.3 ± 1.9	19.6 ± 2.0**	15.7 ± 2.4	15.4 ± 1.2
Dianyu 1	17.6 ± 3.1	17.1 ± 2.0	10.6 ± 1.9	10.3 ± 1.9	25.6 ± 1.7	24.4 ± 2.5	18.3 ± 1.4	18.7 ± 1.9
Khao Nam Jen	8.0 ± 1.1	9.3 ± 2.3	8.5 ± 1.6	8.1 ± 1.1	11.6 ± 2.0	13.3 ± 2.5	10.3 ± 1.9	9.8 ± 2.0
Khau Mac Kho	7.9 ± 1.5	9.6 ± 0.9*	4.6 ± 0.7	5.4 ± 1.0*	11.6 ± 1.2	11.9 ± 1.6	7.6 ± 1.3	7.7 ± 1.9
Indica-1								
Tupa 121-3	15.0 ± 4.0	14.9 ± 4.8	9.6 ± 1.9	7.7 ± 1.7*	25.4 ± 1.7	22.6 ± 2.7*	20.9 ± 1.4	18.3 ± 2.5*
Kasalath	17.9 ± 2.8	16.0 ± 3.0	10.1 ± 1.1	10.6 ± 2.2	31.2 ± 3.6	26.3 ± 2.3**	21.3 ± 2.1	22.4 ± 3.2
Muha	12.2 ± 1.8	14.0 ± 1.7*	13.2 ± 2.3	17.6 ± 4.0*	28.0 ± 4.0	26.7 ± 3.1	20.6 ± 3.4	20.4 ± 2.0
Basilanon	15.8 ± 2.7	14.2 ± 3.2	9.4 ± 1.8	9.9 ± 1.7	40.0 ± 4.9	39.9 ± 6.3	29.0 ± 3.1	27.3 ± 2.5
Indica-2								
Bei Khe	13.4 ± 3.0	18.0 ± 3.9*	16.6 ± 2.3	16.7 ± 4.6	32.4 ± 2.6	27.4 ± 3.8**	28.4 ± 2.7	26.3 ± 3.6
Ryou Suisan Koumai	14.2 ± 3.0	19.2 ± 3.1**	14.3 ± 1.5	17.6 ± 2.7**	29.6 ± 3.6	29.2 ± 3.2	21.3 ± 2.3	23.2 ± 1.9
Deng Pao Zhai	13.7 ± 2.1	19.2 ± 1.4**	15.6 ± 2.9	17.8 ± 3.9	35.0 ± 3.2	37.2 ± 3.1	27.8 ± 3.8	28.0 ± 3.2
Naba	17.2 ± 2.3	20.4 ± 3.4*	14.7 ± 3.3	17.6 ± 1.9*	36.1 ± 5.3	31.4 ± 3.4*	27.0 ± 3.8	25.7 ± 2.0
Milyang 23	13.0 ± 2.8	15.8 ± 1.9*	8.6 ± 0.9	10.1 ± 1.6	26.4 ± 2.9	28.1 ± 2.2	19.4 ± 2.5	18.8 ± 3.2
Bleiyo	8.8 ± 3.7	10.2 ± 1.0	10.0 ± 1.7	9.8 ± 2.5	22.3 ± 2.8	21.8 ± 2.0	19.1 ± 2.5	17.2 ± 2.0

$^*P < 0.05$; $^{**}P < 0.01$ for comparison between control and inoculated samples. †Mean ± SD ($n = 9$). DAT, days after transplanting.

greater number of tillers than those without inoculation, and 2 cultivars (SE and TU) showed a significantly lower number of tillers (Fig. 1d; Table 3).In both the SN and LN fields, the effects of the inoculation on shoot length were less clear compared to the effects on tiller numbers (Fig. 1e–h; Table 4). However, as with tiller numbers, more cultivars showed shoot length responses to the inoculation at 37 DAT (Fig. 1e,f; Table 4) than at 58 DAT (Fig. 1g,h; Table 4) in both the SN and LN fields.

These results suggest that B510 generally has a pronounced influence on rice plant growth under SN fertilization conditions, compared to LN conditions, especially at the early growth stages. Furthermore, the impact of B510 inoculation at the later growth stage seems mostly negative or absent in most cultivars under both SN and LN conditions, except for 3 cultivars (NI, FU, and Akage) that showed a noticeably positive response under LN conditions (Fig. 1c,d). Interestingly, all cultivars belonging to the *indica-2* subgroup showed an increase in tiller number in the SN field following inoculation (Fig. 1a), and 4 of 6 *indica-2* subcultivars in the LN field similarly showed an increase in tiller number, with no negative responses (Fig. 1b). These results imply that the *indica-2* group might have genetic characteristics responsible for the stable and unidirectional reaction to B510 inoculation at the early growth stage, independent of nitrogen fertilization levels, suggesting the possibility of genetic manipulation for rice and B510 interaction.

In the present study, it was shown that the tiller number was more responsive to inoculation than the shoot length, especially at the early growth stage under SN conditions. The *indica-1* and *indica-2* subgroups include different ecotypes: *indica-1* consists mainly of the *aus* ecotype, and *indica-2* includes various ecotypes other than *aus* (Kojima *et al.* 2005). The *aus* ecotype, with early maturity, is adapted to uplands and is expected to have resistance to drought (Tsunoda 1987). There are differences in root characteristics between the two groups: the *indica-1* group has deeper and thicker roots than the *indica-2* group (Uga *et al.* 2009). The genetic and phenotypic differences between *indica-1* and *indica-2* might have contributed to their unique responses to the B510 inoculation observed in the present study, as the initial interaction between a rice plant and B510 takes place in the rhizosphere through root inoculation. Several previous studies have demonstrated a positive correlation between the concentration of low molecular weight dicarboxylic acids, such as succinate and malate in root exudates, and the plant growth-promotion effects in gramineous plants, including rice (Christiansen-Weniger *et al.* 1992; Gyaneshwar

Table 4 Efects of *Azospirillum* sp. B510 inoculation on shoot length of rice plants

Variety group/ cultivar name	37 DAT				58 DAT			
	Standard nitrogen		Low nitrogen		Standard nitrogen		Low nitrogen	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
japonica								
Nipponbare	41.2 ± 1.7†	40.2 ± 2.2	41.4 ± 1.9	39.1 ± 1.0**	65.8 ± 1.9	63.8 ± 1.8*	66.1 ± 2.7	64.3 ± 1.8
Koshihikari	49.1 ± 1.4	47.2 ± 3.1	47.8 ± 1.1	45.0 ± 2.6**	78.6 ± 2.6	78.9 ± 2.6	76.3 ± 2.0	75.5 ± 2.8
Fukkurinko	57.3 ± 2.6	54.1 ± 2.9*	51.6 ± 2.1	51.4 ± 4.0	82.0 ± 3.3	81.0 ± 1.7	75.7 ± 2.6	76.6 ± 4.7
Nanatsuboshi	57.8 ± 2.7	55.7 ± 1.7	54.8 ± 2.2	51.7 ± 4.1	83.0 ± 2.7	82.7 ± 5.0	80.1 ± 1.0	80.6 ± 2.8
Akage	66.6 ± 1.9	66.9 ± 4.0	68.0 ± 4.3	67.4 ± 6.6	93.0 ± 5.2	91.7 ± 4.3	90.2 ± 5.0	89.8 ± 3.0
Sekiyama	54.2 ± 3.0	55.1 ± 2.3	60.7 ± 2.0	58.3 ± 3.7	96.7 ± 2.4	96.2 ± 2.6	90.8 ± 2.7	91.2 ± 4.3
Habataki	50.9 ± 1.3	51.0 ± 1.8	55.4 ± 1.5	51.2 ± 1.0**	70.7 ± 1.6	69.3 ± 2.1	65.5 ± 2.0	65.9 ± 1.7
Dianyu 1	45.2 ± 1.9	45.3 ± 1.2	43.1 ± 1.4	43.3 ± 1.3	75.8 ± 2.0	74.9 ± 4.2	74.5 ± 3.9	75.4 ± 1.7
Khao Nam Jen	59.8 ± 2.8	55.2 ± 4.9*	56.1 ± 3.0	57.4 ± 2.5	100.7 ± 1.8	92.8 ± 2.0**	88.2 ± 3.0	88.9 ± 3.7
Khau Mac Kho	50.3 ± 3.9	50.2 ± 3.2	46.0 ± 2.8	49.4 ± 3.6*	96.1 ± 1.0	93.3 ± 2.1	87.1 ± 4.4	87.5 ± 3.0
Indica-1								
Tupa 121-3	42.8 ± 1.4	41.1 ± 1.7	37.9 ± 0.9	38.7 ± 1.2	71.8 ± 2.2	69.3 ± 1.7*	64.4 ± 1.4	62.7 ± 1.0**
Kasalath	53.7 ± 3.7	51.6 ± 2.3	48.6 ± 2.4	47.6 ± 3.4	81.6 ± 2.9	80.4 ± 1.4	74.0 ± 2.1	76.7 ± 2.8*
Muha	43.3 ± 0.8	43.2 ± 1.3	44.2 ± 1.2	45.5 ± 1.4*	71.0 ± 2.6	71.6 ± 2.0	67.7 ± 1.8	69.1 ± 0.8
Basilanon	45.2 ± 2.6	42.7 ± 1.2*	41.6 ± 1.1	43.7 ± 2.2*	64.1 ± 3.2	57.7 ± 1.5**	56.1 ± 2.2	56.7 ± 2.0
Indica-2								
Bei Khe	51.9 ± 2.5	53.6 ± 5.5	50.4 ± 2.3	53.3 ± 2.0*	80.6 ± 2.8	75.0 ± 3.2**	70.7 ± 2.7	69.1 ± 1.5
Ryou Suisan Koumai	61.2 ± 2.1	65.1 ± 1.6**	60.4 ± 2.8	62.4 ± 3.0	106.7 ± 3.6	07.6 ± 2.7	98.1 ± 4.0	96.8 ± 2.8
Deng Pao Zhai	57.6 ± 2.3	57.8 ± 2.7	53.3 ± 3.2	56.2 ± 2.7	89.1 ± 1.6	86.4 ± 1.4**	77.7 ± 2.5	77.3 ± 1.9
Naba	49.5 ± 2.5	46.7 ± 3.5	44.0 ± 4.1	47.1 ± 1.7	72.9 ± 2.6	71.8 ± 6.1	64.6 ± 3.0	66.6 ± 1.6
Milyang 23	42.1 ± 2.8	38.9 ± 2.0*	37.3 ± 1.1	37.7 ± 1.2	61.0 ± 1.4	54.9 ± 2.4**	55.0 ± 1.9	53.6 ± 1.6
Bleiyo	50.8 ± 3.8	55.6 ± 2.4**	54.1 ± 2.4	53.7 ± 2.5	83.3 ± 2.6	81.6 ± 4.4	75.7 ± 1.7	74.7 ± 2.1

*$P < 0.05$; **$P < 0.01$ for comparison between control and inoculated samples. †Mean ± SD ($n = 9$). DAT, days after transplanting.

et al. 2002; Suzuki *et al.* 2009), and the utilization of dicarboxylic acids is considered one of the metabolic characteristics of PGPR (Rudrappa *et al.* 2008). In addition, the genome of *Azospirillum* sp. B510 possesses multiple symbiotic sugar transporters and a diverse set of malic enzymes, suggesting that B510 can potentially utilize C4-dicarboxylate in its symbiotic relationship with a host plant (Kaneko *et al.* 2010). Thus, root functionality, such as the secretion of dicarboxylic acids, could be affected by both rice genotype and nutrition conditions. Consequently, these environmental factors could synergistically modify and complicate the interaction between *Azospirillum* sp. B510 and rice plants.

Watanabe and Lin (1984) reported that treatment with *Azospirillum lipoferum* strain 34H to the root zone of rice plants promoted early tillering and reproductive growth of the host plants. Similarly, the present study showed that the inoculation of *Azospirillum* sp. strain B510 to rice plants could increase the tiller number. Furthermore, this bacterium has also been shown to increase the tiller number and seed yield of NN under field conditions in Hokkaido, Japan (Isawa *et al.* 2010), possibly because an increase in tiller number directly results in an increase in seed yield in high latitude areas, such as Hokkaido. The complex relationship between rice plants and B510 could be explained by the fact that *Azospirillum* sp. can interact with plants through several mechanisms, including the production of growth hormones and the assimilation of nitrogen compounds from soil and air (Garcia De Salomone and Dobereiner 1996). Correspondingly, different plant genotypes can react positively or negatively with a strain of *Azospirillum* sp. under different environmental conditions. Interestingly, the strong positive reaction under LN conditions was observed for the NI cultivar (Fig 1d). Since the B510 strain was originally isolated from the NI cultivated in the same experimental field used in the present study (Elbeltagy *et al.* 2001), the result might indicate a high affinity between B510 and NI. However, it was observed that tiller numbers of some cultivars decreased by inoculation with B510. Lynch (1982) suggested that inoculation of bacteria inhibited growth of plants to compete nutrition in some cases, although the bacteria contributed to the growth as PGPR under other conditions. It has been shown that inoculation of PGPR reduces root volume, depending on plant genotype (Dubeikovsky *et al.* 1993; Elliott and Lynch 1985). Therefore, the negative effects of inoculation *Azospirillum* sp. strain B510 on some cultivars might cause stress by competing for nutrients between rice and B510, or chang-

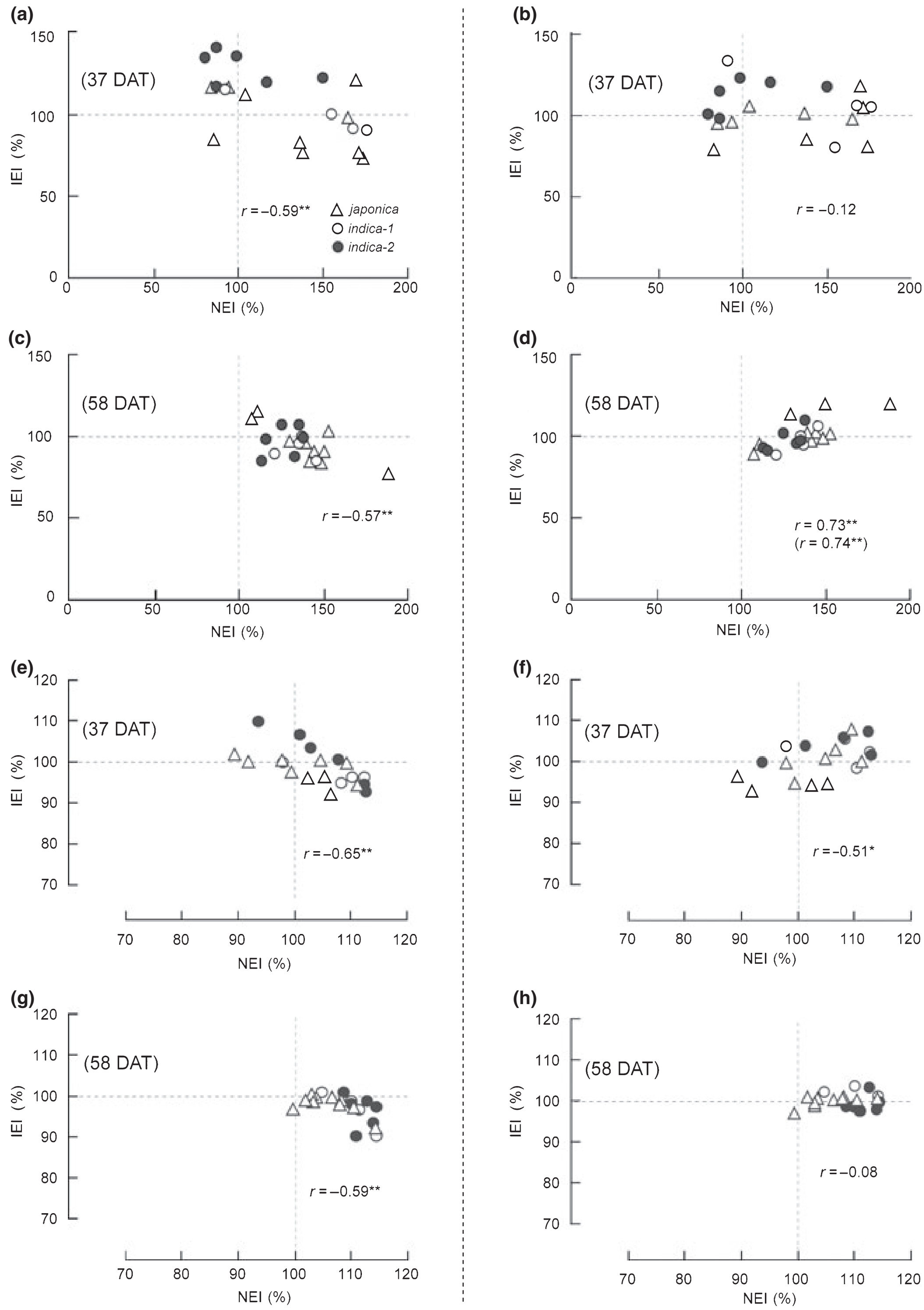

Figure 2 Correlation analysis between the inoculation effect index (IEI, %) under standard nitrogen (SN) and low nitrogen (LN) field conditions and the nitrogen effect index (NEI, %), for tiller number and shoot length in each genotype of rice plant. (a) IEI and NEI for tiller number 37 days after transplanting (DAT) in the SN field, (b) 37 DAT in the LN field, (c) 58 DAT in the SN field, (d) 58 DAT in the LN field. (e) IEI and NEI for shoot length 37 DAT in the SN field, (f) 37 DAT in the LN field, (g) 58 DAT in the SN field, (h) 58 DAT in the LN field. In (d), the correlation coefficient in parentheses was calculated, excluding Nipponbare. IEI (%) = (tiller number of rice plants inoculated with B510)/(tiller number of rice plants without inoculation) × 100. NEI (%) = (tiller number of rice plants in SN field)/(tiller number in LN field) × 100. *$P < 0.01$; **$P < 0.05$.

ing root morphology/functionality under the experimental conditions employed.

A correlation analysis revealed a significant negative correlation between IEI and NEI for tiller numbers at both 37 and 58 DAT in the SN field (Fig. 2a,c). Some cultivars in the *indica-2* subgroups, showing a LN effect, positively responded to inoculation with B510 under SN conditions (Fig. 2a). These results suggest that rice genotypes, which have a weak response to nitrogen fertilization, might be positively affected by inoculation with B510 in the SN paddy field. In contrast, there was a significant, positive correlation between IEI and NEI for tiller numbers at 58 DAT in the LN field (Fig. 2d). Furthermore, there was no significant correlation between IEI and NEI at 37 DAT in the LN field (Fig. 2b). Some *japonica* rice cultivars showing a higher nitrogen effect positively responded to inoculation with B510 under LN conditions (Fig. 2d). This result suggests that rice genotypes, which have a positive response to nitrogen fertilization, might be positively affected by inoculation with B510 in the LN paddy field. Therefore, it is considered that the impact of inoculation with B510 depends on responsiveness to nitrogen fertilization in each rice genotype of rice and the nitrogen level in the paddy field.

In conclusion, the results of the present study suggest that the effects of inoculation with *Azospirillum* sp. strain B510 on the growth of rice plants, especially on the tiller number at the early growth stage, vary depending on the rice genotype, as well as nitrogen level. Moreover, we observed cross-effects between the rice genotype and nitrogen fertilization level. Therefore, it is necessary to consider plant genotype and growth stage, as well as nitrogen fertilization levels, to evaluate beneficial plant-associated bacteria, such as azospirilla, under field conditions.

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