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Effect of *Rhizobium* inoculation to nodulation and growth of soybean [*Glycine max* (L.) Merrill] germplasm

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ABSTRACT
Nitrogen is an important macronutrient mineral that is needed in the largest amount by the plant and is the main limiting factor for plant development. *Rhizobium* is a group of bacteria capable of providing nutrients for soybean crops. When symbiotic with legume crops, this group of bacteria is able to infect plant roots and form root nodules. The study aimed to investigate the effect of *Rhizobium* on nodulation and growth of some soybean germplasm. The results showed no interaction between the inoculation treatment and the genotype. The significant effect of inoculation was shown by the number of nodules, nodule dry weight, root length, and root dry weight. Inoculation did not affect plant height and shoot dry weight. These traits were more influenced by genetic factors. In the condition without inoculation, the root length was higher but the root dry weight was lower. Plant height differed between the genotypes. KPT5 and KPT6 showed the highest plant height than any other genotypes. Both genotypes also had the highest shoot dry weight. The highest shoot dry weight was also achieved by KPT4.

Key words: Nodule, *Rhizobium*, Soybean.

INTRODUCTION

Nitrogen is an essential macro nutrient mineral that is needed in the largest amount by plants and is a major limiting factor for plant development, growth and overall harvest (Simon *et al*., 2014). Effective use of nitrogen fertilizer will increase food security (the amount of food produced) and nutritional safety (e.g., food protein content). Sustainable agriculture requires more efficient use of nitrogen. The efficiency of nitrogen use tends to decrease as the available supply of plant nitrogen increases. The need of nitrogen for supplying soybean seeds during seed filling is very high and has been proposed as the cause of nitrogen remobilization and leaf aging (Golparvar *et al*., 2012). In a study conducted by Flavio *et al*. (2004), fertilization with 80 kg N ha^{-1} at the beginning of the seed filling period extended 3 days seed filling period. To produce 1 t ha^{-1} soybean requires 70 kg N, 7 kg P, and 43 kg K per ha (Manshuri, 2010).

Rhizobium is a group of bacteria capable of providing nutrients for soybean crops. When symbiotic with legume crops, this group of bacteria is able to infect plant roots and form root nodules. Root nodule serves to take nitrogen in the atmosphere and channel it as nutrients needed by host plants. *Rhizobium* can donate N in the form of amino acids to soybean plants (Aminu *et al*., 2015). *Rhizobium* fixing nitrogen occurs when atmospheric nitrogen is converted to ammonia by enzyme nitrogenase, and microbial genes are required to fixate nitrogen to be widely distributed into the environment (Gaby and Backley, 2011). Rhizobia can live in plant residues (saprophytes) or all of them in plants (endophytes) or closely related to plant roots (rhizobacteria) (Mohammadi and Sohrabi (2012). The study of Aminu *et al*. (2015) show that the number of nodules formed is high if the soil is inoculated with *Rhizobium*.

Rhizobium can be symbiotic with soybean plants, especially soybean plants and able to fixate air nitrogen to meet the nutrient needs of host plants and the environment. The interactions between root nodules and symbiotic bacteria have been studied through proteomics during the signal exchange and symbiotic growth (Simon *et al*., 2014). Rhizobia convert atmospheric nitrogen into ammonia, a form directly used by plants for the synthesis of amino acids and nucleotides. Thus there will be a symbiosis. This symbiotic relationship will benefit both sides. According to Furseth *et al*. (2012), soybeans can fixate 175 kg N per year in irrigated areas while in dry areas it is only able to slow as much as 100 kg N per year.

MATERIALS AND METHODS

Study site and plant materials: The study was conducted at the Indonesian Legume and Tuber Crops Research Institute

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(ILETRI) greenhouse, Malang, Indonesia. Plant materials were from Central Lombok, West Nusa Tenggar consisted of 12 soybean germplasm.

Experimental design: The study used factorial design that was arranged in a randomized complete block design. The first factor was the dose of inoculation, which consisted of two levels i.e. without inoculation (T0) and with inoculation (T1). The second factor was the soybean genotype consisting of 12 genotypes.

Planting: Before planting, sand was washed to remove the remnants of soil that can interfere in the process of root observation. The sand was then dried in the sunshine. The dried sand was filtered with a 1 × 1 cm sieve. Sand then put into polybag as much as 4 kg per polybag. Planting was done by making two planting hole as deep as 2 cm. Two seeds were planted in each planting hole. Thinning was done at 14 days after planting with two plants per polybag. Watering was done every day according to the needs of the plant.

Rhizobia inoculation: Inoculation of rhizobia was conducted at the planting using Nodulin™. The dosage used was 8 g for 1 kg seed. Nodulin™ was applied by mixing with soybean seeds that would be planted.

Observation and data analysis: Observation was conducted at 40 days after planting covering number of nodules, nodule dry weight, root length, root dry weight, plant height, and shoot dry weight. The data were analyzed with analysis of variance (ANOVA). The data were significantly re-analyzed using least significant difference. Data analysis is done using PKBT-STAT 2.1 software.

RESULTS AND DISCUSSION

Anova shows inoculation significantly affected nodular traits (number of nodules and nodule dry weight) and root traits (root length and root dry weight), while genotypes significantly affected plant height and shoot dry weight. There was no interaction between genotypes and inoculation on all observed traits (Table 1). The number of nodules produced by the plant without inoculation was lower than the plant with inoculation. (Fig.1). In the condition without inoculation, soybean can produce root nodules, because there is indigenous *Rhizobium* in the soil. Inoculation increases the number of nodules because the existing nodules in the soil are in small quantities. In this study, the not inoculated condition reached 8 nodules per plant, while inoculation condition reached 15 nodules per plant. Triadiati (2013) can obtain nodules of up to 104 per plant using suitable *Bradyrhizobium japonicum* strains.

Inoculation showed a significant effect on nodule dry weight, while the genotype and interaction did not affect this trait (Table 1). The inoculated genotypes produced higher nodule dry weight than not inoculated genotypes. The dry weight of the nodules in this study (Fig. 2) is equivalent to the study of Hungria *et al*. (2015) which reached 24 mg

Table 1: Analysis of variance of soybean root and shoot traits

SV	NN‡	NW†	RL	RW‡	PH	SW
Inoculation (I)	11.132**	13.415**	14.720**	41.822**	2.593	2.672
Genotype (G)	0.234	0.024	1.004	2.056	23.679**	5.052**
G x I	0.472	0.027	0.900	0.412	0.945	0.683
CV(%)	51.95	11.74	12.43	7.44	6.09	12.39

†=transformed by using√(Y+1), ‡=transformed by using √(Y), SV=Source of variation, NN=number of nodules, NW=nodule dry weight, RL=root length, RW=root dry weight, PH=plant height, SW=shoot dry weight

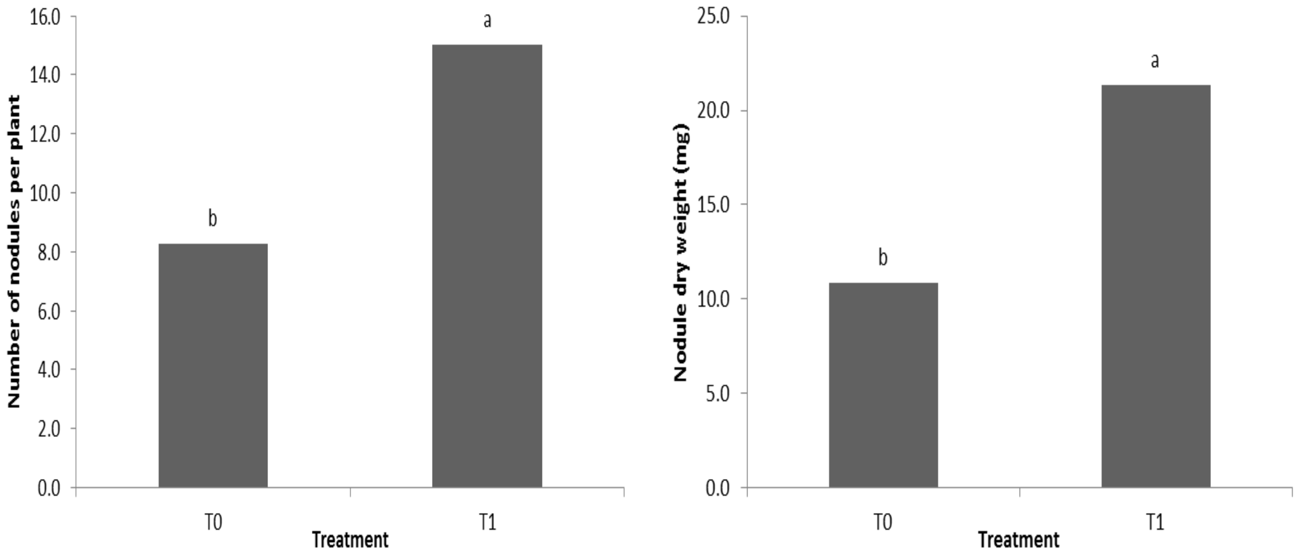

Fig 1: Number of nodule per plant and nodule dry weight of soybean germplasm without inoculation (T0) and inoculation (T1)

nodule dry weight and 18.9 number of nodules, but lower than those reported by Triadiati *et al*. (2013) with 40 mg nodule dry weight and 17 number of nodules. The nodule dry weight, reported by Triadiati *et al*. (2013), varies because in the same study one nodule can reach 40 mg. Heritability of nodule dry weight per plant and individual nodule dry weight is moderate (Hwang *et al*., 2014), which means genetic and environmental factors have an equal effect.

Analysis of variance showed that inoculation had a significant effect on root length, while genotypes and interactions had no significant effect (Table 1). Root length without inoculation was longer than root length with inoculation (Fig. 2). Differences in the availability of nutrients can change the root architecture. Plants that lack a nutrient try to meet it by extending the roots. Conversely, plants that have sufficient nutrient availability will not attempt to get the nutrients so that there is no change in plant architecture. It also occurs in *Arabidopsis*, where the roots become shorter in the availability of more nitrate (López-Bucio *et al*., 2003). Changes in root architecture are also sometimes associated with the availability of other elements that can negatively affect soybean growth (Sheirdil *et al*., 2012).

Unlike the root length, inoculated soybeans had higher root dry weight than soybean without inoculation (Fig. 2). It may be due to the growth of root substance including lateral roots that develops better with the supply of nitrogen resulting from nitrogen fixation by root nodules. López-Bucio *et al*. (2003) reported that the lateral root density remained constant at the treatment with different nitrate concentrations. Therefore, it is assumed that the increasing root dry weight can also be characterized by the addition of root diameter or the multiplying number of lateral roots as a result of the addition of the root mass.

Plant height was influenced by the genotypes, but not influenced by inoculation treatments as well as no interaction between genotype and inoculation (Table 1). The absence of inoculation effects on plant height is presumably due to the inoculation was less, or the soil already had equivalent *Rhizobium* to produce optimal plant height. Moreira *et al*. (2015) also reported no increase in plant height on the addition of nitrogen. KPT6 was the soybean genotype that had the highest plant height (Fig. 3). Plant height heritability is not consistent among the studies, where the values vary from low (Kuswantoro, 2017a), medium (Kuswantoro, 2017b), to high (Baraskar *et al*., 2014). These results indicate that the different genotypes might generate different heritability causing a different response to an environment such as *Rhizobium* inoculation.

Shoot dry weight in this study did not differ between inoculation treatments but differed among the genotypes that indicate the genetic factors play a higher role than inoculation. KPT5 showed the heaviest shoot dry weight followed by KPT4 and KPT6, while GDK5 showed the lowest shoot dry weight compared to other accessions (Fig. 4). A similar result was also reported by King *et al*. (2014), where the use of different genotypes could provide significant or insignificant shoot dry weight depending on the tested genotypes. It may occur because in the treatment without inoculation were also obtained nodules. Nevertheless, Hungria *et al*. (2015) reported inconsistencies in shoot dry weight depending on the location of the study.

Root length and number of nodules per plant formed a regression line with the equation y=-0.086+19.62 in the treatment without inoculation. The root length with number of nodules and nodule dry weight had a positive relationship in inoculation treatment (Fig. 5) that shown by the regression lines of y=0.129x+0.8697 and y=0.348x+57.84 respectively. The angle formed by the relationship between root length

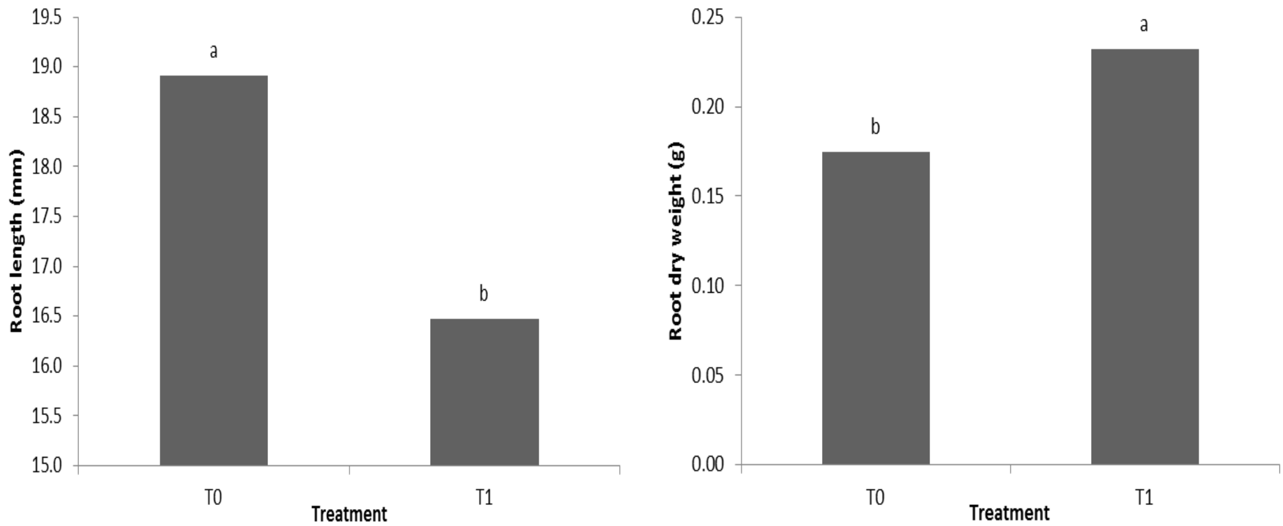

Fig 2: Root length and root dry weight of soybean germplasm without inoculation (T0) and inoculation (T1)

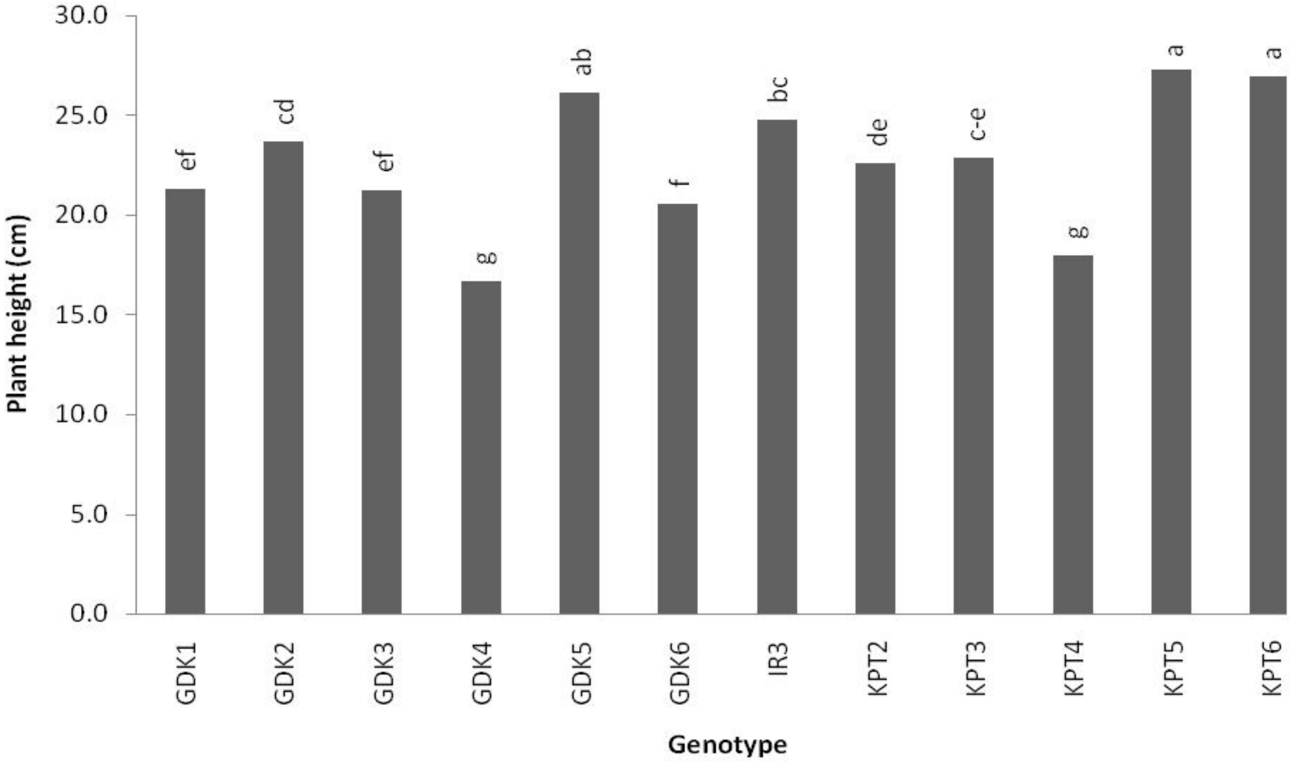

Fig 3: Plant height of soybean germplasm

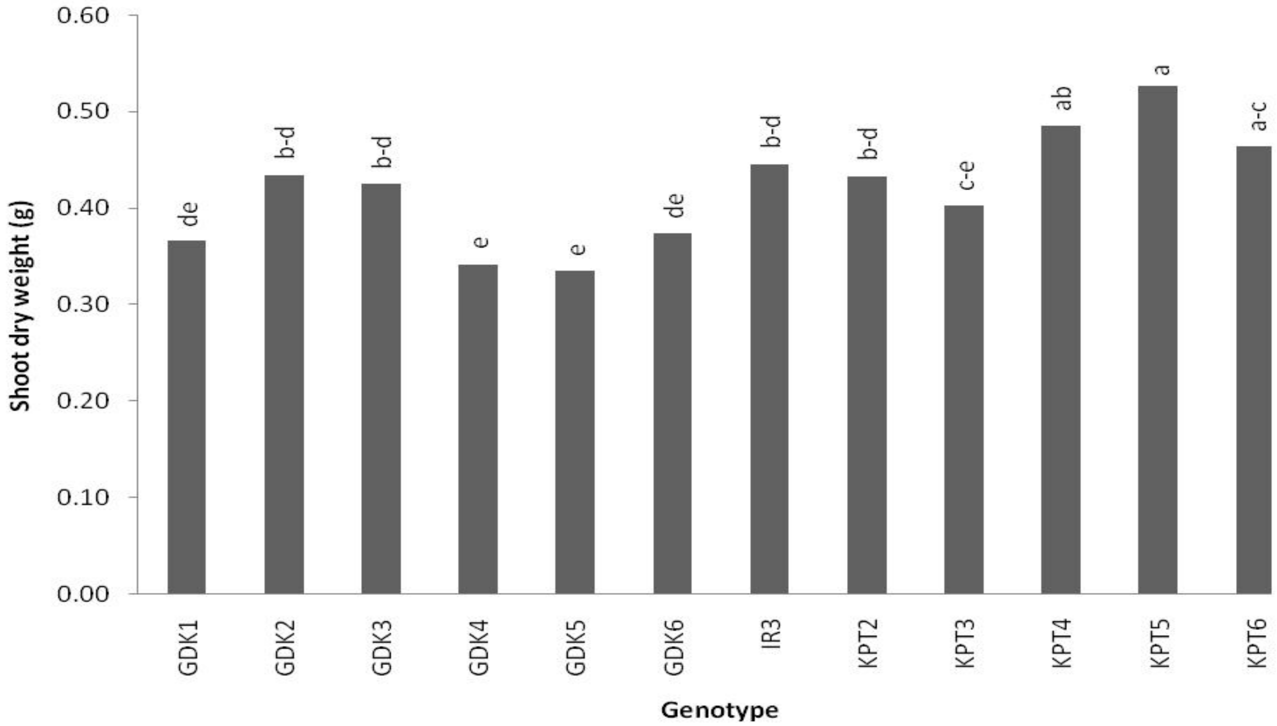

Fig 4: Shoot dry weight of soybean germplasm

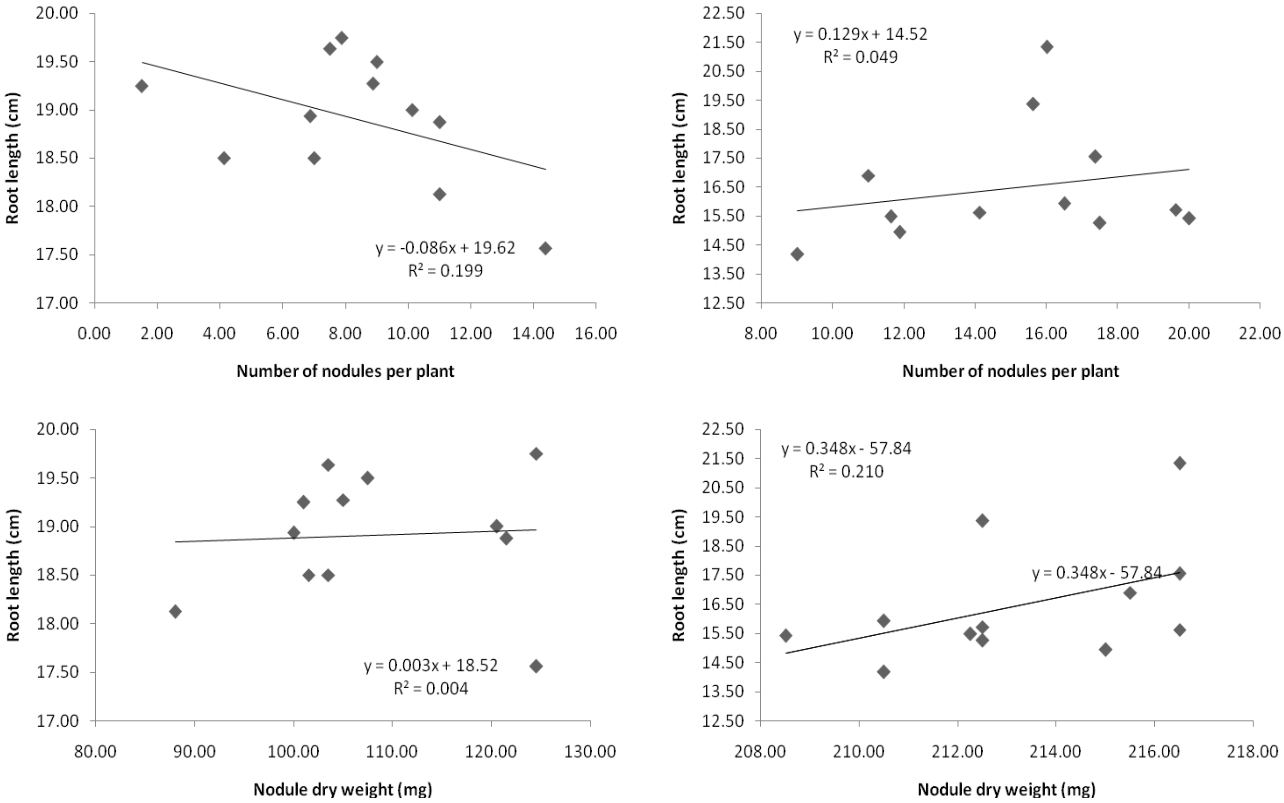

Fig 5: Relationship between root length with number of nodules and nodule dry weight in no inoculation (left) and inoculation (right)

and nodule dry weight was greater than the angle formed by number of nodules and root length. This means that *Rhizobium* inoculation enabled increasing number of nodules and nodule dry weight that caused higher root length. Abbasi *et al*. (2008) reported a significant difference in the number of nodules and nodule dry weight in *Rhizobium* inoculation treatment.

Regression between root dry weight with number of nodules in the treatments without inoculation or with inoculation was positive following the equations of y=0.000x + 0.172 and y=0.002x+0.200, respectively. The relationship between nodule dry weight and root dry weight in the treatment without inoculation tended to be negative with the regression equation of y=-2E-5x+0.177. In contrast, the regression between nodule dry weight and root dry weight was positive when inoculated with the equation of y=0.004x-0.802 (Fig. 6). The number of nodules was negatively correlated with individual nodule weight and size, because nodule number, individual nodule weight, and size contribute to total nodule weight (Hwang *et al*., 2014). There was no significant relationship among shoot dry weight, root dry weight, shoot length and root length (Stajkoviæ *et al*., 2011).

The relationship between number of nodules with plant height tended to be positive either in the treatment without inoculation or inoculation following regression equation y=0.389x+19.74 and y=0.370x+16.77, respectively. In contrast, regression generated from plant height and nodule dry weight tended to be negative (y=-0.037x+26.98). There was a tendency that low dry weight nodules will produce high soybean plant in the treatment without inoculation (Fig. 7). The regression lines formed by nodule dry weight and plant height was positive (y=0.053x+10.94). Nitrogen had a significant effect on plant height (Abbasi *et al*., 2008). If the plant is inoculated, the plant height will increase followed by the increase in shoot dry weight. Stajkovic *et al*. (2011) reported no significant correlation between the number and weight of dry nodule with plant height.

The relationship between shoot dry weight and number of nodules in the treatment without inoculation followed the regression line of y=-0.002x+0.424. Nodule dry weight and shoot dry weight were negatively related to the treatment without inoculation, where the angle was larger compared to the angle between number of nodules and shoot dry weight with the regression line of y=-0.002x+0.691. In the inoculated plants, number of nodules and nodule dry weight had a positive relationship with shoot dry weight following regression lines of y=0.01x+0.281 and y=0.008x+1.287, respectively (Fig. 8). The number of nodules is significantly correlated with yield dry matter (Abbasi *et al*., 2010). Schweiger *et al*. (2014) also obtain a

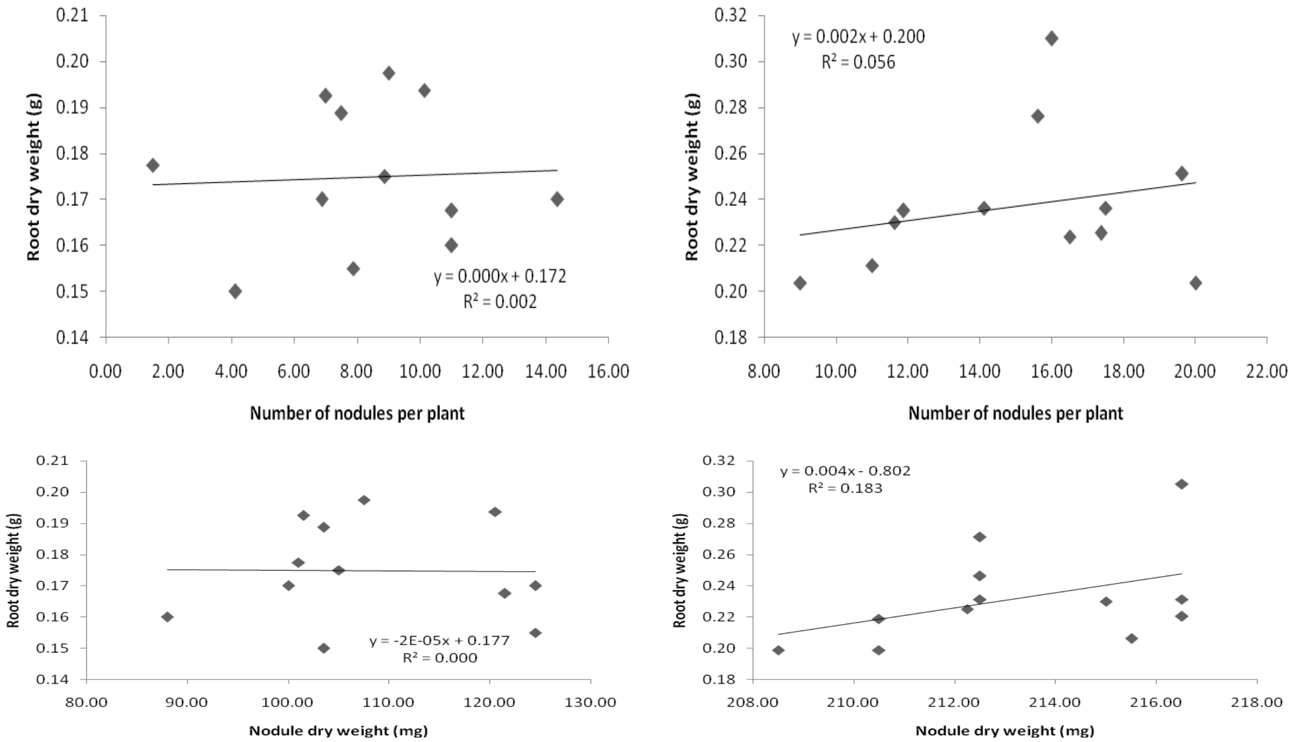

Fig 6: Relationship between root dry weight with number of nodules and nodule dry weight in no inoculation (left) and inoculation (right)

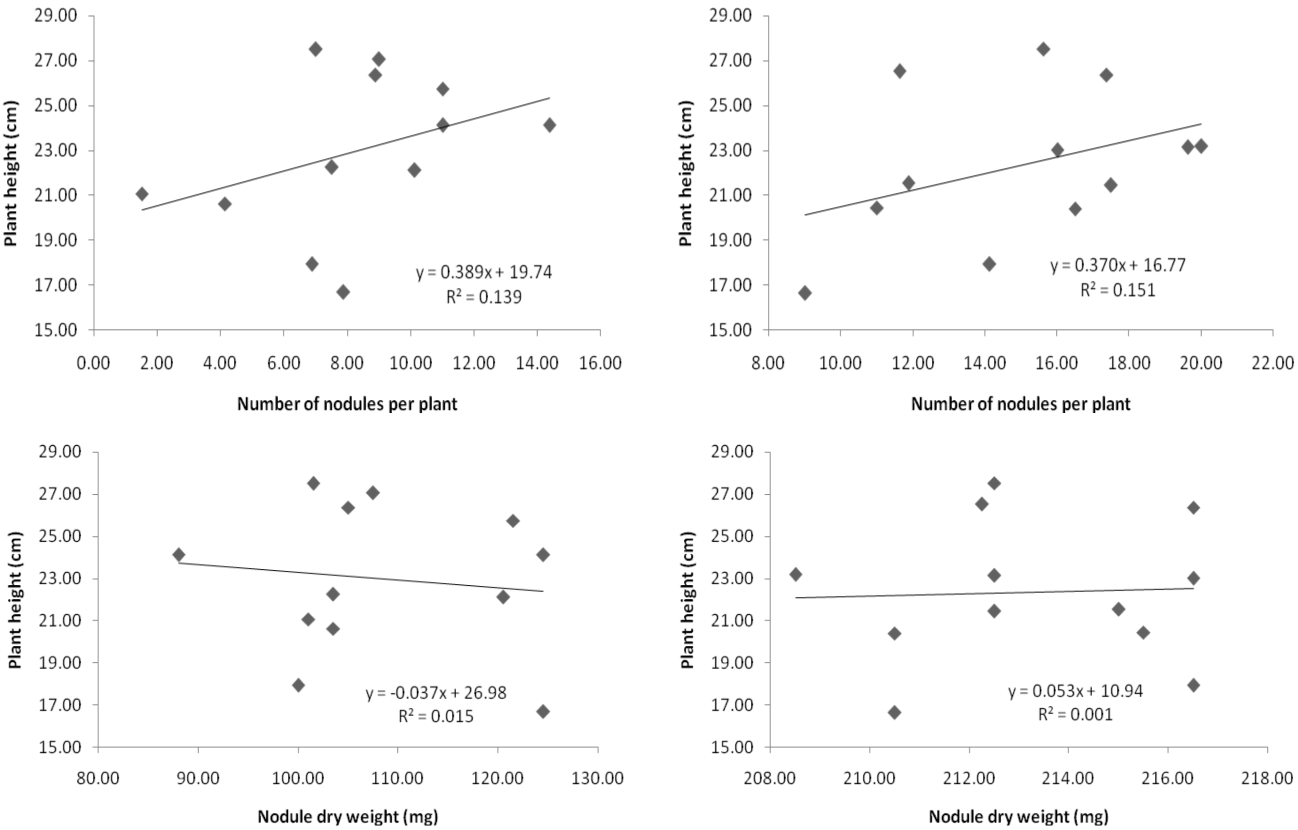

Fig 7: Relationship between plant height with number of nodules and nodule dry weight in no inoculation (left) and inoculation (right)

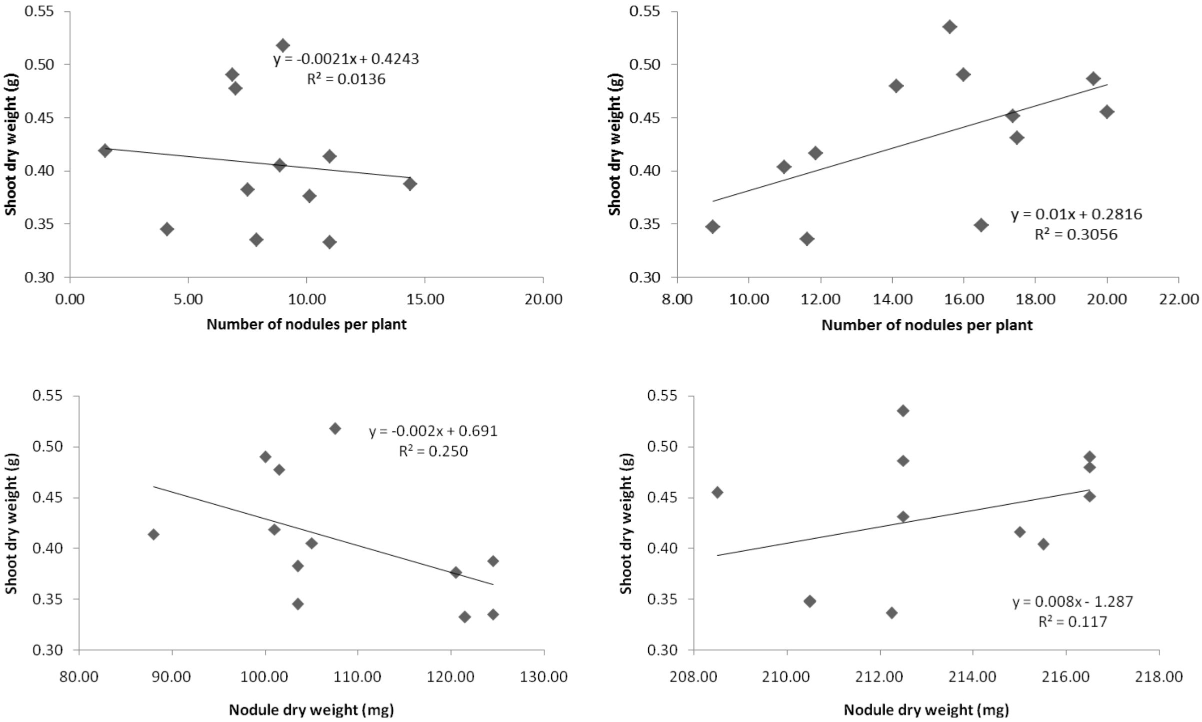

Fig 8: Relationship between shoot dry weight with number of nodules and nodule dry weight in no inoculation (left) and inoculation (right)

weak relationship between the N2-fixation with the N content in plant shoot.

CONCLUSION

The inoculation treatment had a significant effect on number of nodules, dry weight of nodules, root length, and root dry weight, while the genotype had a significant effect on plant height and shoot dry weight. No interaction between genotype and inoculation was observed in all observed characters. Inoculation increased the number of nodules and nodule dry weight. Root length under conditions without inoculation was longer than the condition with inoculation, as the plant attempts to meet the nutrient deficiency by lengthening the roots. The root dry weight was higher in the condition with inoculation than in the condition without inoculation, although the root length was lower in the condition with inoculation. The highest plant height was shown by KPT5 and KPT6. The highest shoot dry weight was obtained by KPT5 followed by KPT4 and KPT6.

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